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

1995 Annual Report

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About the Committees

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

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

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

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

The report contains, at Annex 4, an alphabetical index to subjects and substances considered in previous reports. A second index, at Annex 5, contains details of the subjects on which the COT has given advice since 1987 as part of its consideration of the results of surveillance for chemicals in the UK diet. These considerations are published in the Food Surveillance Papers which report this surveillance work, rather than in the Committee's annual reports.

The usual way in which committee reviews are conducted is that the relevant secretariat critically assesses all the relevant data and prepares papers for the Committee. These normally consist of appendices giving detailed summaries of the studies reviewed – methodology and results – and a covering paper in which the available data are briefly summarised, the most important points highlighted

and recommendations presented for discussion by the Committee. Although original study reports are not routinely circulated to members, they are made available on request, and are circulated if the study is particularly complex. Definitive summaries are necessary because documentation on any one chemical can amount to many hundreds of pages. The Committees cannot undertake to review information provided by industry that has not been forwarded through or discussed with the appropriate Secretariat.

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

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

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Preface

In the past, the main role of the Committee on Toxicity of Chemicals in Food , Consumer Product and the Environment (COT) has been to advise on the safety-in-use of food additives and on contaminants in food. In recent years the work on food additives has lessened as the European Union has assumed responsibility for legislation in this area and assessment of the safety of food additives has increasingly been undertaken by the European Commission's advisory body, the Scientific Committee for Food (SCF). Nevertheless, there has been no reduction in the COT's workload. Instead, the expertise of committee members was challenged in 1995 by requests for advice on a diverse range of chemicals, as indicated in this year's report.

During the year the Committee was asked by the Department of Health to review of the effects of alcohol on pregnancy, reproduction and infant development, as part of a review of the Sensible Drinking Limits. This proved to be a challenging task and, given the nature of studies available for review, one in which only broad recommendations could be formulated. Another major task was an update of the Committee's advice on the toxicity of dioxins, which was undertaken following the publication of a health assessment of these environmental pollutants by the US Environmental Protection Agency (EPA). This review marked a new initiative for the Committee. Because of the specialised nature of the information, reviews of individual chapters of the EPA document were undertaken and presented by a committee member with expertise in the relevant area, rather than by the secretariat. I am indebted to those members for their work and, indeed, to all members for the hard work and commitment they have shown during the year.

Among other topics covered in 1995 was a review of the artificial sweetener cyclamate, of the potential adverse effects to operating theatre personnel of the anaesthetic gas nitrous oxide, and of draft Food Surveillance Papers on pesticide residues and on metals in food. Requests for advice on these and other issues came from a variety of sources.

The Committee is grateful to its secretariat and other officials from the Department of Health and MAFF and from other Government Departments, who have provided the high quality information needed to facilitate its work during the year.

FRANK WOODS

Boron in Drinking Water and Food

1.1 The COT was asked by the Department of the Environment to advise whether, on public health grounds, it was advisable to reduce actual exposure, or to limit potential exposure, to boron in drinking water. This request arose following publication by the World Health Organisation (WHO) of a guideline value of 0.3 milligrams per litre (mg/l) for boron in drinking water and a European Commission proposal on the revised Drinking Water Directive 1994, which included a maximum allowable concentration of 0.3 mg/l boron. A substantial proportion of water supplies in the UK exceed this level.

1.2 The Committee reviewed the available toxicity data on boron and boric acid at meetings in 1994 and 1995. It agreed that the critical issue was adverse effects in developmental studies in the rat and derived a No Observed Adverse Effect Level (NOAEL) of about 10 mg per kilogram body weight per day (10 mg/kg bw/day) from these studies. This was close to the NOAEL used by WHO although that had been derived from a series of toxicity studies in dogs which the COT considered to be unreliable due to small animal numbers and inadequacies in the histopathological descriptions. Using the usual safety factor of 100, a Tolerable Daily Intake (TDI) derived from the NOAEL would therefore be 100 micrograms (μg)/kg bw/d. The Committee noted that the WHO guideline value was based on an arbitary but conventional allocation of 10% of the TDI value for intake from drinking water.

1.3 The COT considered that there was no evidence that dietary boron is beneficial. The Committee concluded that there was no evidence to suggest that the current actual or permissible concentration of boron in statutory drinking water supplies was a hazard to health but, in order to ensure a reasonable margin of safety was maintained, the practicability of lowering the maximum permitted concentration in the UK from 2mg/l to 1 mg/l should be explored. It further concluded that there was no evidence that the dietary intake of boron in the UK was hazardous to health.

Cyclamate

1.4 During 1995 the COT reviewed new studies on the artificial sweetener cyclamate and agreed the following statement:

(i) We last considered the safety-in-use of the artificial sweetener cyclamate in 1990, when we reviewed the available data in three areas where concern had previously been expressed either about cyclamate or about its metabolite, cyclohexylamine (CHA), which can be produced by microbial fermentation of unabsorbed cyclamate in the gut. The three areas were: (a) the potential of cyclamate for carcinogenic effects, (b) the potential for cardiovascular effects from CHA and (c) the testicular toxicity caused by CHA in some laboratory animal species.

(ii) We concluded that there were adequate data to conclude that the use of cyclamate in food would not present a carcinogenic hazard to man. We also concluded that there was no evidence of cardiovascular effects due to CHA in man up to the highest dose tested (11 mg/kg bw/day). However, we reiterated our concern about the irreversible testicular atrophy which occurs

in the rat when CHA is fed above a threshold level for 3 weeks or more. We considered that although the rat appears to be particularly susceptible to this adverse effect of CHA compared to the other species which had then been studied, there was no reason to believe that man might not also be sensitive to CHA. We set a temporary Acceptable Daily Intake (ADI) of 0–1.5 mg/kg bw for cyclamate and its sodium and calcium salts, which was based on the NOAEL for CHA in the rat and incorporated a conservative safety factor of 200.

(iii) We have now reviewed the results of new studies on CHA in monkeys. These studies, although limited (because of the high systemic toxicity of CHA in monkeys), indicate that CHA can cause testicular toxicity in this species also, but the severity of effect is less than in the rat. In the principal study, CHA was administered in capsules twice daily to monkeys at doses which increased from 34 mg/kg bw/day to 100 mg/kg bw/day over a 7 week period. There was a decrease in testicular size and in testosterone levels in the monkeys receiving CHA which we consider to be a specific effect of treatment. In terms of the plasma concentrations of CHA achieved at these doses, we conclude that monkeys are as sensitive to CHA as rats (ie effects occur at similar plasma concentrations) but the severity of effect is less in the monkey and the type of effects seen are likely to be reversible. We regard this as reassuring and it has enabled us to reconsider the ADI for cyclamate, using the monkey data to adjust the safety factor (see below).

(iv) In deriving the new ADI, we have continued to use the well-established NOAEL for CHA in the rat, of 100 mg/kg bw/day. We also consider it appropriate to continue to use a figure of 60% for the percentage conversion of cyclamate to CHA (40% of the ingested dose of cyclamate is absorbed before it reaches the lower gastrointestinal tract, and so only 60% is available for conversion to CHA). We recognise that this is a conservative approach in that less than one percent of the population is likely to exhibit such a high conversion rate, nor is it likely to be maintained consistently by any individual. Therefore, as in 1990, we have compensated for use of a very high conversion rate by using a safety factor of 5, rather than the usual value of 10, to account for variability between individuals in the human population. We have continued to use a factor of 10 to allow for variability in response between the animal model and man.

(v) Since the response of monkeys to CHA was less severe than that seen in the rat, we no longer consider it necessary to use an extra safety factor of 2 for severity of effect; nor do we consider that it is necessary to set only a temporary ADI. Thus we have derived an ADI_{1995} for cyclamate of 0–6 mg/kg bw. The derivation of the ADI is shown in detail below. We have also reviewed the available data on plasma concentrations of CHA in the rat and monkey at the NOAEL and at effect levels and compared these with data on plasma CHA concentrations in man given various doses of both CHA and cyclamate. These data provide additional assurance that an ADI of 0–6 mg/kg bw is appropriate.

(vi) In conclusion, therefore, we ***recommend*** an ADI_{1995} for cyclamate and its sodium and calcium salts of 0–6 mg/kg bw, expressed as cyclamic acid.

(vii) *Derivation of the COT ADI_{1995} for cyclamate:* the ADI for cyclamate is derived by applying an appropriate safety factor to the NOAEL for cyclamate.

However, the adverse effect on the basis of which the ADI is set is caused not by cyclamate, but by its microbial metabolite CHA. The NOAEL for cyclamate can be derived from the known NOAEL for CHA in the rat as follows:

$$\text{NOAEL for cyclamate} = \text{NOAEL for CHA} \times \frac{\text{MW for cyclamate}}{\text{MW for CHA}} \times \frac{100}{\%\ \text{metabolism}}$$

where MW is molecular weight and '% metabolism' is the % conversion of cyclamate to CHA. The NOAEL for CHA is 100 mg/kg bw/day, the ratio of molecular weights is 1.81 and the conversion rate used is 60%.

(viii) Thus, the NOAEL for cyclamate is 302 mg/kg bw/day.

(ix) The following safety factors were used:

- a factor of 10 to allow for extrapolation between rats and man and
- a factor of 5 to allow for interindividual variation.

Thus the ADI_{1995} is $0 - \frac{302}{5 \times 10}$

ie 0–6 mg/kg bw.

Effects of Ethanol Intake on Pregnancy, Reproduction and Infant Development

1.5 An Interdepartmental Group was established in 1994 to review the current health and related advice on ethanol intake, in the light of increasing evidence of some beneficial effect of low doses of ethanol in adults on coronary heart disease. Previously, the Government has advised that if men drink less than 21 "units" per week and women drink less than 14 "units" per week (equivalent to 168g and 112g ethanol per week respectively), they are unlikely to damage their health. As part of this Interdepartmenal Review, the Committee was asked for advice on the harmful effects of ethanol on pregnancy, reproduction and infant development. The Committee's advice is given below. The advice has been published elsewhere[1].

(i) Many studies have been carried out in the last 20 years to try to identify the effects of ethanol in pregnancy and to establish intake levels at which these effects occur. A major problem in interpreting the human studies is the large number of confounding factors, including poor nutrition, licit and illicit drug intake and smoking, each of which can have adverse effects on pregnancy. Other factors also contribute to the variability of these studies; including difficulty in verifying intake of ethanol, different patterns of consumption and polymorphism in ethanol metabolism. In post-natal developmental studies, environmental factors are also critically important.

(ii) Despite all the variables, there is general agreement, from both human and animal studies, that ethanol has the potential to induce the following effects:– abortion; fetal growth retardation; facial and other dysmorphologies; and impaired post-natal physical and mental development.

[1] Department of Health. *Sensible Drinking: The Report of an Inter-Departmental Working Group*. Department of Health, London, December 1995.

(iii) Most studies agree that 2 drinks[1] per day and above may be associated with reduced birthweight which is one of the most sensitive parameters. Some studies have found effects at lower levels, but most have not. However, there is no good evidence that 1 or 2 drinks[1] per week has any adverse effect.

(iv) There are both human and animal data that suggest that binge drinking can also produce the adverse effects listed in paragraph (ii). There is evidence that adverse effects can be induced at all stages of pregnancy.

(v) The full spectrum of physical and mental handicaps known as Fetal Alcohol Syndrome is only seen in the offspring of alcoholic women. On the other hand, adverse effects on cognitive and behavioural development might be observed as indicators of ethanol-induced damage in the offspring of women with lower ethanol intakes.

(vi) There is limited evidence that ethanol may also impair reproductive function in men and fertility in women, but this evidence is inadequate so far as the identification of the intakes at which these effects are induced.

(vii) The principal studies which have reported adverse effects of ethanol intake on pregnancy, reproduction and infant development have been summarised by our Secretariat in an Annex[2]. These studies give a balanced presentation of the data available and demonstrate the often conflicting results . This Annex also reports the effects of ethanol in animal reproduction studies and on the disposition of ethanol.

(viii) After due consideration, the Committee *recommends* that:–

(a) any new advice to pregnant women should be in terms of "units" of alcohol per day, since "binge drinking" can also affect the fetus.

(b) to any new advice which may be formulated on sensible drinking limits, a caveat should be added to the effect that:–

women who are pregnant or who are likely to become pregnant should keep their alcohol intake substantially below limits suggested for non-pregnant women.

Hemicellulase Preparations for us in Breadmaking

1.6 During 1995, the COT considered submissions from a number of companies on preparations of the enzyme hemicellulase produced by three different microorganisms – *Aspergillus niger, Bacillus subtilis* and *Trichoderma longibrachiatum*. Two of the companies used genetically modified microorganisms (GMOs). Whereas the COT considers the toxicity of the enzyme preparation, the Advisory Committee on Novel Food Processes (ACNFP), a joint committee of the Department of Health and the Ministry of Agriculture, Fisheries and Food (MAFF), considers the safety and use of GMOs. The production of hemicellulase preparations is relatively simple, involving the fermentation of the microorganism, extraction of extracellular

[1] Where one drink is defined as 8g of ethanol equivalent to one unit in the present "sensible drinking message".
[2] Available on request from the Secretariat.

enzymes, removal of microbial debris, ultrafiltration and formulation into the final commercial product. All of the enzyme preparations were intended for use in the manufacture of bread. The submission on each preparation was considered with respect to the Committee's guidelines for the safety assessment of microbial enzyme preparations used in food. In one case, the submission consisted of additional information requested by the COT when it originally reviewed the preparation in June 1994. The Committee was satisfied with the safety-in-use of this and the three other preparations and agreed to give temporary approval for use in bread-making. In most cases, the Committee asked for additional information on minor points, which did not preclude the preparation being given approval, or asked for confirmation from the company concerned of deposition of a sample of the production strain in a national culture collection – this being a requirement of the COT's guidelines.

1.7 Several manufacturers expressed concern with regard to confidentially if they deposited the production strain in culture collections and argued that their in-house culture collections could be accessed by the regulatory authorities if the need arose. However, the COT was made aware that a sample of any patented microorganism had to be deposited with a culture collection recognized as an International Depositary Authority under the 1960 "Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures and Patent Depositary Facilities in Europe". It was also informed that it was now a requirement under European legislation that samples of production strains of microorganisms used to produce enzyme preparations intended for animal feeds must be deposited in culture collections recognized under the Budapest Treaty. The COT decided to maintain this requirement in their guidelines. After being informed of the COT's decision, manufacturers of enzyme preparations which had received COT temporary approval deposited the production strains in recognized culture collections.

Lupins

1.8 The COT was asked in 1990 by the ACNFP for advice on the interpretation of the histological finding of altered liver foci in certain rats in a 90-day study to investigate the toxicity of alkaloids present in the seeds of *Lupinus angustifolius*. The use of these seeds as a novel food had been submitted for clearance by the ACNFP.

1.9 The COT was of the view that it was not possible to reach any conclusion regarding the significance of the liver foci in this study (the incidence did not follow any obvious dose relationship) because of problems with the mixing of the lupin alkaloid extract into the rat diet, and therefore asked that a further study be conducted. The company agreed to conduct a second study and the results from this were considered by the COT in 1995.

1.10 In addition, the COT considered other aspects of the toxicology of lupin alkaloids, including metabolism and excretion, and pharmacological activity. The COT also reviewed data on possible mycotoxin (phomopsin) contamination of lupin seeds, and the human data relating to possible allergic reactions to lupin-based foods.

1.11 The COT completed its evaluation of all the data presented to it in September 1995 and passed its advice to the ACNFP, for consideration as part of the overall safety evaluation of this novel food.[1] The COT's conclusions were as follows:

(i) the first toxicity study on lupin alkaloids was flawed for the purposes of determining a NOAEL, due to problems encountered with the mixing of the alkaloids in the diet and the resulting uncertainty regarding the actual doses consumed by the animals. Nevertheless, the study was still considered suitable for detecting potential target organs of toxicity;

(ii) the mixing of the diets was satisfactory in the second 90-day rat study on lupin alkaloids and this study is therefore acceptable as a contribution to the database on which the safety of lupin alkaloids can be assessed;

(iii) there were no significant adverse effects observed in the second study other than reduced food intake and bodyweight gain seen in the high dose group animals (5000 ppm lupin alkaloids in the diet), particularly at the beginning of the study, and slight decreases in erythrocyte counts and haemoglobin levels seen in the top dose group females only at 13 weeks; thus 1000 ppm lupin alkaloids in the diet can be taken as a NOAEL;

(iv) the disposition data demonstrate that the elimination of lupin alkaloids is not dependent on their metabolism and is thus unaffected by the hydroxylation phenotype of the individual;

(v) pharmacological effects of lupin alkaloids (blocking of ganglionic transmission, decreased cardiac contractility and contraction of uterine smooth muscles) have been seen in anaesthetised animals receiving bolus intravenous doses of 1–2 mg/kg bodyweight. However, in man, single oral doses of 10mg lupin alkaloids had no effect on cardiovascular parameters. Furthermore, it was noted that the maximum likely intake of lupin alkaloids in man from a variety of lupin-based food products that might be consumed during the day was estimated to be 4–6 mg/person. A multigeneration study in the rat indicated no effect on the uterus at doses of lupanine up to 7mg/kg bw/day;

(vi) a limit should be set for phomopsin in final lupin products intended for human consumption;

(vii) there have been occasional reports of allergic reactions to foods containing lupin flour, and allergenicity could arise in an occupational context via the inhalation of flour dust. Most individuals who reacted to lupin flour had known allergic reactions to other foods and there may be a cross-reaction with soya and pulses. The only real allergenic hazard is likely to be in an occupational context and this would probably be less of a problem than is the case with soya.

[1] Further information on lupins can be found in the 1995 Annual Report of the Advisory Committee on Novel Foods and Processes available from the ACNFP Secretariat at Ministry of Agriculture, Fisheries and Food, Room 239C, Ergon House, c/o Nobel House, 17 Smith Square, London SW1P 3HX

Malachite Green

1.12 Malachite Green is a substance which has been used for many years in the trout industry, as a water treatment, to treat fungal infections on eggs and for the control of certain diseases in fish. The COT considered it in 1993[1]. During 1995 the Committee saw more data on levels of malachite green residues in trout and gave further advice, in the form of the following statement:

(i) When we last considered this issue, we reached a number of conclusions, which were published in our annual report for 1993. In summary, we considered that there were insufficient data to permit a full safety assessment of malachite green and, therefore, that it had not been proven safe for use in fish farming. Nevertheless, we considered that the occasional trace residues found in farmed trout at that time would probably not adversely affect the health of consumers.

(ii) We have now considered further residue data from 406 samples of trout analysed as part of three surveys conducted between July 1993 and March 1995. These show that the incidence of malachite green residues is increasing and that the mean concentration found in the surveys was from 2 to 4-fold higher than that found in the 1992 survey. However, the potential exposure estimates for humans for malachite green *per se* continue to be very low (less than 1µg/kg bw/day).

(iii) No new data have become available on the toxicity of malachite green and there remains uncertainty about how the material used in fish farming compares to that used in those toxicological studies which have been carried out on this substance. We note that there are data suggesting that the predominant residue in fish treated with malachite green is leucomalachite green rather than parent substance and we *welcome* the steps being taken by the Veterinary Medicines Directorate to validate a methodology which will permit the contribution to the residue in trout from leucomalachite green to be assessed.

(iv) We consider that there are still insufficient data to reach a conclusion about the safety of this substance and we are concerned that the residue levels appear to be increasing. Although the residues found are unlikely to adversely affect the health of consumers, if this material continues to be used we *recommend* that toxicity data are obtained and that, in the first instance, appropriate genotoxicity studies should be carried out on malachite green and its major conversion products (the carbinol derivative and leucomalachite green).

