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

1993 Annual Report

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ANNUAL REPORT

OF THE

COMMITTEES ON

TOXICITY
MUTAGENICITY
CARCINOGENICITY

OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

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About The Committees

This is the third 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.

Continuing the practice begun last year, this report also publishes the commercial interests of committee members. Members are required to declare any commercial interests on appointment and, again, during meetings if a topic arises in which they have an interest. If the Chairman so deems, members whose outside interests may be considered to be too close to the topic under discussion can be excluded from discussion and from decision making.

The usual way in which committee reviews are conducted is that the relevant secretariat critically assesses all the relevant data and prepares papers for the Committee. These normally consist of appendices giving detailed summaries of the studies reviewed – methodology and results – and a covering paper in which the available data are briefly summarised, the most important points highlighted and recommendations presented for discussion by the Committee. Although original study reports are not routinely circulated to members, they are made available on request, and are circulated if the study is particularly complex. Definitive summaries are necessary because documentation on any one chemical can amount to many hundreds of pages.

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

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

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Preface

This year's report contains conclusions and advice to Ministers which illustrate very well the nature and range of work carried out by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT). The advice laid out in this report was formulated after lengthy review of substantial amounts of complex data and built upon the Committee's consideration of specific topics in previous years. This underlines the value of the history of the COT which was set up in **1978.**

The membership of the Committee is such as to include expert opinion in a wide range of subject areas and, from time to time, sub-groups review particular topics, co-opting individuals with special knowledge to aid the decision making process. This is illustrated in the section on perchloroethylene.

Readers of this report will be familiar with the setting of an acceptable daily intake (ADI) for food additives and contaminants based on data derived from chronic animal toxicity studies. This quantitative estimate of acceptable intakes of chemicals in food was first used by the COT in **1989** and, as this and other annual reports have shown, is of considerable value in the assessment of toxicity of chemicals in man. The COT is well versed in the derivation and application of an ADI and is engaged in the "fine tuning" of its use as a tool in safety assessment.

Although an annual report is of necessity retrospective we should consider the future. Some of the responsibility for the formulation of advice in relation to the chemical safety of food now rests with bodies advising the European Commission. For example, the consideration of a new additive is in the hands of the Scientific Committee for Food (SCF). However, as our terms of reference show, much work remains for the COT and there is a considerable challenge to be faced in relation to the formulation of methods of both risk assessment and risk management of chemicals against the background of the Government policy to promote increasing standards of food safety.

Lastly, I wish to acknowledge, on behalf of the Committee, the very high standard of administration and documentation provided by the Secretariat.

FRANK WOODS

Carrageenan

1.1 Carrageenan is the generic term for a group of similar chemicals obtained by aqueous extraction of certain types of red seaweeds. It is used in food as a gelling agent and stabiliser and is particularly useful in milk products.

1.2 Carrageenan had been reviewed by the COT as part of the review of a class of food additives termed emulsifiers and stabilisers. In its report of this review[1], the COT recommended that consideration should be given to tightening the specification for carrageenan to restrict the proportion of lower molecular weight material (degraded carrageenan) in food-grade material, so as to minimise the absorption of this material from the gut. Carrageenan is well known to have effects on the immune system of laboratory animals if administered parenterally and some studies have shown effects following oral administration. The Committee was concerned that even limited uptake of carrageenan by humans could result in immunological effects and, in particular, that it might enhance the likelihood of allergic-type reactions. Therefore, it also asked that studies be carried out further to investigate the extent of absorption and bioavailability of orally administered food-grade carrageenan, particularly by the immature gut, and of possible effects on the immune system due to uptake by the gut-associated lymphoid tissues. In the meantime, the COT altered its classification of carrageenan from fully acceptable to provisionally acceptable, pending the provision of the data requested.

1.3 Following the publication of this advice in 1992, submissions were received from the International Food Additives Council and from the Centre de Liaison des Industries de Traitement des Algues de la CEE containing data not previously seen by the COT, including an unpublished report of a long-term study in monkeys, and arguing that these data answered the COT's concerns and that the classification should therefore revert to fully acceptable. The submissions were reviewed by the Committee, which also considered recommendations from the Ministry of Agriculture, Fisheries and Food (MAFF) about how the specification could be tightened.

1.4 The COT concluded that the submissions did not fully consider the potential for immunological effects of carrageenan acting via the gut-associated lymphoid tissue and still considered it possible that orally administered carrageenan could influence the development of allergic food intolerance. Further work is still, therefore, required. The Committee also recommended that the specification for carrageenan should be altered to introduce a maximum of 10% of a molecular weight fraction of less than 100,000.

Florfenicol

1.5 Florfenicol is a new, broad spectrum antibiotic which has been developed for veterinary use. The Veterinary Products Committee (VPC) had received an application for the licensing of the UK use of a florfenicol-containing product,

1. See: Food Advisory Committee Report on the Review of the Emulsifiers and Stabilisers in Food Regulations. FdAC/REP/11. HMSO, London, 1992.

and the European Commission's Committee on Veterinary Medicinal Products (CVMP) had been asked to consider setting a maximum residue limit (MRL) for florfenicol. The COT was asked to advise these committees on the toxicology of florfenicol. The main concern which the COT was asked to address was the potential of florfenicol to cause blood dyscrasia in humans. Although there was no experimental evidence to indicate that this drug could cause these effects, there was concern that florfenicol might have similar toxicological properties to a chemically-similar drug: chloramphenicol, which is known to cause serious blood dyscrasia (including leukaemia and fatal aplastic anaemia) in a very small proportion of exposed people.