Methylcyclopentadienyl Manganese Tricarbonyl

1.13 Methylcyclopentadienyl manganese tricarbonyl (MMT), has been widely used in Canada, and to a lesser extent, in Australia and the USA, as an octane improver in petrol instead of lead, although it has never been used in the UK. The primary health concern is not with MMT itself but with the manganese oxides, which are the major products found in exhaust emissions.

[1] Committees on Toxicity, Mutagenicity, Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. 1993 Annual Report. HMSO, London, 1994

1.14 A new product containing MMT (HiTec), has been developed as an additive for diesel fuel, for which it has been claimed that its addition results in significant reductions in emissions of certain gaseous and particulate pollutants. While the reduction in pollutant emissions from diesel-engined vehicles would be welcomed from a health perspective, it is important that emissions of manganese from combustion of MMT should not present any new risks to health. The Committee was asked to consider the toxicological aspects of any increased exposure to manganese arising from the use of this additive. The Committee's conclusions and recommendations were as follows:

(i) Occupational groups, such as garage workers, may be at risk of significant exposure to MMT via the lung and skin if the MMT-based additive, HiTEC, is added to diesel fuel. However, since MMT is decomposed rapidly in air, any environmental exposure is likely to be transient. Emissions of manganese to the environment from the combustion of HiTEC will be in the form of inorganic manganese compounds. The toxicological databases for assessing the risks to health of both MMT and inorganic manganese compounds are incomplete.

(ii) Predictive models developed in North America suggest that the increase in ambient concentrations of manganese from the introduction of HiTEC in diesel fuel would be small. The low levels predicted by the models are consistent with concentrations of particulate manganese in a number of Canadian cities measured over a period when MMT completely replaced the use of lead in petrol. In London, for example, ambient concentrations of manganese are predicted to increase by only 4% (0.0008 μg Mn/m^3) above current levels of about 0.02 μg Mn/m^3. Exposure to manganese arising from the use of HiTEC would not, therefore, add significantly to current total exposure from the diet, drinking water and air. These models may not, however, be entirely suited to the UK because of differences in climatic and driving conditions compared with Canada and the USA. Furthermore, the models are based on the use of MMT in petrol engines which emit manganese in a different chemical form than diesel engines.

(iii) Sub-clinical effects have been demonstrated in workers exposed to high concentrations of inorganic manganese compounds in respirable dust (PM_{5-7}). Performance in tests of neurobehavioural function (eg visual reaction time, eye-hand coordination, hand steadiness) was adversely affected in a dose-dependent manner with manganese exposure. The critical study is that conducted by Roels *et al* (1992). The NOAEL derived from this study is 32 μg Mn/m^3, which is five orders of magnitude greater than the predicted increase in ambient concentrations of manganese if HiTEC is added to diesel fuel. This provides a considerable margin of safety to allow for uncertainties in the toxicological database and to afford protection for vulnerable groups in the general population.

(iv) Nevertheless, since there are uncertainties in the exposure and toxicity of manganese from the use of HiTEC, we *recommend* that the adequacy of current arrangements for monitoring manganese in air in the UK are reviewed. We also *recommend* that a series of exposure assessment studies for manganese be carried out on groups heavily exposed to vehicle emissions, both before and after the introduction of HiTEC to diesel fuel. Consideration should also be given to monitoring exposure to MMT,

particularly at filling stations and underground carparks, and also among occupational groups such as garage workers.

Nitrous Oxide

1.15 Nitrous oxide is a gas which is used as an anaesthetic in hospitals. In 1993 the Health and Safety Executive (HSE) proposed an occupational exposure standard (OES) for nitrous oxide of 100 parts per million (ppm). This proposal was subsequently criticised by the Association of Anaesthetists as being unnecessarily stringent in that it was derived from animal toxicity studies rather than human studies. In view of the very considerable financial implications to the NHS, the Department of Health asked the COT for independent advice on the scientific basis of the proposed OES. In particular, the Committee was asked about the validity of basing it on a critical NOAEL of 500 ppm derived from developmental toxicity studies in the rat.

1.16 The COT reviewed the available data on the reproductive toxicity of this gas at its February meeting. It concluded that the large number of studies in the rat were consistent in showing adverse effects. At very high exposure concentrations there are teratogenic effects and at lower concentrations (1000 ppm and above) there are fetotoxic effects (reduced fetal size and weight). Mice seem to be far less sensitive to the foetotoxic effects of nitrous oxide, but this conclusion was based on a single study. The Committee agreed with HSE that it was appropriate to use 500 ppm as the critical NOAEL based on the rat data.

1.17 The COT also reviewed the available human data ie the results of studies which had investigated reproductive outcomes in people who had been occupationally exposed to nitrous oxide for prolonged periods. It concluded that these did not allow the animal data to be discounted. Indeed, the Committee considered that the human data, despite limitations, tended to reinforce the concerns which arise because of the effects seen in animals.

Novel Oils for use in Infant Formulae

1.18 The COT was asked by Advisory Committee on Novel Foods and Processes (ACNFP) for advice on the significance of a number of lesions reported in animal toxicity studies on novel oils for use in infant formulae. The novel oils contain high levels of the long chain polyunsaturated fatty acids arachidonic acid (ARA) and docosahexaenoic acid (DHA) which are important in neural development and visual cortical function in infants, particularly pre-term infants.

1.19 In a 9-week and a 13-week study on the novel oils ARASCO (ARA single cell oil) and DHASCO (DHA single cell oil) liver, kidney and gastric lesions were reported in both rats dosed with the oils and the positive control group. The lesions consisted of tubular basophilia and mineralization in the kidney, fatty change in the liver and gastritis and mucification in the stomach. In addition, an increased incidence of renal variations i.e. undeveloped renal papilla and dilated renal pelvis were reported in foetuses from rats dosed with ARASCO and DHASCO in a teratology study.

1.20 The Committee noted that the kidney changes reported in the 9 and 13-week studies were relatively common in rats and was satisfied that the kidney and the liver lesions reported in these studies were not caused by the administration of ARASCO and DHASCO *per se* but were due to the administration of a synthetic diet. The Committee was less certain about the gastric lesions since such lesions had not been reported in other studies which used synthetic diets and there was no obvious mechanism. However, the COT concluded that these lesions were probably also due to the synthetic diet.

1.21 The COT was also satisfied that the renal variations seen in the teratology study did not result from the administration of ARASCO and DHASCO. It had been suggested that the increased incidence of these renal variations was due to natural random variation. However, the COT considered that the difference probably resulted from inconsistent procedures used when examining the fetuses. The COT noted that it would have been more appropriate to carry out a study investigating the effects of exposure to these oils during parturition and early postnatal development than a teratology study.

1.22 The advice of the COT was passed to the ACNFP, which is awaiting advice from the Committee on Medical Aspects of Nutrition (COMA) on the nutritional implications of using these oils in infant formulae before deciding whether food safety clearance can be given[1].

2,3,7,8–Tetrachlorodibenzo-P-Dioxin [TCDD]

1.23 Various aspects of the toxicology of TCDD and related compounds (commonly known as dioxins) have been considered on several occasions by the COT, COM and COC. Dioxins are ubiquitous environmental contaminants and are generally present at very low concentrations in all foods, especially fatty foods and cows' milk. The COT has reviewed the health risks of dioxins twice in recent years. The first review was published in the Department of Environment Pollution Paper No.27, *Dioxins in the Environment 1989* and the second in MAFF's Food Surveillance Paper No. 31, *Dioxins in Food 1992*. The Committee completed a third review during 1995 which was prompted by the publication of a draft health assessment document from the US Environmental Protection Agency (US EPA). The purpose of the COT's latest review was to identify whether there were any new data or arguments in the US EPA document which would necessitate changing the risk assessment of dioxins and related compounds and/or revising the TDI of 10 picograms (pg)/kg bw/day. This TDI was originally recommended by an expert group convened by the World Health Organisation Regional Office for Europe in 1991, and was later endorsed by the COT. Following its 1995 review of the US EPA document on dioxins, the COT made the following statement:

(i) There is continuing public concern about the health hazards of polychlorinated dibenzo-p-dioxins (PCDDs) and related compounds in the

[1] Further information on the use of novel oils in infant formulae can be found in the 1995 Annual Report of the Advisory Committee on Novel Foods and Processes available from the ACNFP Secretariat at Ministry of Agriculture, Fisheries and Food, Room 239C, Ergon House, c/o Nobel House, 17 Smith Square, London SW1P 3HX.

environment. One of the most extensively studied PCDD congeners, 2,3,7,8–tetrachlorodibenzo-p-dioxin (2,3,7,8–TCDD), exhibits a broad range of toxic effects in laboratory animals, some at very low doses. In addition, these compounds are persistent and tend to accumulate in biological systems. These factors have inevitably led to concern about the human health significance of these compounds in the environment.

(ii) In 1989 we made a comprehensive statement about the health hazards of PCDDs and polychlorinated dibenzofurans (PCDFs), which was published. We made a second statement in 1991 when UK exposure data on these compounds from food became available. On this occasion, we endorsed the TDI of 10 pg/kg bw/day 2,3,7,8–TCDD recommended by an expert group convened by WHO Regional Office for Europe (WHO/EURO) and we recommended that, when considering mixtures of PCDDs and PCDFs, the TDI can be regarded as 10pg/kg bw/day 2,3,7,8–TCDD Equivalents. We further stated that, in view of the estimated long elimination half-lives of this class of compounds, it would be more appropriate to regard the TDI as a time-weighted average tolerable intake. It was also recommended that the toxicology of PCDDs and PCDFs are subject to continuing review.

(iii) The US Environmental Protection Agency (US EPA) recently released a draft health assessment document on 2,3,7,8–TCDD and related compounds which was made available for public comment. This exhaustive report, which is the result, in part, of a substantial research initiative by the EPA, reviews the available data on pharmacokinetics and disposition, mechanism(s) of action, acute/subchronic and chronic toxicity, immunotoxicity, developmental and reproductive toxicity, carcinogenicity, dose-response modelling, epidemiology (cancer effects and effects other than cancer) and risk characterization. We have reviewed the EPA report and this has provided us with a suitable opportunity to consider all the available data and to check whether there is a need to revise the current TDI.

General Comments

(iv) The EPA document provides an update on molecular mechanisms of the action of 2,3,7,8–TCDD based on its interaction with the aryl hydrocarbon receptor (Ah receptor) and addresses the potential role played by this receptor in health and PCDD/F–induced disease. In assessing the data on the mechanism of action, it became apparent that the report provides no new conceptual or experimental information in this area which necessitates alteration of the view we expressed in 1991. However, one outcome of the receptor work reported so far is to support the use of "Toxic Equivalency Factor" calculations which have been carried out in the past since there is evidence to suggest that most PCDDs and PCDFs studied so far interact with the same Ah receptor in initiating the end points studied. The TEF values assigned to different PCDD and PCDF congeners are based on toxicity and enzyme induction studies and broadly correlate with the affinity of the Ah receptor for the congener. Calculation of total exposures and the interpretation of TDI values assume that the effects of different PCDDs and PCDFs in a mixture would be additive: this approach may be over-conservative since some constituents may act as antagonists, but synergistic

interactions cannot be excluded. More information in this area would improve the scientific basis of the risk assessment of PCDDs and PCDFs.

(v) In our review of the chapter on disposition and kinetics we focused upon new evidence which might be crucial to the use of toxicity studies in animals for risk assessment in humans. We identified no new relevant data on absorption, metabolism, excretion and half-life which would influence our hazard assessment. The report does present new data on the time– and dose-dependence of the distribution of 2,3,7,8–TCDD in rats. The WHO/EURO evaluation used a correction factor for differences in the liver/adipose tissue ratio of 2,3,7,8–TCDD in rats and humans since rats have a much higher ratio and, therefore, have a higher "target organ dose" for hepatocarcinogenicity, which was the critical endpoint used in the risk assessment. The correction factor was derived from experimentally obtained, steady-state data and we still consider it to be appropriate for the method of risk assessment used. However, the question about the liver/adipose tissue ratio in relation to both time and dose introduces an additional variable and complication in the new low-dose extrapolation used in the EPA risk assessment.

(vi) Studies in laboratory animals have demonstrated that 2,3,7,8–TCDD affects the immune system and reproduction and development and that it is a carcinogen in rodents. In 1989 we reviewed these areas in detail and we note that the EPA report now also acknowledges these as the three critical areas. It was with particular reference to these toxicological end-points that we questioned whether there was a need to change the risk assessment of 2,3,7,8–TCDD and related compounds and/or to revise the current TDI.

Developmental and reproductive toxicity

(vii) The EPA document's review of the many studies in reproductive and developmental toxicity of 2,3,7,8–TCDD is of high quality. Although a number of new studies have been published since our last review, only a few have a direct bearing on the setting of a TDI.

(viii) Some newly published studies in rats report on the induction of testicular toxicity in male offspring when 2,3,7,8–TCDD is administered to pregnant rats at a single dose of 64 nanograms (ng) 2,3,7,8–TCDD/kg bw (equivalent to a body burden of 64ng/kg) during the perinatal period. The body load achieved at this dose would be only slightly higher than that achieved in rats given repeated doses of 1 ng 2,3,7,8–TCDD/kg bw/day which we previously identified as the no adverse effect level for reproductive toxicity. However, maternal blood concentrations and fetal exposure would be much higher in the new studies since the entire dose would enter the bloodstream prior to distribution to maternal tissues and the fetus. In contrast, most of the body load due to chronic intake would have already distributed to maternal tissues, such that blood concentration and fetal exposure would be much lower, despite the similarity in body load. Therefore, overall, we consider the results of the new studies to be consistent with the previously agreed TDI.

(ix) Another new study, which reports a decrease in testosterone levels in occupationally exposed individuals associated with serum 2,3,7,8–TCDD

concentrations greater than 20pg/g blood lipid is considered too limited for the results to be reliable.

(x) We extended our review to include an assessment of a report of an increased incidence of endometriosis in rhesus monkeys 10 years after a past exposure (from 1977 to 1982) to up to 25 ppt 2,3,7,8–TCDD in the diet. Although this finding is interesting and merits further investigation, the data are not adequate to enable us to come to any conclusion currently, in view of the small number of the animals involved and the apparently high spontaneous background incidence of endometriosis in rhesus monkeys.

(xi) It has long been assumed that the reproductive and developmental effects of PCDDs and PCDFs is mediated by the Ah receptor. The EPA document reviews many studies which support this contention. The natural ligand for the Ah receptor is not known, but is very likely a normal component of development, given the spatial and temporal expression of the Ah receptor during development. If the natural ligand for the Ah receptor is a developmental signalling molecule, then, in principle, a small exogenous activation of cells could lead to abnormal fate and maldevelopment. Alternatively, it is possible that homeostatic mechanisms are capable of correcting a low level of exogenous activation of the Ah receptor. There is currently no evidence to test either of these views.

Immunotoxicity

(xii) The immunotoxic potential of 2,3,7,8–TCDD and related compounds has received considerable attention in recent years. Our 1989 review contained a comprehensive summary of the available immunotoxicity data and on the basis of that review an estimate of 60pg/kg bw/day was derived as the level above which there may be concern about adverse effects on immune function in man.

(xiii) The US EPA draft report contains some new material relevant to the immunotoxic potential of 2,3,7,8–TCDD but there are no new substantive data reported that are inconsistent with our previous reviews and interpretation of 2,3,7,8–TCDD-induced immunotoxicity data. It is apparent that one of the most sensitive endpoints for 2,3,7,8–TCDD-induced rodent immunotoxicity is altered host resistance to pathogenic microorganisms. An enhanced susceptibility to viral infection following exposure of mice to a single intraperitoneal dose of 0.1µg/kg 2,3,7,8–TCDD was reported in 1990. Another report (cited in the EPA report but not as yet published) endorses the latter study and documents an enhanced mortality to influenza virus at 0.01µg/kg. The no effect level (NOEL) in terms of daily dose derived from this study is in the order of 0.7ng/kg bw/day which, after applying a safety factor of 100, yields a value of 7pg/kg bw/day. Although this value is slightly lower than the current TDI of 10pg/kg/day, it is derived from one, as yet unpublished, study in mice showing an unusual sensitivity to 2,3,7,8–TCDD and represents a worst case which would not, by itself, justify a reduction of the TDI.

Carcinogenicity

(xiv) The Committee on Mutagenicity (COM) and the Committee on Carcinogenicity (COC) considered 2,3,7,8–TCDD in 1988/9 and concluded

that this compound was carcinogenic in rodents but that this was unlikely to be due to a mutagenic mechanism. No new data are available to warrant alteration of the conclusions regarding the mutagenic potential of TCDD.

(xv) The COC gave further consideration to the carcinogenicity of 2,3,7,8–TCDD in 1993, when more epidemiological data were available. The Committee concluded that the new data strengthened the possibility of an epidemiological link between occupational exposure to 2,3,7,8–TCDD and an increase in total cancers in humans, although there was no consistent association with cancer at any specific anatomical site(s). It was considered that there was insufficient evidence for a clear causal link but it would be prudent at present to regard 2,3,7,8–TCDD as a possible human carcinogen.

(xvi) The COC has now considered the EPA review and has agreed that there is no new information in that review which requires the Committee to modify its previous conclusions with regard to the carcinogenicity of 2,3,7,8–TCDD.

(xvii) The COC also gave specific consideration to the EPA risk assessment model based on the assumed non-mutagenic, receptor-mediated carcinogenesis. It was noted that many assumptions are made in the application of this model to liver cancer in rats (the endpoint used in the EPA risk assessment) which cannot be scientifically justified. These include the assumption of a linear dose-response relationship at all dose levels for Ah receptor binding and subsequent steps leading to cancer development, and that the biochemical changes induced by the Ah receptor are toxic effects and, furthermore, that these are responsible for all the observed toxicity of 2,3,7,8–TCDD. It was concluded that this model is an interesting research tool but that it is not appropriate for use in risk assessment as proposed by the EPA. The exposure and cancer incidence data for humans obtained from epidemiological studies were not considered sufficiently reliable for dose-response analysis as proposed by the EPA.

Exposure from human milk

(xviii) With regards to human background exposure levels, the values reported in the EPA document were very similar to those we previously considered in 1989. For certain groups of the population such as breast-fed babies the margin of exposure between the no effect levels determined in animals and the human levels is rather small. We reiterate our previous view, that the period of breast-feeding is short compared with the long half-lives of 2,3,7,8–TCDD and related compounds and, in consequence, there will be insufficient time for the body burden in babies to rise to a high level. Taken over a lifetime, the eventual steady-state body burden of PCDDs and related compounds will not be significantly increased by the short period of higher intake. Breast-feeding confers considerable benefits on the young infant in terms of immunological protection, nutrition and mother-infant bonding. We continue to support the recommendation made by the WHO Working Group which considered this issue in 1988 that, despite the presence of PCDDs and related compounds in human milk, breast feeding should be encouraged on the basis of convincing evidence of the benefits of human milk to the overall health and development of the infant. However, we would not wish to see an increase in the level of PCDDs and related compounds in human milk and we *recommend* that appropriate measures to be taken to

reduce inputs of these compounds to the environment, thus reducing human exposure. We welcome the results of a recent study in Germany which reported that PCDD and PCDF levels in human blood and breast milk are decreasing

Conclusions

(ixx) We *welcome* the US EPA initiative to investigate further the health hazards of 2,3,7,8–TCDD and related compounds. The document provided a thorough review of the literature on the effect of these compounds on various biological systems. However, we do not consider it provided any new information or analysis that necessitates the alteration of the previously agreed TDI of 10pg/kg bw/day 2,3,7,8–TCDD Equivalents or of our previous advice.

(xx) We *accept* the COC's view that, although there is insufficient evidence for a causal link between exposure to 2,3,7,8–TCDD and cancer in humans, it would be prudent at present to regard 2,3,7,8–TCDD as a possible human carcinogen.

(xxi) We *recommend* that both the toxicology of PCDDs, PCDFs and related compounds and the TDI are subject to continuing review.

(xxii) We *reiterate* our previous view that, despite the levels of PCDDs and related compounds in human milk, breast feeding should be encouraged and promoted on the basis of convincing evidence of its benefits to the overall health and development of the infant.

(xxiii) We *reiterate* our 1989 and 1991 recommendations that the most useful action which could be taken to reduce exposures to these undesirable compounds is, as far as possible, to identify the continuing major sources of PCDDs and related compounds and to take appropriate measures to reduce inputs to the environment in the long-term, with the aim of reducing levels in food and in human tissues.

Thiabendazole

1.24 Thiabendazole (TBZ) is a permitted preservative used to prevent the growth of moulds on the skin of citrus fruit and bananas. It is also used as a fungicide in agriculture and in human and veterinary medicine as an anthelmintic.The COT reviewed the toxicology of TBZ on several occasions between 1988 and 1991 and decided that the critical adverse effect of TBZ was on the development of the fetus. Although there is a considerable data base on TBZ, many of the studies are rather old. The manufacturers have been updating the toxicological data and at the time of the last COT review in 1991, a number of studies were still in progress and the COT had only considered interim reports of these. Therefore, the Committee recommended a temporary $ADI_{1991-1994}$ of 0–0.05mg/kg bw/day which was derived by applying a safety factor of 200 to the NOAEL of 10mg/kg bw/day obtained from a gavage teratology study in the rat. Full reports of the outstanding studies, as well as results from new investigations, became available during 1995. These studies included an oral developmental toxicity study in rabbits, a two generation dietary reproduction study in rats, a 106–week dietary toxicity/carcinogenicity

study in rats, a 53–week oral toxicity study in dogs and an oral developmental study in mice.

1.25 The COT reviewed these new studies in 1995 and recommended an extension of the temporary ADI of 0.05mg/kg bw/day for a further year. The Committee did not recommend a full ADI mainly because a number of recent mutagenicity studies had been published which were referred to the COM for evaluation. In addition, an increased incidence of thyroid adenomas was noted in the intermediate (30 mg/kg bw/day) and high-dose (90 mg/kg bw/day) groups of the above mentioned 106–week dietary toxicity/carcinogenicity study in rats. The Committee recommended that the manufacturer should provide details of a mechanistic study to substantiate claims that this type of tumours in rats occur commonly with compounds that increase liver weight and cause changes in the clearance of thyroid hormones, and that the results of such a study would be needed before TBZ is considered further.

Food Surveillance Papers

1.26 The Steering Group on Chemical Aspects of Food Surveillance is a committee which advises government on the food chemical safety, nutritional adequacy and authenticity of the UK diet and which keeps under review the possibility of chemical contamination of any part of the national food supply. It has 11 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.

1.27 During 1995 the COT considered a report from the Working Party on Pesticide Residues (Food Surveillance Paper No. 50, in press) and two progress reports from the Working Party on Inorganic Contaminants in Food on Metals in Food (Food Surveillance Paper No. 51, in press; and Food Surveillance Paper No. 52, in press). The COT's advice in included in these Food Surveillance Papers as an appendix.

Topics Still Under Consideration

1.28 The following topics, which were discussed by the COT at meetings held in 1995, are under review:

Acetyl Tributyl Citrate
alpha-Solanine and alpha-Chacoline
Certain microbial enzyme preparations
Sucralose

1995 Membership of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

Professor H F Woods BSc MB BCh DPhil FRCP(Lon) FFPM FRCP(Edin) Hon FFOM

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N R Lazarus MB BCh BSc PhD MRCPath (Medical) (Until March 1995)
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J Norman BSc DPhil (Scientific MAFF)
Mrs D Davey (Administrative)

D E Jaeger BSc PhD CBiol MIBiol
J Shavila BSc MSc PhD
H A Walton BSc DPhil

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 Investment Trust Foreign & Colonial Investment Trust Hanson Smith & Nephew	Share Holder Share Holder Share Holder Share Holder Share Holder	Wide range of national & international food & chemical companies.	Dean of the Faculty of Medicine, University of Sheffield, which has extensive activity in teaching and research in nutrition and toxicology and in topics related to and supported by many companies in the food and chemical industry. Trustee of Hallamshire Therapeutic Research Trust Ltd, Harry Bottom Charitable Trust and Special Trustees for the former United Sheffield Hospitals.
Prof P J Aggett	NONE	NONE	Nestlé Nutricia Milupa SMA Nutrition	Research Support Research Support Research Support Research Support
Dr P N Bennett	Glaxo Wellcome Grand Metropolitan Hanson ICI Marks and Spencer Medeva Tomkins Unilever	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	Research Institute for the Care for the Elderly plc Bath Institute for Clinical Sciences Trading Co	Director Director
Dr V Beral	NONE	NONE	NONE	NONE
Dr N A Brown	Glaxo Shook, Hardy & Bacon Styrene Information Research Control Tate & Lyle	Consultancy Consultancy Consultancy Occasional Fee	EC (DGXII) Glaxo Wellcome Trust	Research Support Research Support Fellowship & Research Support
Dr J K Chipman	Pharmaco LSR Shook, Hardy & Bacon	Teaching Fee Consultancy	Astra STD Pharmaceutical Ltd Water Research Centre Zeneca, Safety of Medicine Zeneca, Central Tox Lab	MRC Collaborative Research Award Research Support Research Support CASE Research Award MRC Collaborative Research Award
Prof A D Dayan	British Petroleum Glaxo-Wellcome Schering Plough Smith Kline Beecham TI	Consultant Pension Consultant Consultant Share Holder	Glaxo Unilever	Research Fellowship Research Fellowship
Prof G G Gibson	NONE	NONE	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**
Prof G Hawksworth	NONE	NONE	Glaxo Wellcome Pfizer Central Research Servier Research & Dev Smith Kline Beecham Solvay Pharma Zeneca	Research Support Research Support Research Support Research Support Research Support Research Support
Dr I Kimber	British Airways British Petroleum ICI ICI Zeneca Zeneca	Share Holder Share Holder Share Holder Consultant Share Holder Employee	Unilever	Grant for Research
Dr D E Prentice	Athena Neurosciences Ciba Gensia Gilead Sciences Hoechst Veterinär Hoffman-La Roche Nestlé Rhone-Poulene Rorer Sandoz Schering-Plough Synthelabo (LERS) Wyeth-Ayerst	Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant	NONE	NONE
Dr A G Renwick	International Sweetners Association	Consultant	Hoffmann-La Roche Unilever	Research Support Research Support
Mr F M Sullivan	Borax Ltd Cocensys Frauenhofer Research Rank Hovis McDougal Rothmans International Wyeth-Ayerst	Consultant Occasional Fee Consultant Consultant Occasional Fee Occasional Fee	NONE	NONE
Dr A Thomas	NONE	NONE	NONE	NONE
Prof D Walker	Amersham Bayer British Petroleum Grampian Pharmaceuticals Rhône Mérieus Sandoz Solvay Duphar	Share Holder Occasional Fee Share Holder Occasional Fee Occasional Fee Occasional Fee Occasional Fee	NONE	NONE
Prof R Walker	Barlow, Lyde & Gilbert Cadbury Beverages Europe Coffee Science Info Centre Colloïdes Naturels International, France Food Safety Advisory Centre IDV Ltd Nutricia	Occasional Fee Occasional Fee Consultant Consultant Consultant Consultant Occasional Fee	Nestlé	Research Studentship

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

Professor J M Parry
(Chairman)
BSc PhD DSc

Preface

The COM provides advice to Government Departments and Agencies in response to questions concerning the potential genetic effects and consequences of exposure of the population to chemicals of diverse origins. During the course of the year the opinion of the Committee has been sought on topics as various as the potency ranking of specific groups of chemicals to the current status and suitability for use of new and developing test methods. A comprehensive evaluation was undertaken of the published work concerning the potential mutagenic activity of alcoholic beverages in support of the work of the Intergovernmental Working Group. Analysis of specific chemicals included the pesticides lindane and carbaryl, environmental pollutants such as the polycyclic aromatic hydrocarbons, the by-products of water chlorination; the trihalomethanes, industrial products such as hydroquinone and specific veterinary medicines such as anthelminic oxibendazole.

To ensure that the advice provided represents the "state of the art" in the field of safety evaluation, the COM undertakes a continual evaluation of test methods. These included methods for measuring DNA damage, such as the so called "Comet Assay" and the use of genetically engineered cultured cell lines expressing specific enzymes involved in the metabolism of genetically active chemicals.

JAMES PARRY

Alcoholc Beverages

2.1 The Government established an Interdepartmental Working Group to carry out a review of its sensible drinking message in the light of the latest evidence that drinking alcohol might give protection from Coronary Heart Disease (CHD). As part of the health input into this group the COM was asked to advise on the mutagenicity of ethanol and alcoholic beverages. Consumption of alcoholic beverages is expressed in terms of units of alcohol. A unit of alcohol contains 8 grammes of ethanol which is roughly the amount in half a pint of ordinary beer or larger or in a small glass of wine or in a standard measure of spirits. (Statements on the carcinogenicity of alcoholic beverages and also their harmful effects on pregnancy, reproduction and infant development can be found in the appropriate COC and COT sections of this Annual report.)

2.2 The COM gave detailed consideration to comprehensive reviews of the available data on the mutagenicity of ethanol, its principal metabolite acetaldehyde and alcoholic beverages per se. The main components of alcoholic beverages are water, ethanol and, in the case of sweet liquors, sugar. It was therefore felt that the review, covering data on alcoholic beverages themselves plus ethanol and its metabolite acetaldehyde was adequate to enable conclusions to be drawn regarding the mutagenic potential of the range of alcoholic beverages consumed by the general public.

2.3 It was recognised that alcoholic beverages contain small amounts of a significant number of volatile and non-volatile organic compounds formed during production storage and maturation. The Committee agreed that it was not essential or practical to review these constituents individually for their mutagenic potential.

2.4 The conclusions reached by the Committee which considered all relevant information available up to May 1995 are reproduced in full below.

Ethanol

2.5 The mutagenic potential of ethanol was summarised as follows:–

(i) *In vitro* studies do not suggest that ethanol has any mutagenic potential. Negative results were consistently obtained in studies using Salmonella typhimurium TA 1535, 1537, 1538, 98 and 100 and in more limited studies using TA 102. Negative results were also obtained in mammalian cells using the mouse lymphoma assay and in metaphase analysis studies for the investigation of clastogenicity. Conflicting results were obtained in studies to investigate SCE induction with some positive results reported at high concentrations of ethanol.

(ii) Negative results were obtained in the sex linked recessive lethal assay, and also in the Somatic Mutation and Recombination Test (SMART assay) in Drosophila melanogaster.

(iii) Negative results were consistently obtained from *in vivo* studies in rodents (rats, mice and hamsters) designed to detect clastogenicity (using either metaphase analysis or the micronucleus test) in bone marrow despite the use of very high dose levels equivalent to several grams/kg per day by gavage or in the drinking water. An increase in SCEs has been reported in

fetal cells when pregnant animals were given very high dose levels of ethanol (5 g/kg/bw or more).

(iv) Ethanol has been fairly extensively investigated for genotoxic effects in germ cells, mainly using the dominant lethal assay. Negative results were obtained in the majority of cases. A number of poorly described experiments have been reported using very high dose levels with inconsistent results obtained. The Committee considered that data from these studies could not be extrapolated to realistic exposure conditions. It was concluded that there is no evidence that ethanol induces germ cell mutations *in vivo*.

Acetaldehyde

2.6 The Committee noted that significant amounts of acetaldehyde occurred in the body as a product of intermediary metabolism but that these were transient due to the rapid conversion to acetic acid. The following conclusions were reached with regard to the mutagenic potential of acetaldehyde.

(i) The Committee noted that the data set available on acetaldehyde was generally of poor quality.

(ii) Negative results have been obtained with acetaldehyde using the Salmonella assay in tests that were not specifically designed to prevent vapour loss but the compound has been shown to produce gene mutations in mammalian cells. It has been more extensively investigated for its ability to induce chromosome aberrations and SCEs in mammalian cells and positive results have consistently been obtained in the absence of exogenous metabolic activation. These *in vitro* studies indicate that acetaldehyde has mutagenic potential.

(iii) Data from *in vivo* studies were too limited to draw definite conclusions. Negative results were obtained in one study using an adequate protocol to investigate SCE induction in bone marrow following administration of dose levels up to 0.5 mg/kg bw (associated with marked toxicity) using the i.p route. Acetaldehyde was reported to produce DNA-protein cross-links in the nasal mucosa of rats in an old study of limited value, following exposure by inhalation to levels of 1000 ppm and above but not at (or below) 300 ppm. These data provide only preliminary information that covalent binding occurred at a high dose level.

(iv) The mutagenic profile of acetaldehyde is very similar to that of formaldehyde. The compound has direct acting mutagenic potential *in vitro*, but would only be expected to have the potential of *in vivo* activity at sites where it is not rapidly metabolised to acetic acid (see COC statement for information on the animal carcinogenicity bioassays with acetaldehyde).

Alcoholic Beverages

2.7 The Committee considered the published data on alcoholic beverages which mainly consisted of experimental studies, both *in vitro* and *in vivo*, on concentrated extracts of these products. In addition an unpublished report[7] on HPRT mutant frequency in circulating T cells in humans with known levels of intake of alcoholic beverages was considered. The following conclusions were agreed:–

[7] (recently published. Cole J & Green MHL (1995). Mutagenesis, 10, 449–452.)

(i) There is some evidence that concentrated extracts of wines and spirits (covering a wide range of types) may have mutagenic activity in bacteria. Activity was inconsistently seen across all types of wines or spirits, with about 10–20% of commercial samples showing some activity. In some cases this was 'direct-acting' in others S–9 was required. A higher proportion of the concentrated extracts of home made wine and spirits had mutagenic activity than commercial samples. These results suggest the presence of low and variable amounts of different compounds with mutagenic potential. In view of the enormous number of organic compounds known to be present in low levels (many hundreds) no conclusions can be drawn regarding the identity of the compounds involved.

(ii) The *in vivo* mutagenicity of alcoholic beverages has not been adequately investigated and no definite conclusions can be drawn. There was no convincing evidence that concentrated extracts from alcoholic beverages have *in vivo* activity in the bone marrow of animals. No data are available in animals for tissues other than the bone marrow.

(iii) There are a limited amount of data from both *in vitro* and *in vivo* studies to suggest that concentrates from alcoholic beverages have some mutagenic potential. This is probably due to the presence of unidentified mutagenic contaminants which have been concentrated to high levels during the extraction process. The significance of the results of studies using concentrates in relation to the consumption of alcoholic beverages is questionable.

(iv) There are no adequate published studies in humans which have investigated the mutagenic effects of drinking different alcoholic beverages. There are some unpublished data available which suggest that any effects are small. Analysis of HPRT mutant frequency in circulating T cells of 143 adults whose alcohol consumption was known (0–56 UK units) did not reveal any relationship between alcohol consumption and HPRT mutant frequency.[1] Furthermore, registered alcohol dependent individuals did not have an elevated mutant frequency.

Overall conclusions

2.8 It was agreed that the consumption of alcoholic beverages does not present any significant concern with respect to their mutagenic potential.

Carbaryl

2.9 Carbaryl is an insecticide used in a number of pesticide and veterinary products and also in human medicines used for the treatment of lice. The Advisory Committee on Pesticides (ACP) initiated a review of carbaryl because the manufacturers had submitted new study reports some of which indicated that long term exposure to carbaryl could cause an increased incidence of tumours in rats and mice. The COM was asked to advise on the mutagenicity of this compound by the Scientific Committee on Pesticides (SCP) who provide technical advise relating to the safety assessment of pesticides to the ACP. The COM considered the

[1] (recently published. Cole J & Green MHL (1995). Mutagenesis, 10, 449–452.)

new studies and all the available published data on mutagenicity. The following statement was agreed and forwarded to the relevant advisory bodies, namely the SCP and ACP, the Veterinary Products Committee (VPC) and the Committee on the Safety of Medicines (CSM).

2.10 The COM made the following statement on Carbaryl

Introduction

(i) Carbaryl is an anticholinesterase carbamate pesticide. It is absorbed from the gastrointestinal tract and via inhalation exposure. The extent of dermal absorption appears to depend on the solvent used to dissolve carbaryl. Absorbed carbaryl is rapidly metabolised (by hydrolysis or hydroxylation and subsequent conjugation) and eliminated predominantly in the urine as water soluble metabolites.

Adequacy of available studies

(ii) There are a large number of mutagenicity studies using carbaryl available. Those undertaken by Rhone Poulenc have generally been conducted to acceptable standards, with the exception of the gene mutation assay in CHO cells for 6–thioquanine resistance (paragraph iv below). The standard of the published mutagenicity studies is generally more variable and some studies have been poorly conducted and/or reported. Additionally, there are some positive results documented in the published literature which lack adequate confirmation (see paragraph viii below). The following conclusions can be drawn;

In vitro data

(iii) Carbaryl has been tested in a large number of *in vitro* tests for a range of genetic end points. Most tests have been conducted using material containing 97% carbaryl.

(iv) Carbaryl gave negative results in bacterial assays for gene mutation. There is no convincing evidence that carbaryl causes gene mutations in mammalian cells. However, a fully satisfactory study in mammalian cells has not been performed.

(v) An adequately conducted study of unscheduled DNA synthesis using rat hepatocytes gave a negative response. A limited study using human lymphocytes gave a negative result.

(vi) Carbaryl was clearly clastogenic in CHO cells *in vitro* in the presence of S–9 and induced spindle disturbances (including aneuploidy), chromosomal damage and SCEs in V–79 cells in the absence of an exogenous metabolising fraction.

In vivo data

(vii) Carbaryl was not clastogenic in bone marrow assays in rats and hamsters or in micronucleus tests in mice under the dosing regimes used. Of these studies, only one bone marrow assay in rats undertaken by Rhone-Poulenc was conducted to contemporary standards. It is concluded that there is no

evidence that carbaryl has clastogenic activity in the bone marrow. There was no evidence of covalent binding to liver DNA or chromatin proteins in mice in a single dose, limited radiolabel study, which used ^{14}C-naphthyl labelled carbaryl.

(viii) Evidence of carbaryl induced disturbances of mitosis and aneuploidy (at the site of contact in the small intestines) has been reported in one old East European study in rats using technical grade carbaryl of low purity (85%). There are no studies to current standards available to support these findings. Additionally the available bone marrow assays were not designed to detect aneuploidy.

Evaluation

(ix) Carbaryl is mutagenic *in vitro* in mammalian cells inducing clastogenic effects and disturbances of mitosis leading to aneuploidy. There is no evidence of *in vivo* mutagenicity in the bone marrow or of covalent binding to liver DNA. The data suggest that carbaryl is not a DNA reactive *in vivo* mutagen.

(x) The Committee is aware that a rat micronucleus assay in the rat using kinetochore antibodies has been requested by the EPA. This study may provide additional data to assess the *in vivo* aneugenic potential of carbaryl. The Committee would wish to review this study when it is available.

Polycyclic Aromatic Hydrocarbons (PAHS)

2.11 Polycyclic aromatic hydrocarbons (PAHs) are a group of highly lipophilic chemicals that are present ubiquitously in the environment as pollutants. Humans are exposed to a mixture of PAHs from inhalation of air pollution and ingestion through food and drinking water. Workers in industries such as aluminium production, coal gasification, coke production and iron and steel founding are exposed to higher levels occupationally. Cigarette smoke is also a major source of PAHs for those individuals who smoke. The COM and the COC were asked for advice by DoE and MAFF on evaluation of the individual compounds so that priority or representative compounds could be identified for monitoring and/or surveillance. In 1994, the Committee had allocated a hazard ranking into one of four classes for 15 PAHs based on the available mutagenicity data. The Committee considered the available data on 9 remaining PAHS of interest to DoE and MAFF.

2.12 The same criteria were used to assess these PAHs. Definitions of the four categories are reproduced below for ease of reference.

1 – Clear *in vivo* mutagens.

2 – Compounds with some indication of mutagenic potential.

3 – Compounds with no significant *in vivo* mutagenic potential.

4 – Compounds on which there were inadequate data and thus no decision could be made.

2.13 With regard to category 2, it was felt that *in vitro* assays using exogenous metabolic activation systems such as 'S–9' could not be used as definitive information in the ranking system for PAHs and that category 2 should be used when there was some indication of *in vivo* activity or *in vitro* activity using cell mediated activation assays.

2.14 The groupings for the remaining PAHs considered by the COM, based on mutagenicity data were as follows:

Group 2: Benzo(c)phenanthrene, Cholanthrene

Group 4: Acenaphthalene, Acenaphthene, Benzo(b)fluorene, Coronene, Fluorene, Perylene, Triphenylene

Fluoranthene

2.15 The Department of the Environment (DoE) asked for advice on this polycyclic aromatic hydrocarbon (PAH) which is the largest single PAH contaminant in drinking water.

2.16 The COM had reviewed the available mutagenicity data on fluoranthene in 1993 as part of the ranking scheme for a number of polycyclic aromatic hydrocarbons. The COM had concluded that fluoranthene had some mutagenic potential *in-vitro*, but had been inadequately investigated *in-vivo*. The Committee had recommended that *in-vivo* mutagenicity studies were required to clarify the situation, namely an *in-vivo* bone marrow assay for clastogenicity and a liver UDS assay. These studies were contracted by the DoE Drinking Water Inspectorate and the reports were considered by the COM. Both studies were considered by the Committee to have been performed to an acceptable protocol and were negative.

2.17 The following conclusions were agreed;

(i) Fluoranthene gave negative results when investigated in an assay for micronuclei induction in the mouse bone marrow and for DNA damage in liver of rats (UDS assay). Both tests were performed to acceptable protocols.

(ii) Fluoranthene can be regarded as having no significant genotoxic potential *in vivo*.

Lindane

2.18 Lindane (ã-hexachlorocyclohexane) is an insecticide used in a number of agricultural and non agricultural pesticide products. The Department of Health asked the Committee for a view on the recently published positive findings in the 'Comet' assay (see section 2.37 below for review of this assay). The Committee considered these findings as well as mutagenicity data reported in the comprehensive IPCS Environmental Health Criteria document on lindane (published in 1991) and mutagenicity studies on lindane published since 1990.

2.19 The Committee concluded:

(i) The mutagenicity of lindane has been extensively examined in both in vitro and in vivo studies. Although, many of these studies were not to the currently recommended protocols, they were of adequate quality and negative results were consistently obtained.

(ii) Studies of DNA damage tests with lindane using single cell gel electrophoresis (referred to as the "Comet assay") undertaken to a number of different protocols for both *in-vitro* and *in-vivo* tests in animals and *in-vitro* tests with primary cultures of human gastric mucosal and nasal mucosal cells gave inconsistent results. The Committee agreed that data from "Comet assays" could not be used for the evaluation of chemicals or for regulatory purposes as further work on optimising test methods and validation of the assay is needed. No conclusions can be drawn from these data regarding the potential genotoxicity of lindane.

(iii) Lindane does not have mutagenic potential of significance for human health.

Hydroquinone and Phenol

2.20 The Committee had at the request of HSE provided advice on the interpretation of the mutagenicity data on hydroquinone and phenol in 1994. The COM had agreed that both hydroquinone and phenol should be regarded as potential somatic cell *in-vivo* mutagens. The Committee was persuaded for these compounds that by the oral route there was potential for a threshold of activity as there was good evidence that two protective mechanisms (namely rapid conjugation and detoxification via the glutathione pathway) would substantially reduce systemic exposure to any active metabolites formed. However, Members agreed that there were insufficient data regarding inhalation and dermal exposure and it was not possible to assume that a threshold existed for activity when exposure was via the respiratory tract or the skin. A submission from industry provided some additional studies on the metabolism of hydroquinone and phenolic derivatives in the lung and skin to the HSE, who requested the Committee's view.

2.21 The Committee agreed that the new data on the metabolism of hydroquinone and phenol in animals and in humans were valuable but appropriate studies to determine the extent of pre-systemic metabolism following either inhalation or dermal exposure had not been undertaken. It was agreed that the following studies would provide useful information:

(i) Further *in vivo* studies in rats or dogs using administration of hydroquinone or phenol via a bronchoscope with very early sampling for free and conjugated test substance in the blood.

(ii) It was essential that the method be sensitive enough to measure both free and conjugated substance.

(iii) Additional investigations in volunteers following dermal administration would also be useful but should be undertaken using higher doses of hydroquinone and early sampling times. (Members acknowledged that the skin irritancy of phenol would limit the dose level of this compound that could be studied.)

Flouride

2.22 The fluorination of water has been the subject of considerable public interest for many years. The COM completed reviews of the mutagenicity data on fluoride in 1987 and in 1990 and had concluded on both occasions that fluorination of water was unlikely to represent a mutagenic risk to the consumer. A number of new studies had been published since 1990 and it was considered timely to review these data.

2.23 The following conclusions were reached.

(i) There was no evidence of a mutagenic effect at the HPRT locus in V79 cells.

(ii) The results of *in-vitro* clastogenicity tests using primary bone marrow cultures from rats, established cell lines from a number of animal species and human fibroblasts show no convincing evidence of clastogenic activity.

(iii) The results of most recent *in-vivo* tests for clastogenic activity in mice were negative. The Committee also considered a study which evaluated the clastogenic effects of a mixture of chemicals in sheep. Members agreed that no significance could be attributed to the results of this latter investigation.

(iv) The Committee considered that two new epidemiological studies of SCE frequency and the incidence micronuclei in individuals resident in areas where fluorosis was endemic provided inadequate information to draw any conclusions. Of more importance was the negative result obtained in a study of SCE induction in patients given high dose levels of fluoride as treatment for osteoporosis, when compared to a well matched control group.

(v) The COM concluded that genotoxicity studies published after 1990 provided no convincing evidence that fluoride had any *in-vitro* or *in-vivo* genotoxic activity. The conclusions drawn in 1990 remained valid. The Committee confirmed that fluoridation of water was unlikely to represent a genotoxic risk to humans.

Trihalomethanes

Bromodichloromethane, Chlorodibromomethane, and Bromoform.

2.24 In 1994, the Department of the Environments' (DoE) Drinking Water Inspectorate (DWI) asked the COC for advice on the carcinogenicity of these 3 trihalomethanes and on a further trihalomethane, chloroform. The question of mutagenicity had been referred to the COM who concluded there was no convincing evidence that chloroform was genotoxic *in-vivo*. The Committee had also agreed that *in-vitro* studies had indicated that bromodichloromethane, chlorodibromomethane, and bromoform should be regarded as having mutagenic potential, but the available *in-vivo* data provided no convincing evidence of mutagenicity. The COM advised that further data were required to provide adequate reassurances that these 3 trihalomethanes had no significant *in-vivo* genotoxic activity namely, an *in-vivo* liver UDS assay for all compounds and in addition in the case of bromoform an *in-vivo* bone marrow assay for clastogenicity.

The DWI had contracted these studies and the data were reviewed by the COM. In all cases the studies were conducted to acceptable protocols and were negative.

2.25 The following conclusions were reached.

(i) Bromodichloromethane and chlorodibromomethane have both given negative results when each compound has been investigated in a well conducted *in-vivo* liver UDS assay.

(ii) Bromoform has given negative results in a well conducted *in-vivo* bone marrow micronucleus assay and a liver UDS assay.

(iii) The data requested by the COM has been provided and is clearly negative. Bromodichloromethane, chlorodibromomethane, and bromoform are unlikely to have any significant *in-vivo* genotoxic activity in humans.

Oxibendazole

2.26 Oxibendazole is a benzimidazole anthelmintic used in veterinary medicines in cattle, sheep and pigs. Results obtained from cytogenetic tests showed that this compound appeared to be a particularly potent inducer of polyploidy *in-vitro*. The Department of Health requested advice on the further testing required to assess the significance of the polyploidy observed with oxibendazole in order to assist in discussions at a Working Group of European Unions' Committee on Veterinary Medicinal Products regarding the significance of residues of oxibendazole in food.

2.27 The COM agreed that oxibendazole was a very potent inducer of polyploidy in CHO cells. It was noted that the compound had given negative results in two bone marrow micronucleus studies in mice which suggested that the compound had no *in-vivo* aneugenic potential in the bone marrow, although some concerns about potential aneugenicity remained. In addition, the potential effect of polyploidy on the germ cells might need further consideration. The Committee agreed a test strategy for oxibendazole (see below) but commented that other chemicals which induced polyploidy should be considered on a case-by case basis. The requirement for further testing would be dependent on the potency of the compound in question at inducing polyploidy in relation to its uses, the likely human exposure and the mechanisms by which polyploidy is induced.

2.28 The following recommendations regarding the further evaluation of oxibendazole were reached.

(i) An assessment of the relationship between polyploidy and the results of dominant lethal studies of structurally related benzimadazoles would assist in identifying further testing requirements for oxibendazole.

(ii) The following testing strategy was recommended specifically for oxibendazole in view of its potent induction of polyploidy.

(a) In the first instance, oxibendazole should be tested for its ability to induce polyploidy in human lymphocytes *in-vitro*, since the results obtained in CHO cells might be specific to this particular cell line.

(b) If oxibendazole was not a potent inducer of polyploidy in human lymphocytes, then no further work would be necessary.

(c) If positive results were obtained in human lymphocytes, further work would be required to investigate potential aneugenicity using the *in-vitro* micronucleus test, and possibly also germ cell effects. (The latter would be dependent on the consideration of the dominant lethal assay data noted above).

2.29 The Committee agreed to give this compound further consideration should appropriate data become available. The Committee also agreed to alter the advice to Government Departments on the health significance of chemicals that induce polyploidy, in order to reflect the possibility that chemically induced polyploidy in germ cell precursors might result in the occurrence of non-disjunction during reduction division and hence the formation of unbalanced gametes. (An effect that may not be detected by a bone marrow micronucleus assay.)

Test Strategies and Evaluation

2.30 The Committee continued to provide advice on test strategies and interpretation of genotoxicity tests particularly in the context of the ongoing discussions of the Genotoxicity Working Party of the International Conference on Harmonisation of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The Committee also commented on the results of a Japanese interlaboratory collaborative study on the use of the mouse lymphoma study to identify chemicals that induce chromosomal aberrations. The Committee agreed that some of the experiments should be repeated and the interpretation of the results needed further consideration by the authors before a finalised paper was submitted for publication.

2.31 The Committee provided advice to the HSE on its sponsored research programme relating to chemical mutagenesis. A paper was also drafted for UK regulatory departments, which outlined some of the general principles for evaluating genotoxicity assays drawn from the Committee's discussion of items during 1994. Dr Cole also prepared a more detailed paper, for use by regulatory officials, on the interpretation of *in-vitro* mammalian cell gene mutation tests using the CHO/HPRT assay.

Thresholds for Aneuploid Inducing Chemicals

2.32 The Committee considered test strategies for evaluating thresholds for aneuploid chemicals in 1993 where it was agreed that it was reasonable to assume that aneuploidy inducing chemicals (particularly those that function by damaging the cell division spindle) have a threshold of action. After extensive discussions, the Committee reached a series of conclusions concerning the nature of the experimental data which might be used to demonstrate a threshold of action for aneugenic chemicals (which have been published in the 1993 Annual Report). Essentially the Committee was of the view that it was not possible to determine thresholds for aneugenicity using the currently available *in-vivo* assays. The most suitable experimental system for the determination of the presence or absence of a threshold would be a cultured cell system, preferably involving the use of human cells such as human lymphocytes. MAFF PSD had now asked for further

clarification regarding a number of issues concerning the testing methods, in the light of new technological developments in screening for aneugenicity.

2.33 With regard to evaluating thresholds the following general conclusions (of which ii relates specifically to an assay that has been validated) were agreed and should be considered in combination with the advice given in 1993 Annual Report.

(i) The Committee agreed that sufficient good centromere specific probes for the analysis of interphase stages were now available and hence it was not necessary to undertake an analysis of both metaphase and interphase cells.

(ii) The use of one positive control substance should be sufficient to demonstrate the biological efficacy of a validated test in the absence of metabolic activation.

(iii) Piece-wise linear regression of the dose response curve might not be optimal to determine a threshold of action and that polynomial methods as used to determine thresholds for low dose radiation effects might need to be considered.

2.34 The Committee also reaffirmed that *in-vitro* studies could be used to screen for aneugenicity, but it would still be necessary to confirm that aneugenic activity could be expressed *in-vivo* in animals.

Use of Cell Lines Expressing Human Xenobiotic Metabolising Enzymes in Mutagenicity Testing.

2.35 The use of cell lines expressing human xenobiotic metabolising enzymes in mutagenicity testing had been the subject of a symposium at this years United Kingdom Environmental Mutagen Society (UKEMS) meeting. The Committee reviewed the relevant literature in order to consider whether mutagenicity assays using human xenobiotic metabolising enzymes could be used as part of a screening test strategy.

2.36 The following conclusions were reached:

(i) Much interest has been expressed in the use of genetically engineered cell lines containing the human enzymes responsible for the metabolism of foreign compounds. Several reviews of this area were published over the period 1989–1992 and it was hoped that this approach would provide supplementary tests for refining the hazard assessment to humans. However relatively little progress has been made since then with the limited additional data being provided by a small group of research workers.

(ii) Methods for validation of the tests and for integrating the results from test cells where the relative amounts of enzyme may differ from those found *in vivo* needed to be developed. The Committee recognised that it would be necessary to integrate data from enzymes which de-activate as well as activate chemicals. The currently available methods tend to lack the phase II conjugation enzymes usually involved in detoxication, although recent research has focused more on phase II enzymes, for example the expression of sulphotransferases, UDP-glucuronosyltransferases and N-acetyltransferases.

(iii) The qualitative and quantitative distribution of biotransformation enzymes is dependent upon (i) organ specificity (ii) interspecies differences and (iii) interindividual variability. The Committee agreed it was very unlikely, therefore, that a single genetically engineered cell line with multiple enzyme activities will provide an alternative to S9-mediated assays. A panel of cells lines, perhaps representing an individual organ might be a more realistic goal. However, the human population shows tremendous variation in biotransformation potential (both between and within populations), and this has been correlated to increased or decreased susceptibilities to specific cancers. These interindividual differences may therefore confer increased sensitivity to particular chemical carcinogens.

(iv) It is questionable however whether it is appropriate to look to *in vitro* assays, however refined, to mirror the *in vivo* process. The current COM guidelines indicate that additional studies following the conclusion that a chemical is an *in vitro* mutagen, should be *in vivo* in order to assess whether the observed activity can be detected in the whole animal and thus indicate that at a potential target site sufficient active metabolite is present to interact with DNA.

(v) The Committee concluded that the use of human enzymes in *in-vitro* mutagenicity tests represented an interesting research area but it was highly doubtful if there were a general role for such methods in testing strategies for investigating the mutagenicity of chemicals.

The Comet Assay

2.37 The 'Comet assay' has been proposed by several research groups as a rapid *in-vivo* assay for detecting genotoxicity at multiple sites. The Committee assessed all the available published literature on the use of this assay to detect chemical mutagens. The Committee agreed that the methods used in the 'Comet assay' represented an interesting research area but further work on the mechanisms of and characterisation of the type of DNA damage observed in the assay and distinguishing this from cytotoxicity and apoptosis was required before the significance of results could be interpreted. The Committee agreed that data from 'Comet assays' could not be used for regulatory purposes at the present and further work on optimising test methods and validation of the assay is needed. Thus no definitive conclusions can be drawn from the data currently available regarding the genotoxicity of chemicals.

Topics Under Consideration

2.38 The following topics, which were discussed by the COM at meetings held in 1995, are under review:

Health significance of chemicals that induce polyploidy.

1995 Membership of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

Professor J M Parry BSc PhD DSc

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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 J M Parry (Chairman)	Albright and Wilson Astra British Telecom Compass Catering JIB Insurance National Power Pharmakopius Powergen Smith Kline Beecham South Wales Electricity Wellcome Foundation	Share Holder Consultant Share Holder Share Holder Share Holder Share Holder Consultant Share Holder Consultant Share Holder Consultant	Glaxo Pfizer Smith Kline Beecham Welsh Water	Grant Grant Studentship Grant
Prof J Ashby	ZENECA	Employee	NONE	NONE
Prof R L Carter	British Airways Johnson Matthey Marks & Spencer Morrison (Wm) Supermarkets Powergen RTZ Corp Thames Water Unilever	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	NONE	NONE
Dr J Cole	NONE	NONE	NONE	NONE
Dr C Cooper	NONE	NONE	NONE	NONE
Prof D S Davies	ICI ML Laboratories Scherring Plough, USA Zeneca	Share Holder Non Executive Director Consultancy Share Holder	Astra Glaxo Wellcome Hoechst Kali Chemi Lilly Merck, Sharpe & Dohme ML Laboratories Pfizer Phacmacia Rhone-Poulenc Rorer Roche Products Sanofi Winthrop Servier Smith Kline Beecham Upjohn Zeneca	Fellowship Research & Studentship Research Research Research Research Fellowship & Research Research Research Adviser Research Research Research Travel grant Research Research
Prof H J Evans	British Petroleum British Telecom Croda ICI Merck, Sharpe & Dohme National Grid RTZ Scottish Power Southern Electric Y Water W Water Zeneca	Share Holder Share Holder Share Holder Share Holder Consultant Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	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 R F Newbold	NONE	NONE	NONE	NONE
Dr D J Tweats	Glaxo	Salary Share Option Holder	NONE	NONE
Dr S Venitt	Abbey National	Share Holder	NONE	NONE

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

Professor R L Carter
(Chairman)
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Preface

The COC evaluates chemicals for their human carcinogenic potential at the request of the Department of Health and other Government Departments. A large proportion of this years work has concerned the evaluation of epidemiological and other data on the carcinogenic risk of drinking alcohol. The Committee's statement to the Inter Departmental Working Group on alcohol has been reproduced in full in this report. The Committee also provided advice to Government Departments on a wide range of other topics. The Department of Health requested a view on a draft risk assessment of 2,3,7,8–tetrachlorodibenzo-p-dioxin (TCDD) prepared by the Environmental Protection Agency (EPA) risk assessment, advice on the claimed association between certain organochlorine insecticides and breast cancer, and guidance on ranking carcinogenic potencies of nitrosamines present in tobacco smoke. The HSE asked for an evaluation of the carcinogenicity of refractory ceramic fibres and for advice on their classification according to European Union criteria. Final conclusions on the significance of the levels of four trihalomethanes and fluoranthene in drinking water were provided for use by the Department of the Environment. The Committee was also asked to consider the carcinogenicity of carbaryl, an insecticide used in pesticides, veterinary products and human medicine.

On a more general issue, the Committee heard a presentation from Dr D P Lovell from the British Industrial Biological Research Association (BIBRA) on the use and limitations of the linearised multistage model (LMS) in quantifying carcinogenic risk.

RICHARD CARTER

Alcoholic Beverages

3.1 The Government established an Interdepartmental Working Group (IWG) to carry out a review of its sensible drinking message in the light of evidence that drinking alcohol might promote protection from Coronary Heart Disease (CHD). As part of the health input into this group, the COC was asked to advise on the carcinogenicity of alcoholic beverages. The Committee considered all the available literature published up to June 1995. The COC statement was drafted using units of consumption in terms of grams ethanol consumed per day. The Sensible Drinking Message uses the term 'unit of alochol' to express consumption. A unit of alcohol contains 8 grammes of ethanol which is roughly the amount in half a pint of ordinary beer or lager or in a small glass of wine, or in a standard measure of spirits. The COC statement for the IWG is reproduced in full below. A list of references used to draft this statement has been published in the Interdepartmental Working Group Report on Sensible Drinking. [1]

3.2 Statement for the Interdepartmental Working Group on the Sensible Drinking Message.

Introduction

3.3 We have been asked by the Interdepartmental Working Group on the Sensible Drinking Message to review the current literature relating to the carcinogenicity of alcoholic beverages, and to interpret these data in relation to the current Sensible Drinking Message. The Sensible Drinking Message states that if men drink less than 21 units of alcohol per week and women 14, they are unlikely to damage their health (A unit of alcohol contains 8 grammes of ethanol – the amount in half a pint of ordinary beer or lager or in a small glass of wine, or in a standard measure of spirits). Thus <21 units per week is equivalent to < 24 g ethanol/day and <14 units per week is equivalent to < 16 g ethanol/day. It is also stated that drinking in excess of 50 units a week for men (ie > 57 g ethanol/day) or 35 units for women (>40 g ethanol/day) is definitely dangerous.

3.4 The World Health Organisation's International Agency for Research on Cancer (IARC) published a monograph entitled "Alcohol Drinking" in 1988. The IARC Working Group concluded that there was sufficient evidence for the carcinogenic effects of drinking alcoholic beverages in humans. The occurrence of malignant tumours of the oral cavity, pharynx, larynx, oesophagus and liver was causally related to the consumption of alcohol. No definite conclusions were drawn in respect of cancer of the colon, rectum, stomach, breast and lung. No association was found for cancers of the urinary bladder, ovary, prostate and lymphatic and haematopoietic systems.

Objectives of review

3.5 The COC review has been based on the IARC monograph (including critical studies cited by the Working Group) and on the extensive literature relating to epidemiology and animal studies published from 1988 to June 1995. We reviewed these data with four main objectives.

[1] Department of Health. Sensible Drinking: The Report of an Inter-Deaprtmental Working Group. Department of Health, London, December 1995.

First to estimate relative risks of developing cancer at each of the sites considered by the IARC Working Group to be causally associated with alcohol drinking.

Secondly, to examine the nature of the trends in the frequency and duration of drinking required to increase the risks of these cancers; to inquire whether abstinence reduces such risks; to identify (if possible) which particular components of alcoholic beverages are carcinogenic in humans; and to evaluate the degree to which the association between cancer and drinking alcohol is confounded by other factors, notably smoking.

Thirdly, to review the available epidemiological data for cancer at other sites in order to determine whether alcohol drinking has a causal role.

Fourthly, to consider whether studies in animals suggested a plausible mechanism for the carcinogenic effects of alcohol observed in humans.

Evaluation of epidemiological data on alcohol

3.6 The results of epidemiological studies reviewed in this statement clearly demonstrate that moderate to heavy drinking of alcohol causes malignant tumours of the oral cavity, pharynx, larynx and oesophagus; but assessing the magnitude of the relative risk (ie risk compared with non drinkers), particularly at low levels of drinking, is very difficult. Because cancers caused by drinking alcohol occur at sites where cancer is relatively rare in the UK while consumption of alcoholic beverages is common, it follows that the actual risk of developing cancer at low levels of drinking is very small (see paragraphs 3.8 and 3.9 below). Factors which complicate the assessment of the epidemiological data can be grouped into three main areas.

First, estimates of alcohol intake are very imprecise, mainly because it is difficult to obtain an accurate history of drinking from individuals over the long period of time required for the development of alcohol-associated cancers. Problems involved in collecting and interpreting this information include:

(i) Inaccurate or biased recall of drinking leading to under-reporting of alcohol consumption.

(ii) Changes in individual drinking patterns over time.

(iii) Cultural and regional variations in drinking habits.

(iv) Differences in quantifying alcohol intakes in separate studies – for example total beverage consumption (usually as gram ethanol/day or as units per week) or as consumption of specific types of beverage such as wines or beers.

(v) Inadequate and inconsistent stratification of exposure groups. Many authors included both moderate and heavy drinkers in the same exposure group.

Secondly, the accuracy of diagnosis and reporting of tumours may vary considerably. The prime sites at risk from drinking alcohol are in the head and neck. The relevant tumour type (squamous carcinoma) is generally easy

to diagnose, but the detailed topography of some areas, particularly the subdivisions of the pharynx and to a lesser extent the larynx, is complicated and may vary in different accounts. It is sometimes difficult to determine the precise site of origin of tumours in these regions. The accurate diagnosis of tumours in other susceptible organs such as the liver is more problematic where different modalities (eg clinical examination, radiology, serology, biopsy, autopsy) with differing diagnostic sensitivities and specificities have been used to identify tumours. For example, the essential distinction between primary hepatocellular carcinoma and metastatic carcinoma may be difficult without histopathological evaluation.

Thirdly, the assessment of dose response data reported in epidemiological studies is difficult, particularly at lower levels of drinking where the numbers of individuals are often too low for the small elevations in relative risk to reach statistical significance at the conventional 5% level. This issue might be resolved by undertaking a meta-analysis of the data for each site, although the problems of dosimetry would still remain.

3.7 We have not defined a threshold level of drinking associated with an increased risk of cancer since, implicit in the statistical analyses of dose-response data from the epidemiology studies reviewed in this paper, is the general assumption that such a threshold level does not exist. Instead, we have attempted to estimate the level of alcohol drinking where there is convincing evidence of an increase in the relative risk of cancer at susceptible sites. There may be a very small risk of cancer at lower levels of drinking, but the studies reviewed were not capable of demonstrating an increased risk at such low levels of alcohol consumption.

Consumption of Alcohol in the UK.

3.8 A brief comment on the national patterns of alcohol drinking is necessary to provide a context in which the epidemiological findings can be interpreted. The most extensive published survey of drinking habits in the UK was conducted by the Office of Population Censuses and Surveys (OPCS) between April 1992–March 1993. Overall, 6% of male respondents and 12 % of female respondents were non-drinkers. 27% of male respondents drank more than 24 g ethanol/day (6% above 57 g ethanol/day) and 11% of female respondents consumed more than 16 g ethanol per day (2% above 35 g ethanol/day). These figures have remained relatively constant over the period from 1984 to 1992. The only changes reported during this time were a 2–3% increase of women at all age groups drinking more than 16 g ethanol/day and a 4% increase of men aged 45–64 drinking more than 24 g ethanol/day. Per capita estimates of ethanol intakes have also been published for the period 1960–1993, based on the sale of alcoholic beverages (See Figure 1 (Annex 1). These data suggest that alcohol drinking increased substantially during the late 1960's and 70's to a level equivalent to about 9 litres of 100% ethanol/person/year (approximately 20 g ethanol/day) which has remained relatively constant during the 1980's to date. It is likely that the rise in popularity of drinking wine was mainly responsible for the reported increase in overall alcohol consumption.

Incidence of newly diagnosed cancer cases in UK

3.9 Table 1 (Annex 2) presents information on the number of new cases of cancer registered in England and Wales (1989) and in Scotland (1990) at a number of sites. Combined data for England, Wales and Scotland are presented in Figure 1 (Annex 2). Cancers at sites generally considered to be causally associated with alcohol drinking – oral cavity, pharynx, oesophagus and larynx and possibly primary liver cancer – are relatively uncommon, so that the numbers of individuals likely to develop cancer at lower levels of drinking will be very small.

3.10 Cancers at certain other sites, mainly large intestine and breast, may also be associated with alcohol, but the IARC Working Group was unable to determine whether the association was causal. Some of these cancers, most notably breast cancer, are common. There would be serious public health implications if even a small proportion of common cancers was attributable to alcohol consumption, and we have examined this aspect in considerable detail to determine whether the original conclusions reached by the IARC Working Group in 1988 are still valid in the light of the further information now available.

Cancers of the Upper aerodigestive tract and certain specific head and neck cancers.

3.11 The most recent epidemiological studies published after the IARC monograph continue to demonstrate a dose-related association between the consumption of alcohol, expressed as grams ethanol consumed per day, and squamous carcinomas of the oral cavity, pharynx, larynx, and oesophagus. The association between cancer at these sites and drinking alcohol has been reported in studies of both smokers and non-smokers. The epidemiological findings are consistent with the view that drinking alcohol is causally associated with head and neck cancers independent of smoking, although the latter is recognised as an important potential confounding factor. (The interaction between smoking and drinking is discussed later in paragraph 3.42 - 3.44). Most investigators have adjusted estimates of relative risk associated with alcohol intake for the confounding effects of smoking, although the adequacy of data on tobacco consumption varies in the different accounts. Most of the dose-response data have accumulated from studies of men, with considerably less information available for women (in whom these tumours are much less frequent). There are only a few reports, for example, which deal exclusively with cancers of the larynx in women. The available data for women are, however, broadly compatible with those derived for men.

Cancers of the Upper aerodigestive tract

3.12 There is clear evidence from both cohort and case-control studies that heavy consumption of alcohol is associated with an increase in the incidence of squamous carcinomas of the upper aerodigestive tract, combining the oral cavity, pharynx, larynx and oesophagus. There are insufficient data available from prospective studies to draw any conclusions regarding dose-response relationships. Most case-control investigations found a dose-related association, although there is considerable variation in the slope of the dose-response between the different reports. No evidence of a dose-response was found by Merletti et al (1989), in their population-based investigation of cancer of the oral cavity and pharynx in patients living in Turin. Discrepancies in the quantification of alcohol consumption and

smoking, and the inclusion of different combinations of anatomical sites and subsites in the various reports, may explain the apparent lack of consistency in estimates of relative risk. It is therefore difficult to quantify the magnitude of the relative risk of upper aerodigestive tract cancer at any particular level of consumption; for example at 75 g ethanol/day there was a 10 fold variation in the estimates of relative risk with a range between 2–20.

3.13 We *conclude* that most case-control studies show a dose-response relationship. It is not possible from the available data to derive precise estimates of relative risk for upper aerodigestive tract cancer, analysed as a single entity, associated with specific levels of consumption. On balance, the data support the view that there is convincing evidence of an increase in relative risk at intakes above approximately 40 g ethanol/day. The evidence is less convincing at intakes between about 20–40 g ethanol/day. It is not possible to exclude a small increase in relative risk at lower intakes of alcohol below 20 g /day. There are fewer studies in women, but the data are compatible with those obtained for men.

Cancers of the Oral cavity

3.14 Results from both cohort and case-control studies are consistent with a dose-related association between drinking alcohol and cancer of the oral cavity.

3.15 We *conclude* that there is convincing evidence of an increase in relative risk at intakes above approximately 40 g ethanol/day with a large increase in relative risk of the order of 8–15 fold associated with intakes in excess of about 70–100 g ethanol/day. The evidence is less convincing at intakes between about 20–40 g ethanol/day. Most studies show a clear dose-response relationship. It is not possible to exclude a small increase in relative risk at lower intakes of alcohol below 20 g/day. There are fewer studies in women, but the data are compatible with those obtained for men.

Cancers of the Pharynx

3.16 The epidemiological data, based mainly on case-control studies, demonstrate a dose-related association for cancers of the pharynx, analysed either as a single entity or subdivided into oropharynx and hypopharynx.

3.17 We *conclude* that there is convincing evidence of an increase in relative risk at intakes above approximately 40 g ethanol/day with a 5–12 fold increase in relative risk associated with intakes in excess of 80 g ethanol/day. The evidence is less convincing at intakes between about 20–40 g ethanol/day. Most studies show a clear dose-response relationship. It is not possible to exclude a small increase in relative risk at lower intakes of alcohol below 20 g/day. There is no convincing evidence of a variation in sensitivity to alcohol in respect of cancer of the oropharynx, hypopharynx or pharynx (analysed as a single entity). Once again, there is less information in women, but the data are compatible with those obtained for men. (The Committee noted the special category of hypopharyngeal (post cricoid) cancers associated with the Plummer-Vinson syndrome which occurs almost exclusively in women, but there is no evidence that the tumours are causally associated with alcohol).

Cancers of the Oesophagus

3.18 Findings from both cohort and case-control investigations are consistent with a dose-related association between drinking alcohol and squamous carcinoma of the oesophagus. A few epidemiological studies have investigated a potential association between the consumption of alcohol and adenocarcinoma of the lower oesophagus and oesophagogastric junction, but it is not possible to draw any conclusions from the results. The evidence is inconsistent, both positive and negative associations with alcohol being reported, and it is often difficult to assess the point of origin and local extent of these cancers.

3.19 We *conclude* that there is convincing evidence of an increase in relative risk at intakes above approximately 30–40 g ethanol/day with a 3–8 fold increase in relative risk associated with intakes between 40–100 g ethanol/day. The evidence is less convincing at intakes between about 20–30 g ethanol/day. Most studies show a clear dose-response relationship. It is not possible to exclude a small increase in relative risk at lower intakes of alcohol below 20 g/day. There are fewer studies in women, but the data are compatible with those obtained for men. There is insufficient information to draw any conclusions regarding an association between adenocarcinoma of the oesophagogastric junction and drinking alcohol.

Cancers of the Larynx

3.20 The epidemiological data, derived primarily from case-control studies, show a dose-related association for cancer of the larynx. The dose-response relationship is relatively shallow compared with other head and neck cancers. Some authors have reported results which suggest that the relative risk of supraglottic cancer associated with drinking alcohol is greater in comparison to the risk of cancer of the glottis. The precise anatomical localisation of tumours in different regions of the larynx is, however, sometimes difficult to determine.

3.21 We *conclude* that there is convincing evidence of an increase in relative risk at intakes above approximately 40–70 g ethanol/day with a 3–9 fold increase in relative risk associated with intakes in excess of 70–100 g ethanol/day. The evidence is less convincing at intakes between about 20–40 g ethanol/day. Most studies show evidence of a dose-response relationship. It is not possible to exclude a small increase in relative risk at lower intakes of alcohol below 20 g/day. It is not possible to draw any definite conclusions with respect to subsite variation within the larynx.

p.t.o for summary table.

SUMMARY TABLE OF RISKS OF CANCERS OF UPPER AERODIGESTIVE TRACT (COMBINED) AND INDIVIDUAL HEAD AND NECK CANCERS

Cancer site	Evidence for dose response	Intake for convincing evidence of increases in RR* (g ethanol/day)	RR at heavy drinking
Upper aerodigestive tract	Most case-control studies. Insufficient evidence from cohort studies	≈40 g/day	Not determined
Oral cavity	Most studies show clear dose response	≈40 g/day	1–5 (≈40–70 g/d) 8–15 (≈70–100 g/d)
Pharynx	Most studies show clear dose response	≈40 g/day	5–12 (≈80 g/d)
Oesophagus	Most studies show clear dose response	≈30–40 g/day	3–8 (≈40–100 g/d)
Larynx	Most studies show evidence of a dose response	≈40–70 g/day	3–9 (≈70–100 g/d)

*Level of intake where there is convincing evidence from epidemiology studies of a small but statistically significant increase in relative risk (RR). The data do not exclude a small increase in relative risk at lower levels of alcohol drinking.

Cancer of the Liver (Hepatocellular carcinoma)

3.22 The most recent studies on alcohol and primary liver cancer are consistent with those reported by the IARC, showing an association at high levels of consumption; but concurrent infection with viral hepatitis B or C (HBV, HCV) in patients with primary liver cancer complicates the assessment of the association between drinking alcohol and liver and cancer.

3.23 Most prospective and case-control investigations support the conclusion that heavy drinking of alcohol is associated with hepatocellular carcinoma. No association was reported in one recent prospective study from Japan of drinking alcohol and liver cancer. Estimates of relative risk in men derived from case-control studies which controlled for HBV infection varied between 2–7 for intakes of more than 80 g ethanol/day. There is little evidence of a dose-response when all the available epidemiological data are considered. There are fewer studies in women, but the data are compatible with those reported in men. The great majority of hepatocellular carcinomas associated with heavy alcohol consumption occur in those patients with established liver cirrhosis. Chronic HCV infection has recently been demonstrated to be a risk factor for cirrhosis and hepatocellular carcinoma. This information was not available to the IARC Working Group in 1988, but the IARC have now concluded that chronic infection with HCV is causally associated with primary liver cancer. Preliminary evidence from one small case-control study of Japanese alcoholics with hepatic cirrhosis suggests that a high intake of alcohol (>130 g/day for at least 10 years) may enhance the development of hepatocellular carcinoma associated with HCV infection. The data from this study also indicate that hepatocellular carcinoma in alcoholics (with accompanying cirrhosis) who do not have co-existing HBV or HCV infection appears to be quite rare. There is currently no published investigation of alcohol drinking and primary liver cancer undertaken in the UK which also considered HCV infection, and the implications of the Japanese study should be treated with some caution since the prevalence of HCV infection in Japan (1.14% of blood donors) has been reported to be higher than in the UK (0.08% of blood donors).

3.24 We *conclude* that heavy drinking of alcohol is associated with primary liver cancer (hepatocellular carcinoma). Most tumours occur in individuals who have cirrhosis. It is not possible, in view of the results of recent investigations concerning the aetiology of liver cancer (particularly with respect to HCV infection) to draw any definite conclusions about the size of the relative risk of liver cancer associated with alcohol drinking.

Cancer sites not considered by IARC to be causally associated with alcohol.

3.25 We have evaluated the epidemiological data published since the IARC review to determine whether the conclusions reached by the IARC working group are still valid in the light of the more recent information now available. Conclusions for individual cancers are given below.

Cancer of the Stomach

3.26 The IARC Working group concluded that there were little aggregate data to suggest a causal role for alcohol in the aetiology of gastric cancer. We *conclude* that the results of the small number of epidemiological studies published after the IARC review are still consistent with the conclusion that there is no association between drinking alcohol and cancer of the stomach.

Cancer of the colon

3.27 The IARC considered that it was not possible to derive a conclusion for cancer of the colon: the epidemiological findings were inconsistent and many reports were uncontrolled for confounding dietary factors. Since the IARC review, a weak association between the consumption of alcoholic beverages and cancer of the colon was reported in women in one prospective study and in men in one case control study. No evidence was documented, in either men or women, in three further prospective studies and in four other case-control studies. A recent meta-analysis documented a weak association between the consumption of 24 g ethanol/day and cancer of the colon (RR = 1.10 (CI 1.03–1.17).

3.28 We *conclude* that the results of epidemiological investigations of colon cancer are inconsistent. The size of the relative risk documented in studies reporting positive results is small and the data have not always been fully adjusted for confounding dietary variables. Thus there is insufficient evidence to associate drinking alcohol with cancer of the colon. The potential interaction between alcohol and the diet in the aetiology of colon cancer is discussed in paragraph 3.45.

Cancer of the rectum

3.29 The IARC concluded that some epidemiological surveys provided suggestive but inconclusive data for a causal link between alcohol, most often beer, and cancer of the rectum. Four prospective studies have been published since the IARC review. A small dose-related increase in the relative risk of rectal cancer in men which was particularly associated with consumption of beer has been documented in a number of studies. Relative risks were between 2–4 at the highest levels of intake (ca > 30 g ethanol/day). It is possible that confounding variables were not fully taken into

consideration. The results of case-control studies published since the IARC review are inconsistent. Estimates of relative risk do not exceed 2 at the highest levels of intake (ca > 30 g ethanol/day). A recent meta-analysis also documented a weak association between the consumption of 24 g ethanol/day and cancer of the rectum (RR = 1.10 (CI 1.02–1.18) and with the consumption of beer (2 drinks/ day) and cancer of the rectum (RR = 1.26 (CI 1.13–1.41)).

3.30 We *conclude* that the results of epidemiological investigations of rectal cancer are inconsistent. Some studies have documented a weak association between cancer of the rectum and alcohol consumption, particularly beer in men. The magnitude of the risk is small and inconclusive in respect of a causal role for alcohol or beer.

Cancer of the Breast

3.31 There is an extensive literature available on the epidemiology of breast cancer. Known risk factors for breast cancer include age, ethnic group, family history of the disease, age at birth of first child, menarche and at menopause, history of biopsy for benign breast disease, socioeconomic status, obesity and, in premenopausal breast cancer, history of lactation. Other proposed risk factors have been cited, such as parity (in addition to age at birth of first child), use of oral contraceptives and hormone replacement therapy but whether they are involved in the aetiology of breast cancer remain controversial. The IARC reviewed 4 large prospective studies and 13 case control studies with respect to alcohol. These studies provided evidence of a consistent dose-response relationship, generally with up to 1.5–2 fold risk. Confounding due to recognised factors was controlled in most of them, but the Working Group did not reach a firm conclusion as to whether a causal association had been established between drinking alcohol and breast cancer. We have reviewed 7 additional prospective studies, 17 new case-control studies and two meta-analyses. We have also considered a formal analysis of the data from six case-control studies which controlled for known dietary confounding factors and a recent authoritative review of the association between alcohol and breast cancer. An additional qualitative review of the design and conduct of 38 case-control study reports published between 1980 and 1992 has also been considered.

3.32 The Committee agreed that the adequacy of control for confounding by known and/or alleged risk factors varied in the different accounts. A weak dose-related association was reported in most cohort studies and in some hospital based case-control studies. The results of population based case-control investigations did not generally support an association. A small statistically significant dose-related increase in relative risk was reported in the two meta-analysis reports (RR at 3 drinks/day 1.38 (CI 1.23–1.55)). The Committee noted that small increases in relative risk documented in the epidemiological studies ranging between approximately 1.2 to 3 and were associated with a highly variable consumption of alcohol (ca 1–60 g ethanol/day). It was agreed that clear evidence of causality had not been demonstrated.

3.33 We *conclude* that while there is no decisive evidence that breast cancer is causally related to drinking alcohol, the potential significance, for public health, of even a weak association between alcohol and breast cancer is such that we recommend, in particular, that this matter is kept under review.

Lung cancer

3.34 The IARC Working Group concluded that there was no evidence that drinking alcohol had a causal role in lung cancer. A weak association was found in one prospective cohort study which analysed a relatively small number of cases over a short follow up period. Two more recent case-control investigations did not report any evidence of an association.

3.35 We *conclude* that the data are consistent with the view reached by the IARC that lung cancer is not associated with drinking alcohol.

Cancer of the Pancreas

3.36 The IARC Working Group considered that the consumption of alcohol was unlikely to be causally related to cancer of the pancreas. A large number of epidemiological investigations on the possible association between pancreatic cancer and alcohol have been published since the IARC monograph. It is difficult to undertake such studies because pancreatic cancer is rapidly fatal and a large number of proxy interviews have to be used. Most epidemiological investigations do not report an association between alcohol and cancer of the pancreas. Evidence of a weak association has been documented in two of the three recently published prospective cohort studies and in one study of alcoholics.

3.37 We *conclude* that the balance of available evidence supports the view that drinking alcohol is not associated with cancer of the pancreas.

Cancer at other sites

3.38 Literature searches have identified some new publications which investigated the possible association between alcohol and cancer of organs and tissues included in this category by the IARC. There are no studies published since the IARC review which alter the conclusions reached by the Working Group.

3.39 We *conclude* that there is no convincing evidence of an association between drinking alcohol and cancer of the urinary bladder, ovary, prostate and lymphatic and haematopoietic systems, the skin, corpus and cervix uteri, vulva, testis, brain, thyroid and soft tissues.

Carcinogenicity of specific beverages in humans

3.40 For cancers causally related to alcohol, risk estimates tend to be highest for the most popular beverage consumed in the geographical area under study. For example the highest risk of oesophageal cancer in studies from Northern France, Uruguay, and Northern Italy were associated with cider/apple jack, wine/hard liquor, and wine (not otherwise specified) respectively. The available data support the view that variation in risk estimates for specific types of beverages reflect cultural and regional preferences in drinking patterns rather than differences in the composition of beverages.

3.41 We *conclude* that epidemiological findings are consistent with the view that the carcinogenic risk associated with the consumption of alcohol is proportional

to ethanol consumption. For cancers causally related to alcohol, the observed variations in relative risks for specific beverages reflect geographical preferences for particular alcoholic drinks. The reported association between consumption of beer and cancer of the rectum (see paragraphs 3.29 and 3.30) has not been consistently reproduced, and hence we *conclude* that there are insufficient data to suggest any beverage-specific effect for cancer of the rectum.

Interaction with smoking

3.42 We have reviewed 1 prospective study and 15 case-control studies which specifically examined the occurrence of cancer in non-smokers who drank alcohol. Evidence of an association between drinking alcohol and increased relative risk of cancer of the oral cavity, pharynx, oesophagus and larynx was documented in 12 of the 15 case-control studies reviewed which examined either non- smokers or minimal smokers. The clearest evidence has been reported for oesophageal cancer. Lifelong non-smokers were examined in 5 of these publications. Thus the epidemiological data are consistent with the view that drinking alcohol is causally associated with head and neck cancers independent of smoking.

3.43 Smoking increases the risk of alcohol-associated head and neck cancers in proportion to the amount of tobacco consumed. The results of case-control studies are conflicting as to whether the interaction between smoking and alcohol results in additive or multiplicative increases in relative risk of specific head and neck cancers at specific sites. It is not possible to determine the lowest levels of smoking required to increase the relative risk of alcohol associated cancer; the numbers of individuals investigated are often inadequate for the small elevations in relative risk of these comparatively rare tumours to reach statistical significance at the conventional 5% level. We *conclude* that it would be prudent to assume that all levels of smoking will increase the risk of alcohol-associated head and neck cancer.

3.44 There is no convincing evidence for an interaction between drinking alcohol and smoking in respect of cancers of the liver, stomach, large intestine, or breast. There is no evidence to suggest that alcohol affects the risk of cancers which are associated with smoking alone, pre-eminently cancer of the lung, but this topic has not been widely investigated.

Effects of alcohol on the diet

3.45 Poor nutritional status has often been described in heavy drinkers consuming 57 g ethanol/day in men and 40 g ethanol/day in women. Reduced intake of fresh vegetables and fruit has been reported in epidemiological surveys documenting higher risks of cancers of the upper aerodigestive tract associated with alcohol. However, the precise role of poor nutritional status induced by heavy drinking in the aetiology of alcohol-associated cancer remains obscure. There is insufficient evidence to draw any conclusions with regard to effects of alcohol on nutrition and primary cancer of the liver in individuals who are not alcohol dependent.

Preliminary evidence from one prospective study has suggested that high intakes of alcohol in individuals whose diet is low in methyl donating groups (such as methionine) may be associated with cancer of the colon. Confirmation from other populations is required before any definite conclusions can be drawn with regard to these data. There is insufficient evidence of a causal association between drinking alcohol and cancer of the colon (see paragraph 3.28).

Mechanism of carcinogenicity in humans

Mutagenicity

3.46 There is no convincing evidence that the carcinogenic effects of alcoholic beverages in humans occurs as a result of a mutagenic effect of ethanol itself, acetaldehyde (the initial metabolite of ethanol) or other beverage constituents. This subject is considered in detail in the statement from the Committee on Mutagenicity.

Carcinogenicity studies in animals

3.47 The carcinogenic effects of ethanol have been examined in a large number of published reports, mainly in mice, rats and hamsters often using very high dose levels. Many of these studies are flawed in terms of their design, interpretation or both. Examples include the use of too few test animals, inadequate control groups, unrealistically high doses of alcohol and a failure to compensate for the resulting acute intoxification, and inadequate documentation of pathology. These studies have, however, given consistently negative results. A carcinogenicity bioassay in rats conducted to currently acceptable standards has recently been published. No evidence of carcinogenicity was reported in male or female rats fed isocaloric liquid diets containing 1% or 3% ethanol for 2 years. We *conclude* that ethanol is not carcinogenic in animals.

3.48 Acetaldehyde, the initial metabolite of ethanol, has been shown to induce malignant nasal tumours in rats and laryngeal carcinomas in hamsters following inhalation exposure to high concentrations which induced intense local inflammation and epithelial degeneration. The carcinogenic profile of acetaldehyde is probably due to sustained localised irritation of the respiratory tract. We *conclude* that the observation of tumours in animals exposed to high inhalation doses of acetaldehyde is not relevant to drinking alcohol.

3.49 The carcinogenicity of a number of individual alcoholic beverages has been examined in laboratory animals. The studies have been inadequately performed and only a very limited range of beverages has been tested. No conclusions can be drawn.

3.50 We *conclude* that there are no data from carcinogenicity bioassays in animals which could explain the observed carcinogenic effects of alcohol in humans.

Studies of mechanisms

3.51 Many research groups have attempted to elucidate potential mechanisms for the carcinogenicity of alcohol. Proposals include modulation of known animal

carcinogens. Both cocarcinogenic and promoting effects have been found and, in one study, tumour suppression. The results of these investigations appear to be highly dependent on the chemicals chosen for study and the protocols used. A number of possible mechanisms for the cocarcinogenic and/or tumour promoting effects of ethanol have been postulated which include ethanol-induced increases in activation of procarcinogens and the concentration of DNA adducts in target tissues, lipid peroxidation in target tissues, increased cell proliferation, and impaired T-lymphocyte activation resulting in impaired cell mediated immunity. Interaction between ethanol and other components of beverages may also be important. For example, enhanced cell proliferation in the rat oesophagus was recorded when a mixture of ethanol and 3–methylbutanol (a fusel alcohol present in spirits) were intubated, compared with ethanol alone. The evidence for ethanol-induced increases in the endogenous levels of oestrogens, cited as a possible mechanism for breast cancer, is inconclusive.

3.52 Despite an extensive literature on alcoholic beverages, ethanol and acetaldehyde, we *conclude* that it is not possible to draw any firm conclusions regarding the mechanism by which alcohol causes human cancer.

Evaluation of Sensible Drinking Message

3.53 We have evaluated the extensive epidemiological data linking cancer with drinking alcohol. There is a dose-related association for cancer of the upper aerodigestive tract (as a whole) and for cancers of the oral cavity, pharynx, larynx and oesophagus. There is no convincing evidence of a dose-response for primary cancer of the liver, which appears to be associated only with heavy drinking and cirrhosis. We have used data derived mainly from studies using groups of men or men and women. There is less information for women alone, largely owing to the comparatively smaller number of women who are heavy drinkers, but the available evidence does not suggest that the risks to women are substantially different from those in men. We have estimated the relative risk for causally associated cancers in heavy drinkers which varies between 3–15 fold depending on tumour site. Convincing evidence of an increase in relative risk of cancer is difficult to obtain at low levels of alcohol consumption. On balance the data support the following conclusions.

(i) the evidence is convincing for an increased relative risk of cancer at susceptible sites at intakes above about 40 g ethanol/day.

(ii) the evidence for an increase in relative risk is less convincing at lower intakes between about 20–40 g ethanol/day.

(iii) there may be a very small risk of cancer at still lower levels of drinking, but the studies reviewed were not capable of demonstrating an increased risk at such low levels of alcohol consumption.

It should be noted that cancers caused by drinking alcohol are relatively rare in the UK. Moreover, the relative risks for such cancers at low levels of intake are likely to be very low. Therefore, the actual risks of developing these cancers must also be very low. (see paragraphs 3.8–3.10 above).

3.54 The relative risk of cancer associated with alcohol increases with the frequency of drinking (ie a higher risk has been noted in daily drinkers). A number of case-control studies have examined the relationship between the duration of drinking and increased risk of oesophageal cancer, but the results are inconsistent. A trend between increasing relative risk and duration has been reported in some investigations whilst others show a similar increase in risk at all time points examined. These studies have focused mainly on evaluating risks in individuals who have drunk alcohol for 20 years or more, and there is little information concerning shorter periods of drinking. It is thus not possible, at present, to define a minimum duration of drinking required at a particular level of consumption which will result in an increased relative risk of cancer at vulnerable sites.

3.55 The results of case-control studies are generally consistent with a reduction in relative risk of head and neck cancers following abstinence from drinking. Most investigators have examined oesophageal cancer. The data suggest that for moderate drinkers the risk declines to that of non-drinkers after about 10 years. In one recent study there was evidence that the period of abstinence required in former heavy drinkers consuming in excess of 85 g ethanol/day is longer (15 years); in former light drinkers consuming up to approximately 30 g ethanol/day the period of abstinence is shorter (5 years). These latter results must, however, be viewed cautiously. Virtually no information on the underlying reasons why individuals gave up drinking was reported in these publications. One prospective study of ex-drinkers documented a higher mortality rate for conditions other than cancer, such as cardiovascular disease and stroke.

Conclusions

3.56 The main conclusions of our review are given below.

(i) We *conclude* that

(a) the epidemiological evidence supports the view that drinking alcohol causes a dose-related increase in the risk of squamous carcinomas of the upper aerodigestive tract as a whole, and for cancers of the oral cavity, pharynx, larynx and oesophagus. There is less information for cancer in these sites in women, but the available information show similar risks in both sexes. The epidemiological data suggest that drinking alcoholic beverages causes cancer independently of smoking. Relative risks, in heavy drinkers (70 g ethanol/day), after controlling for the confounding effects of smoking, vary between 3–15 fold depending on the tumour site. There is convincing evidence of an increase in relative risk at intakes above about 40 g ethanol per day. The evidence is less convincing at intakes between 20–40 g ethanol/day. The epidemiological data do not allow a quantification of relative risk at lower levels of drinking, but it is not possible to exclude a small increase in relative risk at intakes below 20 g ethanol/day. The tumour types causally associated with alcohol are relatively rare in the United Kingdom and thus the number of cases which could be attributed to low levels of drinking would be very small. **(Paragraphs 3.11–3.21, 3.42, 3.53).**

(b) The risk of cancer at susceptible sites increases with the frequency of drinking. The results of studies investigating the duration of drinking

associated with an increased risk of cancer are inconsistent and have generally only considered periods longer than 20 years. No conclusions can be drawn from the available epidemiological data with respect to the minimum duration of drinking which will result in an increased relative risk at vulnerable sites. **(Paragraph 3.54).**

(c) The results of case-control studies are generally consistent with a reduction in relative risk of head and neck cancers following abstinence from drinking. The epidemiological data suggest that abstaining for periods of 10–15 years may reduce the risk of cancer to that of non-drinkers, but it is not possible to draw definite conclusions on this aspect. **(Paragraph 3.55)**

(d) The epidemiological data indicate that risk of cancer associated with drinking alcohol is due to the consumption of ethanol. Differences in risks attributed to particular types of beverage result from cultural and regional differences in drinking habits. There is no convincing evidence of beverage-specific effects. **(Paragraphs 3.40–3.41)**

(e) Smoking increases the risk of alcohol associated head and neck cancers. It would be prudent to assume that all levels of smoking will increase the risk of alcohol associated cancers. **(Paragraphs 3.42–3.44)**

(f) The precise role of poor nutritional status induced by heavy drinking in the aetiology of alcohol-associated cancer remains obscure. **(Paragraph 3.45)**

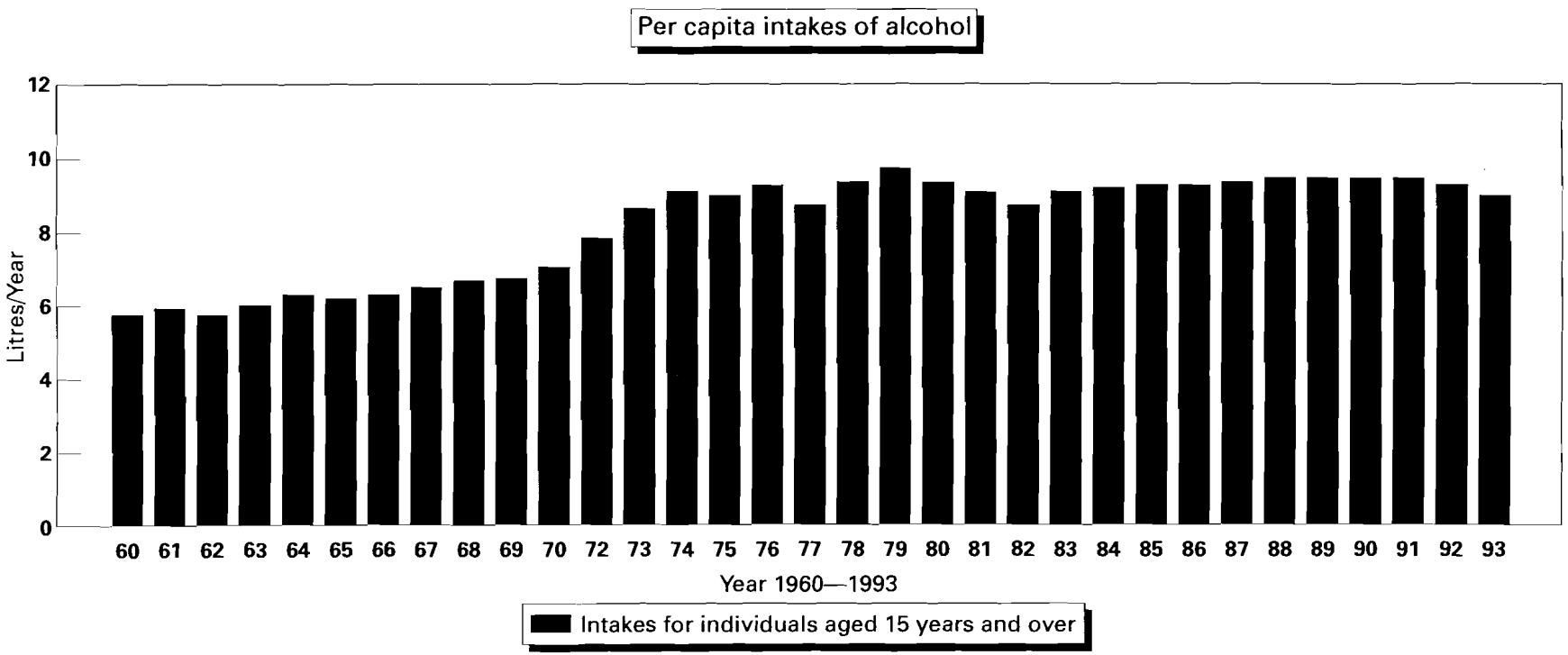
(ii) We *conclude*, that heavy drinking of alcohol is associated with primary liver cancer. Most tumours occur in individuals who have cirrhosis. It is not possible, in view of the results of recent investigations concerning the aetiology of liver cancer, (particularly with respect to HCV infection) to draw any definite conclusions about the size of the relative risk of liver cancer associated with alcohol drinking. **(Paragraphs 3.22–3.24)**

(iii) Some epidemiological investigations have reported an association between alcohol and cancer of the stomach, colon, rectum, lung and pancreas. We *conclude* that the evidence does not support a causal association between cancer at these sites and drinking alcohol. **(Paragraphs 3.25–3.30, 3.34–3.39)**

(iv) We *conclude* that while there is no decisive evidence that breast cancer is causally related to drinking alcohol, the potential significance for public health of a weak causal association between alcohol and breast cancer are such that *we recommend*, in particular, that this matter be kept under review. **(Paragraphs 3.31–3.33)**

(iv) It is not currently possible to draw any firm conclusions from the available studies in animals regarding the mechanism by which drinking alcohol induces cancer in humans. **(Paragraphs 3.46–3.52)**

Figure 1



Data From OPCS

TABLE 1 ANNEX 2

REGISTRATIONS OF NEWLY DIAGNOSED CASES OF CANCER

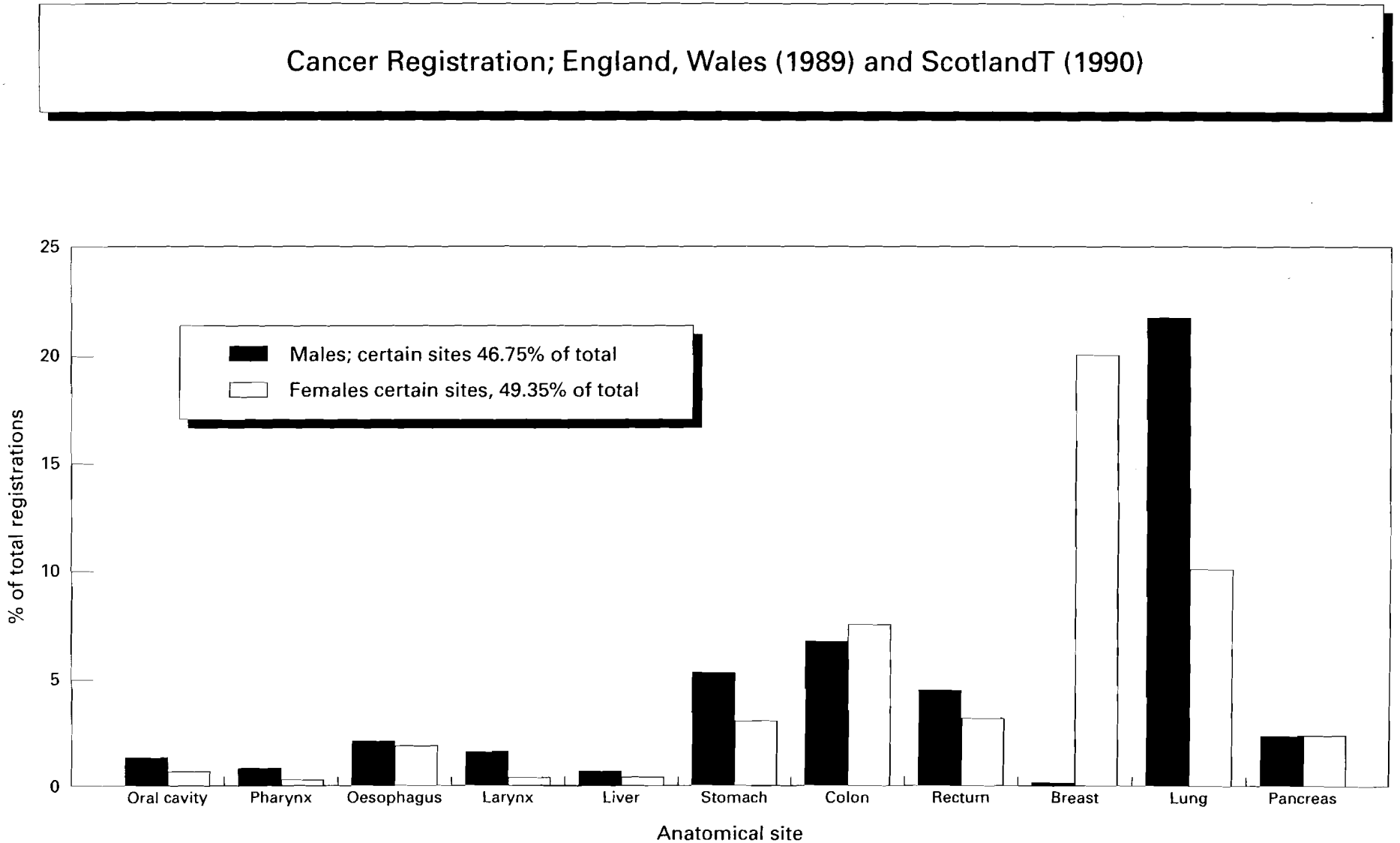
ALL AGES. (SELECTED SITES)

			England/Wales[1] 1989	(%)	Scotland[2] 1990	(%)
All malignant neoplasms	(140–208)**	M	121,529	(100)	13,319	(100)
		F	144,447	(100)	14,132	(100)
Oral Cavity	(140–145)	M	1,264	(1.0)	233	(1.7)
		F	796	(0.5)	126	(0.9)
Pharynx	(146–149)	M	668	(0.5)	105	(1.0)
		F	366	(0.3)	44	(0.3)
Oesophagus	(150)	M	2,957	(2.4)	362	(1.8)
		F	2,224	(1.5)	301	(2.1)
Larynx	(161)	M	1,610	(1.4)	209	(1.6)
		F	372	(0.3)	72	(0.5)
Liver	(155)	M	734	(0.6)	117	(0.9)
		F	475	(0.3)	68	(0.5)
Stomach	(151)	M	6,608	(5.4)	652	(4.9)
		F	4,211	(2.9)	442	(3.1)
Colon	(153)	M	7,802	(6.4)	922	(6.9)
		F	9,102	(6.3)	1,196	(8.5)
Rectum	(154)	M	5,690	(4.9)	524	(3.9)
		F	4,565	(3.2)	470	(3.3)
Breast	(175)	M	215	(0.2)	17	(0.1)
	(174)	F	27,768	(19.2)	2,900	(20.5)
Lung	(162)	M	25,276	(20.8)	2,969	(22.3)
		F	11,533	(8.0)	1,721	(12.2)
Pancreas	(157)	M	2,930	(2.4)	323	(2.4)
		F	3,268	(2.3)	339	(2.4)

** = ICD Ninth edition code

Refs: [1] OPCS, Cancer Statistics and Registrations. Registrations of cancer diagnosed in 1989, England and Wales, Series MBI No 22. HMSO 1994.

[2] Sharp L, Black RJ, Harkness EF et al. Cancer Registration Statistics Scotland 1981–1990. Scottish Cancer Intelligence Unit 1993.



England and Wales (1989 OPCS data) and Scotland (1990)

2,3,7,8–TETRACHLORODIBENZO-P-DIOXIN [TCDD]- COMMONLY KNOWN AS DIOXIN

3.57 The COT asked the COC for advice on the section dealing with carcinogenicity risk assessment section from a draft review prepared by the US Environmental Protection Agency (EPA) review of TCDD, published during 1994. The COT were examining the report with regard to the Tolerable Daily Intake for TCDD established by the World Health Organisation Regional Office for Europe.

3.58 TCDD and other chlorinated dibenzo-p-dioxins and furans are widely distributed in the environment. For individuals not occupationally exposed to dioxins, the main source is the diet. The COC had reviewed the available animal and human carcinogenicity data on TCDD in 1988 and in 1993. In 1988 the COC advised that TCDD was a non-genotoxic carcinogen in rodents at unusually low oral doses. The limited evidence available at that time suggested that the compound acted as a tumour promoter and that a threshold dose level might exist for its carcinogenic effects. In 1993 the COC considered a series of epidemiological investigations of occupational exposure to relatively high levels of TCDD. The Committee concluded that although there was insufficient evidence for a definite causal link with various forms of cancer, it would be prudent at present to regard TCDD as a possible human carcinogen.

3.59 The Committee reached the following conclusions:

(i) The COC considered the EPA review and agreed that there was no new information which would require the Committee to modify the conclusions reached in 1993 with regard to the carcinogenicity of TCDD in humans.

(ii) The Committee also gave specific consideration to the EPA risk assessment model based on the assumed non-mutagenic 'receptor-mediated' carcinogenesis. It was noted that many assumptions were made in the application of this model of liver carcinogenesis in rats which could not be justified scientifically. In particular, these included the assumption of a linear dose response relationship at all dose levels for receptor binding and subsequent steps; also that the biochemical changes induced by binding of TCDD to the Ah receptor represented a toxic effect and furthermore were responsible for all the observed toxicity of TCDD. The experimental and cancer incidence data in humans from the epidemiology studies were not sufficiently reliable to be used as proposed by the EPA. It was concluded that this model was an interesting research tool but that it was not appropriate for use in the risk assessment of TCDD.

Carbaryl

3.60 Carbaryl is an insecticide used in a number of pesticide and veterinary products and also in human medicine for the treatment of lice. The Advisory Committee on Pesticides (ACP) initiated a review of carbaryl because the manufacturers had submitted new reports, some of which indicated that long term exposure to carbaryl caused an increased incidence of tumours in rats and mice. The COC was asked to advise on the carcinogenicity of this compound by the Scientific Committee on Pesticides (SCP) who provide technical advice relating to

the safety assessment of pesticides to the ACP. The COM evaluated the mutagenicity data. The COC considered the newly submitted carcinogenicity studies and all the available published information. The following statement was agreed and forwarded to the relevant advisory bodies: the SCP and ACP, the Veterinary Products Committee (VPC) and the Committee on the Safety of Medicines (CSM).

Statement on Carbaryl

(i) The published results of previous carcinogenicity studies in mice and rats have been reviewed in detail by the International programme on Chemical Safety (IPCS) in an Environmental Health Criteria Document (No 153, published by the WHO, Geneva, 1994). The COC agreed with the conclusions reached by the IPCS that none of them had been satisfactorily conducted (eg, inadequate group sizes, number of dose levels, selection of dose levels, and histopathological investigations). They could not, therefore, be used to evaluate carbaryl. The carcinogenicity of carbaryl has recently been evaluated again, in new investigations in the mouse and rat undertaken for Rhone Poulenc by Hazelton Washington Inc. The results of these studies are given below:

Mouse

(ii) Mice (CD–1) were given diets containing 0, 100, 1000 or 8,000 mg/kg carbaryl for 104–105 weeks.

(iii) The conduct of this study was generally compatible with contemporary standards. The Committee noted, however, that the high dose level (approximately equal to 1250 mg/kg bw/day in males and 1440 mg/kg bw/day in females) resulted in significant toxicity as evidenced by reduced body weight gain. In the high dose males, the mean body weight gain was reduced by approximately 81% at week 4 and by 33% at week 13. In the high dose females body weight gain was reduced by approximately 70% at week 4 and by 19% at week 13. This top dose exceeded the Maximum Tolerated Dose and may compromise the value of the results for human health assessment. No treatment related mortality attributable to carbaryl was documented in this study. The Committee was also aware that the low dose animals were misdosed with the wrong compound on one day during week 93 which resulted in the deaths of 9 male and 8 female mice.

(iv) The COC agreed that a dose related increase in combined haemangiomas and haemangiosarcomas occurred in males which was statistically significant at the mid and high dose levels. A significant increase in combined haemangiomas and haemangiosarcomas was also reported in the high dose female group.

(v) A significant increase in combined renal adenomas and carcinomas was observed in the high dose males, and a significant increase in hepatocellular adenomas and carcinomas was also reported in high dose female mice.

Rat

(vi) Rats (F344) were given diets containing 0, 250, 1500 or 7,500 mg/kg carbaryl for 104–105 weeks.

(vii) The conduct of this study was generally compatible with contemporary standards. The Committee noted that the high dose level (approximately equal to 349.5 mg/kg bw/day in males and 484.6 mg/kg bw/day in females) resulted in significant toxicity as evidenced by reduced body weight gain. In the high dose males, the mean body weight gain was reduced by approximately 57% at week 4 and by 40% at week 13. In the high dose females, the mean body weight gain was reduced by approximately 60% at week 4 and by 52% at week 13. This top dose exceeded the Maximum Tolerated Dose and may compromise the value of the results for human health assessment. No carbaryl related mortalities occurred in this study.

(viii) No evidence of a treatment related increase in tumours was seen in rats at the low and mid dose levels. The COC agreed that a significant increase in the incidence of combined transitional cell adenomas and carcinomas of the urinary bladder occurred in male and female rats consuming the high dose diet.

(ix) A significant increase in the incidence of hepatocellular adenomas was observed in the high dose female rats. A significant increase in the incidence of thyroid follicular adenoma and a single follicular cell carcinoma were also reported in the high dose male group.

Mutagenicity

(x) There is no substantial evidence that carbaryl is a DNA reactive *in vivo* mutagen. No conclusions can be drawn from the available mutagenicity data regarding the mechanism of carcinogenicity of carbaryl.

Conclusion

(xi) The Committee noted that there were important limitations in the design and conduct of both the rat and mouse studies. Additionally there were no explanatory mechanistic data available regarding the observed increase in combined vascular system haemangiomas and haemangiosarcomas in male mice and combined transitional cell adenomas and carcinomas of the urinary bladder in male and female rats. The COC agreed that despite the problems in interpreting these data, it would be prudent for the present to consider carbaryl as a potential human carcinogen.

Organochlorines and Breast Cancer

3.61 The association between exposure to certain weakly oestrogenic organochlorine insecticides, and the development of breast cancer has been the subject of a number of recent scientific papers. It is in this context that the COC was asked by the Department of Health to review the epidemiological data available on three organochlorine (OCs) insecticides and breast cancer. The review focused on lindane, beta-hexachlorocyclohexane (β–HCH) and DDT which are or have been used as insecticides. Residues of these insecticides (particularly DDT and β–HCH) may persist in the environment and in human tissues.

3.62 The Committee agreed the following conclusions.

(i) The epidemiological data available on breast cancer and exposure to OC insecticides were limited. Six case-control studies had documented OC insecticide levels in serum, plasma and breast adipose tissue but the total number of individuals studied amounted to only 302 women with breast cancer and 412 women without the disease. In epidemiological terms this was a relatively small number of individuals (drawn from Canada, Denmark, Finland and the USA). Not all of these studies measured each of the three OC insecticides considered in this review and details regarding characteristics of cases and controls were often inadequately reported.

(ii) Accumulated exposure to the three OC insecticides was estimated by measuring concentrations in plasma, serum or breast adipose tissue making it difficult to compare studies. The preferred estimate of exposure would be derived from measurement of concentrations in breast adipose tissue. The use of biopsy and sometimes autopsy tissue samples also complicated the interpretation of these data. Many of the studies failed to control for known risk factors for breast cancer which include family history of the disease, age at birth of first child, menarche and at menopause, and history of biopsy for benign breast disease.

(iii) Only one prospective investigation had been published. Serum samples were collected from a total of 150 women (a nested case group derived randomly from a cohort of 57,040 women), on average 14 years prior to the diagnosis of breast cancer. This, along with the five other case control studies, looked at DDT levels (measured as the metabolite DDE) in serum or in plasma and breast fat. No association between serum DDE levels and breast cancer was found in the prospective investigation. An equal number of positive and negative associations between DDE levels in breast fat and breast cancer have been reported. The summary odds ratio documented in a meta-analysis report was 1.11 (99% CI 0.97–1.26), which was not a significant association. The two investigations which measured β–HCH levels in breast fat gave conflicting results and no clear association was found. Two studies measured the levels of lindane in breast fat: neither found any difference in lindane levels in tissue from women with breast cancer and tissue from control subjects without the disease.

(iv) The Committee also considered reports documenting a decrease in the breast cancer mortality rate between 1976–1986 in pre-menopausal women resident in Israel. It had been suggested in the published literature that the observed decrease in breast cancer mortality in Israel might be associated with bans on the use of OC insecticides. The Committee noted the short interval between the introduction of the ban and the decline in breast cancer mortality. Members agreed that improved detection and treatment of breast cancer rather than exposure to OC insecticides was more likely to account for this finding.

3.63 The following overall conclusion was agreed.

The available epidemiology indicates that there is no clear evidence of an association between the levels of lindane, β–HCH and DDT in serum and breast fat and breast cancer at the present time. However, the Committee recommend that the matter be kept under review.

Refractory Ceramic Fibres

3.64 Refractory Ceramic Fibres (RCFs) are a type of aluminosilicate fibre which are used predominantly in industrial high temperature applications. The HSE asked for the Committee's evaluation of the available carcinogenicity studies, including new investigations sponsored by an industrial consortium and undertaken at the Research Consulting Company (RCC) laboratories, Geneva, Switzerland. The Committee was asked to express an opinion with regard to the classification of RCFs for carcinogenicity under the criteria laid out in the Dangerous Substances Directive (67/548/EEC).

3.65 The Committee noted that the carcinogenicity bioassays of RCFs published during the 1980's were inadequate to draw conclusions regarding potential carcinogenicity. The Committee agreed that the new RCC studies were of a high standard, providing information on the carcinogenicity of four types of RCF fibres (RCF 1–4) in rats using inhalation exposures to the maximum tolerated dose level (MTD). One of these fibres (RCF 4) had been produced by heat treatment of RCF 1. A separate investigation in rats examined the dose response relationship for the carcinogenicity of RCF 1. The carcinogenicity of RCF 1 was also investigated in hamsters exposed by inhalation to high doses (equivalent to the rat MTD).

3.66 The Committee agreed the following conclusions.

(i) The RCC study in the rat, at the MTD, indicated that RCFs induced bronchoalveolar adenomas and carcinomas in the rat. There was some evidence for the induction of pleural mesotheliomas although, in view of the small numbers involved, their significance was uncertain.

(ii) No lung tumours were found in rats exposed at lower doses in the separate dose response study with RCF 1.

(iii) The RCC study in Syrian golden hamsters using high doses (equivalent to the rat MTD) of one of the RCFs – RCF 1– indicated that this compound induced proliferative lesions in the pleural mesothelium, some of which were considered to be mesotheliomas.

(iv) There was no direct evidence to suggest that the proliferative effects and neoplasia seen in either the rat or the hamster were associated with overload phenomena at the MTD or were artifacts caused by an inappropriate dosing regime.

(v) These data indicate that the RCFs investigated were carcinogenic in the rat and the hamster.

(vi) A category 2 classification was recommended using the criteria for the classification of chemicals in the EU under the terms of the Dangerous Substances Directive. It was noted that classification in this system is on the basis of hazard and not risk in use.

(vii) These recommendations relate to the RCFs under consideration, and the conclusions would not necessarily apply to RCFs of markedly different particle size. Such compounds would need to be considered on a case by case basis.

Trihalomethanes in Drinking Water

Chloroform, Bromodichloromethane (BDCM), Chlorodibromomethane (DBCM) and Bromoform

3.67 The Drinking Water Inspectorate from the Department of the Environment (DoE) asked the COC for advice on the carcinogenicity of these 4 trihalomethanes in drinking water. They arise in drinking water largely as a result of disinfection, although they may also occur as contaminants. In public water supplies in England and Wales, the total concentration of the four trihalomethanes rarely exceeds 100 microgrammes per litre (100 µg/l). Thus for a 60 Kg individual, daily ingestion of each trihalomethane would normally be considerably lower than 3 microgrammes per kilogram body weight (3 µg/kg bw/day). The Committee published interim conclusions on these 4 compounds in the 1994 Annual Report, and agreed to undertake a further review when additional advice on the mutagenicity studies requested by the COM became available. The Committee noted that the COM had concluded that it was unlikely that any of these 4 trihalomethanes had significant *in-vivo* genotoxic activity to humans

3.68 The Committee recalled that it was unlikely that adequate epidemiological data would become available in the near future for the evaluation of these compounds in drinking water; risk assessments would thus have to be based on animal studies. Furthermore in considering concentrations of trihalomethanes in drinking water, it was appropriate to proceed as if each individual compound carried a potential carcinogenic hazard. The Committee reached the following final conclusions on each the trihalomethanes considered.

Chloroform

3.69 Chloroform induced renal adenomas and carcinomas in rats and mice. Administration of 38 mg/kg bw/day or more *via* the drinking water for 104 weeks produced a dose related increase in renal tumours in male but not female rats. The lowest reported carcinogenic dose in mice was 60 mg/kg bw/day in an experiment where male mice were given oral doses of chloroform by gavage, 6 days per week for 80 weeks. The reported increase in hepatocellular adenomas and carcinomas noted in carcinogenicity bioassays where chloroform was given by gavage in corn oil is uninterpretable, with respect to assessing human hazard, due to the pronounced liver toxicity. There is no convincing evidence of genotoxicity. Chloroform should be regarded as an animal carcinogen, the tumours being induced by a non-genotoxic mechanism.

BDCM

3.70 BDCM induced renal adenomas and carcinomas in rats and mice and there is some evidence for induction of adenomas and carcinomas of the large intestine in rats. The lowest reported dose that gave rise to a carcinogenic effect in rats was 100 mg/kg bw/day in male and female rats given oral doses of BDCM by gavage, 5 days per week for 102 weeks. The lowest reported carcinogenic dose in mice was 50 mg/kg bw/day in an experiment where the animals were given oral doses of BDCM by gavage, 5 days per week for 102 weeks. It is noted that there were no data from carcinogenicity bioassays where the compound was administered in

drinking water. BDCM was mutagenic *in vitro* but was negative in two well conducted *in vivo* assays (bone marrow micronucleus assay and liver UDS performed to currently recommended standards). It is unlikely that BDCM will exert significant genotoxic effects in humans. BDCM should be regarded as an animal carcinogen, the tumours being induced by a non-genotoxic mechanism.

DBCM

3.71 DBCM induced a small increase in the incidence of hepatocellular adenomas and carcinomas in male and female mice after gavage administration of 100 mg/kg bw/day, 5 days per week for 104–105 weeks. This result is uninterpretable, with respect to assessing human hazard, due to the pronounced liver toxicity resulting from the administration of DBCM by gavage in corn oil. There was no evidence of carcinogenicity in rats given DBCM by gavage (at up to 80 mg/kg bw/day, 5 days per week for 102 weeks) or in mice given DBCM in drinking water (at up to 400 mg/litre for 102 weeks). DBCM was mutagenic *in vitro* but was negative in two well conducted *in vivo* assays (bone marrow micronucleus and liver UDS performed to currently recommended standards). It is unlikely that DBCM will exert significant genotoxic effects in humans and there is insufficient evidence to regard DBCM as an animal carcinogen.

Bromoform

3.72 Bromoform induced adenomas and carcinomas of the large intestine in male and female rats dosed by corn oil gavage at 200 mg/kg bw/day, 5 days per week for 105 weeks. There are no published studies of bromoform administered in the drinking water which could be used to clarify the significance of this result. Bromoform was mutagenic *in vitro* and gave an equivocal response in an *in vivo* bone marrow micronucleus assay. Two further *in-vivo* genotoxicity assays were undertaken at the request of the COM (bone marrow micronucleus and liver UDS performed to currently recommended standards). These studies were clearly negative. It is unlikely that Bromoform will exert significant genotoxic effects in humans. Bromoform should be regarded as an animal carcinogen, the tumours being induced by a non genotoxic mechanism.

Conclusion regarding levels in drinking water

3.73 The ratio between the lowest dose level giving rise to a carcinogenic effect in animals and the likely human exposure level from drinking water for each of the four trihalomethanes considered by the Committee was in excess of 10,000. Thus the levels of these trihalomethanes in drinking water in the UK are unlikely to provide a carcinogenic risk to humans.

Fluoranthene in drinking water

3.74 In 1994, the Committee provided interim advice to the Department of the Environment on fluoranthene in drinking water. This compound is the major polycyclic aromatic hydrocarbon found as a contaminant in drinking water. The Committee had concluded, in 1994 that the carcinogenicity data for fluoranthene were inadequate but, on the basis of the available *in-vitro* mutagenicity data and the structure of the compound, it was prudent to assume that it had carcinogenic

potential. Additional mutagenicity studies were requested by the COC to clarify whether fluoranthene had mutagenic potential *in-vivo*. The results from these investigations were now available and the COM had advised that fluoranthene should be considered as having no significant genotoxic potential *in-vivo* (see paragraph 2.17 of the COM report).

3.75 The following conclusions were agreed.

(i) The available data are inadequate for a definite conclusion to be drawn on the carcinogenicity of fluoranthene. Data from five papers presenting skin-painting carcinogenicity studies in mice were negative, although numbers tested in each study, where stated, were small. In another study in mice, fluoranthene and benzo(a)pyrene, applied together to skin, increased the combined incidence of skin papillomas and squamous carcinomas above those produced by the same dose of benzo(a)pyrene alone. Positive results were obtained in two short-term lung tumour assays in neonatal mice when fluoranthene was given intraperitoneally at very high doses (greater than 100 milligrams per kilogram bodyweight), but the predictive value of this test is questionable.

(ii) Annual average concentrations for water supply zones in England and Wales are below 1 microgram per litre, although on rare occasions levels in excess of 1.5 micrograms per litre have been recorded. The evidence indicates that consumption of drinking-water containing such low concentrations of fluoranthene does not pose a significant risk of cancer.

Potency Ranking of Nitrosamines in Tobacco Smoke

3.76 The COC was asked by the Technical Advisory Group (TAG) of the Scientific Committee on Tobacco and Health (SCOTH) to rank the carcinogenic potency of a number of *N*-nitrosamines which occur in mainstream and/or sidestream tobacco smoke and have been implicated as potential aetiological agents for tobacco-related cancers. TAG, as part of its remit in relation to the Health of the Nation has been concerned with identifying and prioritising the additives and contaminants present in tobacco to which exposure could be reduced. The COC adopted a similar approach to that endorsed by the Committee for the prioritisation of food carcinogens which involved ranking according to carcinogenic hazard and, for genotoxic carcinogens, a further ranking based on carcinogenic potency. The Committee used a two step strategy: carcinogenic potency was first ranked by using the available published TD_{50} estimations and then refined further by information from comparative potency studies and/or information on organotrophy. The Committee noted that, despite limitations in the quantity and quality of the available data, the initial ranking based on TD_{50}s and the refined ranking were similar.

3.77 The following conclusions were agreed and forwarded to TAG.

(i) The COC was asked to consider the tobacco specific nitrosamines, 4–(methylnitrosoamino)–1–(3–pyridyl)–1–butanone(NNK), nitrosonornicotine (NNN), nitrosoanabasine (NAB), and nitrosoanatabine (NAT) and a further 9 nitrosamines. They are listed in the table below. The Committee noted

that there were many other carcinogenic substances in tobacco smoke and emphasised the selective nature of the current exercise.

(ii) Most of the nitrosamines which were evaluated have been found to be genotoxic carcinogens in animals in oral administration studies. There are a number of problems in using the available animal carcinogenicity data to rank the potency of these compounds mainly because of an absence of appropriate toxicokinetic data in animals and the difficulty in extrapolating carcinogenic potency, estimated from high dose level oral studies in animals to the much lower levels of exposure occurring from the inhalation of tobacco smoke by humans. The few investigations which included comparisons of carcinogenic potency were inadequate to produce a refined ranking. The available carcinogenic potency data (most often reported as TD_{50} estimations) suggested that, as a group, the potency of nitrosamines exhibited a fairly narrow range when compared to the range of potency for known animal carcinogens. A pragmatic ranking of these compounds into 4 groups was agreed.

Group 1 (High potency)	4–(methylnitrosoamino)–1–(3–pyridyl)–1–butanone (NNK) *N*-nitrosodi-*n*-butylamine *N*-nitrosodimethylamine (NDMA) *N*-nitrosomethylethylamine *N*-nitrosodiethylamine (NDEA) *N*-nitrosomorpholine *N*-nitrosodi-n-propylamine
Group 2 (Medium potency)	*N*-nitrosodiethanolamine *N*-nitrosonornicotine (NNN) *N*-nitrosopiperidine (NPD) *N*-nitrosopyrrolidine (NPYR)
Group 3 (Low potency)	*N*-nitrosoanabasine (NAB)
Group 4 (Data inadequate to evaluate)	*N*-nitrosoanatabine (NAT)

Use of T_{25} to Estimate Carcinogenic Potency

3.78 The Committee reviewed the recent proposal to use the T_{25} to estimate the carcinogenic potency of chemicals. The T_{25} can be defined as the daily dose (expressed as mg/kg bw/day) resulting in a tumour incidence of 25% at a specific tissue site, after correction for spontaneous incidence, within the standard study period for that species. It is simple to calculate the T_{25} from carcinogenicity bioassay data and there is no need to undertake an analysis of the dose-response curve; but no allowance is made for the effect of intercurrent mortality during its calculation. Hence the T_{25} is a relatively crude estimate of potency.

3.79 The Committee agreed that, at present, there was little information to ascertain the value of using the T_{25} to assess carcinogenic potency, particularly with respect to whether it is possible to identify different levels of carcinogenic potency using the T_{25}. There was no information on the concordance between T_{25} and TD_{50} rankings. It was agreed that the T_{25} should be used with caution in the ranking of chemical carcinogens.

3.80 The Committee endorsed its previous view that potency indices can be used as part of the information required to rank genotoxic chemical carcinogens, but

are not appropriate to the ranking of non-genotoxic carcinogens. The Committee concluded that the TD_{50} was still the most practical quantitative estimate of carcinogenic potency available for the ranking of genotoxic carcinogens.

Presentation

Quantitative risk assessment and the limitations of the Linearised Multistage model

3.81 Dr D P Lovell from the British Industrial Biological Research Association (BIBRA) gave a presentation on the use and limitations of the linearised multistage model (LMS) in quantifying carcinogenic risk. He explained the basis of the model and assumptions used when applying the model to data from carcinogenicity bioassays. He noted that estimates of the parameters of the model used to provide estimates of low dose risk to humans had no direct relationship to the specific biological events in the carcinogenic process. *(Lovell P and Thomas G. Quantitative risk assessment and the limitations of the linearised multistage model. Human and Experimental Toxicology, (1996), 15,87–104).*

Topics Still Under Consideration

3.82 The following topics, which were discussed by the COC at meetings held in 1995, are under review.

Advice on studies undertaken by the Small Area Health Statistics Unit.
Consideration of the number of species employed in carcinogenicity bioassays.
Prioritisation of polycyclic aromatic hydrocarbons present as contaminants in food.

1995 Membership of the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

Professor R L Carter MA DM DSc FRCPath FFPM

MEMBERS

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I F H Purchase BVSc MRCVS PhD FRCPath CBiol FIBiol
A G Renwick BSc PhD DSc
S Venitt BSc PhD
Professor G T Williams BSc MD FRCP FRCPath

SECRETARIAT

J M Battershill BSc MSc (Scientific)
K N Mistry (Administrative)

R J Fielder BSc PhD Dip RCPath

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

DECLARATION OF INTEREST DURING THE PERIOD OF THIS REPORT

	Personal Interest		Non Personal Interest	
Member	**Company**	**Interest**	**Company**	**Interest**
Prof R L Carter (Chairman)	British Airways Johnson Matthey Marks & Spencer Morrison (Wm) Supermarkets Powergen RTZ Corp Thames Water Unilever	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	NONE	NONE
Prof P G Blain	NONE	NONE	ICI (ended 10/95) Unilever	Research Studentship Research Studentship
Prof C E DChilvers	British Gas ICI Tomkins Zeneca	Share Holder Share Holder Share Holder Share Holder	Merck, Sharp & Dohme	Research Support
Dr C Cooper	NONE	NONE	NONE	NONE
Prof A D Dayan	British Petroleum Glaxo-Wellcome Schering Plough Smith Kline Beecham TI	Share Holder Pension Consultant Consultant Share Holder	Glaxo Unilever	Research Fellowship Research Fellowship
Dr P B Farmer	British Gas National Power Powergen	Share Holder Share Holder Share Holder	Amersham BAT DOW Farmos Smith Kline Beecham Zeneca	Research Support Research Studentship Research Studentship Research Support Collaborative - Studentship Collaborative - Studentship
Prof D Forman	NONE	NONE	NONE	NONE
Prof R F Newbold	NONE	NONE	NONE	NONE
Prof J M Parry	Albright and Wilson Astra British Telecom Compass Catering JIB Insurance National Power Pharmakopius Powergen Smith Kline Beecham South Wales Electricity Wellcome Foundation	Share Holder Consultant Share Holder Share Holder Share Holder Share Holder Consultant Share Holder Consultant Share Holder Consultant	Glaxo Pfizer Smith Kline Beecham Welsh Water	Grant Grant Studentship Grant
Dr I F H Purchase	ICI Zeneca	Consultant Employee	NONE	NONE
Dr A G Renwick	International Sweetners Association	Consultant	Hoffman-La Roche Unilever	Research Support Research Support
Dr S Venitt	Abbey National	Share Holder	NONE	NONE
Prof G T Williams	NONE	NONE	NONE	NONE

Terms of Reference

To advise at the request of:

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

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

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

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

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

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

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

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

Declaration of Interests: A Code of Practice for Members

Introduction

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

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

Definitions

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

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

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

   b. trade associations representing companies involved with such products

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

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

Different Types of Interest

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

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

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

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

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

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

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

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

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

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

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

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

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

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

the annual declaration form following the change. (Non-personal interests involving less than £1000 from a particular company in the previous year need not be declared to the Department.)

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

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

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

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

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

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

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

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

Record of Interests

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

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

Glossary of Terms

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

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

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

ADENOMA (see tumour).

ADI Acceptable daily intake, 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'.

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

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

ANEUGENIC Inducing aneuploidy (qv).

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

ANTHELMINTIC A chemical used to destroy parasitic worms.

ANTICHOLINESTERASE A chemical that prevents the breakdown of acetylcholine by the enzyme acetylcholinesterase, thereby permitting high levels of acetylcholine to accumulate at reactive sites.

ANTIMICROBIAL The ability to destroy microbes.

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

ATROPHY Wasting away of an organ tissue due to degeneration of cells.

BASOPHILIA A property of a microscopic structure whereby it shows an affinity for basic dyes.

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

BRONCHOSCOPE An instrument for inspecting the interior of the tracheobronchial tree and removing tissue for culture and biopsy.

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

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

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

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

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

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

CELL DIVISION SPINDLE See microtubules.

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

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

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

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.

COHORT A defined population.

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

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

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

CYTOGENETIC Concerning chromosomes, their origin, structure and function.

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

DOMINANT LETHAL ASSAY See Dominant Lethal mutation.

DYSMORPHOLOGY Abnormal development of form or structure of the body or its parts.

ENDOMETRIOSIS A condition in which the tissue lining the womb (endometrium) is present at other sites in the body. The tissue undergoes the periodic changes similar to the endometrium and causes pelvic pain and painful periods.

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

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

ERYTHROCYTE Red blood cell.

EXOGENOUS Arising outside the body.

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

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

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

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

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

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.

GUIDELINE VALUE (For Drinking water) A guideline value represents the concentration of a constituent that does not result in any significant risk to health of the consumer over a lifetime of consumption.

HAEMANGIOMA/HAEMANGIOSARCOMA Tumours of the endothelial cells of blood vessels. Haemangiomas are benign. In haemangiosarcomas, cytological and histological features of malignancy are present such as cellular pleomorphism, increased mitotic activity, tissue invasion or metastases.

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

HAEMOGLOBIN A pigment contained within red blood cells which is the medium by which oxygen is transported within the body.

HEPATOCARCINOGENICITY Ability to induce liver tumours.

HEPATOCELLULAR Relating to the cells of the liver.

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

HISTOLOGY The study of the minute structure of tissues.

HOMEOSTASIS The physiological process by which the internal systems of the body are maintained at equilibrium, dispite variations in the external conditions.

HPRT ASSAY Hypoxanthine phosphoribosyl transferase (HPRT) is an enzyme involved in the salvage of nucleic acid breakdown products for re-use in cellular metabolism. It is not essential for cell survival but cells will die if incubated in the presence of a toxic nucleic acid base analogue (such as 6–thioguanine) unless HPRT is rendered inactive by a mutation in the gene coding for the enzyme. The basis of HPRT mutation assays is the selection of cells containing chemically induced mutations by culturing the cells in the presence of a toxic nucleic acid base analogue.

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

INTERPHASE The period between mitoses (qv) when a cell is not undergoing division.

INTRAPERITONEAL Within the abdominal cavity.

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

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

LIGAND A molecule which binds to a receptor.

LYMPHOCYTE Type of white blood cell.

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

MALIGNANCY See 'tumour'.

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

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

META-ANALYSIS A statistical procedure to summarise quantitative data from several different epidemiological studies. It is most commonly used in summarising epidemiological evidence with respect to disease incidence.

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.

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

MICRONUCLEI Isolated or broken chromosome fragments which are not expelled when the nucleus is lost during cell division, but remain in the body of the cell forming micronuclei.

MICRONUCLEUS TEST See Micronuclei.

MICROTUBULES Filaments which make up the cell division spindle and are responsible for the movement of chromosomes during nuclear division.

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

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

MUCIFICATION The presence of mucus.

MUCOSAL Regarding the mucosa or mucous membranes, consisting of epithelium (qv) containing glands secreting mucus, with underlying layers of connective tissue and muscle.

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

MYCOTOXIN Toxic compound produced by a fungus.

NEOPLASM See 'tumour'.

NON-GENOTOXIC See 'carcinogens'.

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

PATHOGENIC Capable of causing disease.

PHASE II ENZYMES (Also known as conjugation enzymes). These enzymes catalyse the addition of a readily available polar endogenous substance to the foreign compound (either directly or after initial oxidative metabolism of the compound (ie phase I metabolism)). The polar moiety renders the compound more water soluble and thus more readily cleared from the body.

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

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

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

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

PROCARCINOGEN An inactive carcinogen which is metabolically converted, via proximate carcinogens, to the electrophilic ultimate carcinogen that reacts with DNA.

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

RELATIVE RISK The ratio of the risk of disease or death among the exposed to risk among the unexposed; this usually is synonymous with risk ratio. Alternatively, the ratio of the cumulative incidence rate in the exposed to the cumulative incidence rate in the unexposed, ie the cumulative incidence ratio.

RENAL Relating to the kidney.

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

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

TDI Tolerable daily intake.

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

TERATOLOGY The study of development abnormalities and their causes.

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

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

TRANSFORMATION The process by which a normal cell acquires the capacity for neoplastic growth. Complete transformation occurs in several stages both *in vitro* and *in vivo*. One step which has been identified *in vitro* is 'immortalisation' by which a cell acquires the ability to divide indefinitely in culture. Such cells do not have the capacity to form tumours in animals, but can be induced to do so by

extended passage *in vitro*, by treatment with chemicals, or by transfection with oncogene DNA. The transformed phenotype so generated is usually, but not always, associated with the ability of the cells to grow in soft agar and to form tumours when transplanted into animals. It should be noted that each of these stages of transformation can involve multiple events which may or may not be genetic. The order in which these events take place, if they occur at all, *in vivo* is not known.

TRANSISTIONAL CELL ADENOMA/CARCINOMA Tumours of the epithelium lining the renal pelvis, ureter and bladder (urothelium).

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

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

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

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

XENOBIOTIC A chemical foreign to the biologic system.

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

1992 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 3216041 Price £11.70.

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