1.6 In 1992, the COT had been asked to address a similar question about the safety of thiamphenicol which is another drug with a structure similar to those of florfenicol and chloramphenicol. In this case, the COT was able to refer to data from human use of the drug, and concluded that there was no convincing evidence to suggest that thiamphenicol could cause blood dyscrasia. However, as florfenicol has never been used as a human medicine, there were no human data to consider in this case.

1.7 The COT considered limited evidence in support of a hypothesis that there was a risk of human blood dyscrasia only when the drug in question had a para-nitro group in its chemical structure (ie. as is the case for chloramphenicol, but not for thiamphenicol or florfenicol). The Committee regarded this hypothesis as unproven, and agreed that there were insufficient data to allow them to conclude whether or not florfenicol could cause blood dyscrasia in humans.

1.8 This advice was passed on to the VPC and to the CVMP's Working Group on Safety of Residues. In the absence of reassurance about the safety of florfenicol, the VPC did not issue a product licence and the Working Group could not set any maximum residue limit (MRL). Consequently, there are no permitted florfenicol-containing products available for human or veterinary use within the EC.

Gellan Gum

1.9 In 1993 the COT completed its review of a new polysaccharide gelling agent, gellan gum. The gum is produced during the fermentation of ***Pseudomonas elodea*** bacteria and has a range of food applications. Several forms of gellan gum are available with differing clarities and textures.

1.10 The COT considered a full toxicological data set on gellan gum and was reassured that there were no significant adverse toxicological effects. Long-term and reproductive studies in rats and a one-year study in dogs gave no observed adverse effect levels (NOAELs) of 3 and 2 g/kg bw/day respectively, the highest doses administered to each species. A rabbit teratology study gave a NOAEL of 900 mg/kg bw/day [a lower NOAEL was anticipated in this species given the enhanced sensitivity of the rabbit gut to gum-like compounds]. The results of two short-term human feeding studies using daily doses up to 200 mg/kg bw gave no cause for concern. However, the Committee did initially have some

reservations about the technical aspects of gum production and asked for more details on the process specification and control. It also wanted reassurance that any toxins which might theoretically be produced during fermentation would be destroyed/denatured by the purification process. The manufacturers of the gum subsequently provided data which addressed all the COT's concerns.

1.11 The Committee was asked by the Secretariat to consider whether it would be prepared to set an ADI non-specified (ADI ns) for gellan gum. The term ADI ns is defined by the World Health Organisation/Food and Agriculture Organisation Joint Expert Committee on Food Additives (JECFA)as "applicable to substances of very low toxicity which, on the basis of available data (chemical, biochemical, toxicological and other), the total daily intake of the substance, arising from its use at the levels necessary to achieve the desired effect and from its acceptable background in food, does not, in the opinion of the Committee, represent a hazard to health. For this reason, and the reasons stated in the individual evaluations, the establishment of an ADI in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice ie it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal inferior food quality or adulteration and it should not create a nutritional imbalance". When an ADI ns is set, it is usually stressed that the evaluation only covers the substance when used as a food additive according to good manufacturing practice and not special dietary or medicinal uses. Also, when new uses of the additive which would significantly increase intake are envisaged, the additive should be brought forward for re-evaluation. Similar gum materials eg locust bean gum, tragacanth gum and xanthan gum, when evaluated by international committees such as JECFA and the European Commission's Scientific Committee for Food (SCF) have been allocated ADI ns.

1.12 The COT reviewed projected intake estimates for gellan gum by adults and by infants aged 6–12 months. Extreme intakes were estimated as 7.7 and 38.6 mg/kg bw/day, respectively, with average intakes considerably lower. The COT decided that, in view of this, and since gellan gum was a relatively inert material, poorly absorbed and of low toxicity, it would be appropriate to set an ADI ns for this gum. However, the Committee asked to see estimates of actual intake data 3 years after gellan gum's introduction to the market in case these were substantially different from the projected intakes.

Malachite Green

1.13 The VPC requested the advice of the COT on the possible risk to consumers from residues of malachite green which had been detected in UK retail samples of farmed trout. Malachite green is manufactured for use as a dyestuff, but has also been widely used for treating fungal and protozoan infections of fish.

1.14 A voluntary sampling programme, initiated under Directive 86/469/EEC, detected residues of malachite green in muscle samples from 6 out of 82 farmed trout. Concentrations of 1 to 7.4 µg/kg were found. No residues were detected in 144 farmed salmon. The limit of determination of the method was initially 1 µg/kg but was raised to 3 µg/kg when the method was automated.

1.15 The COT reached the following conclusions about the risk to consumers from the residues identified in farmed trout:

(i) Poor chemical characterisation of the malachite green used in toxicological studies and that used in fish farming, along with the near absence of data on absorption, distribution, metabolism and excretion, made it difficult to accurately relate the doses given in experimental studies to those applied in fish farming.

(ii) *In vitro* mutagenicity data indicated that malachite green has mutagenic potential, although the limited *in vivo* mutagenicity data suggested that this potential is not expressed in the whole animal. The COT noted with concern that there were mutagenic dyes (eg. gentian violet) which were chemically similar to malachite green. Further work, including a fuller investigation of possible *in vivo* mutagenicity, would be needed for the mutagenic risk from malachite green residues to be fully clarified.

(iii) There was no reliable evidence that malachite green is carcinogenic, but neither were there adequate data to demonstrate a lack of carcinogenicity. However, the COT expressed concern about the observation that several dyes (eg. gentian violet, brilliant blue FCF, guinea green B and benzyl violet 4B), which are closely similar in chemical structure to malachite green, have been shown to be carcinogenic in laboratory animals. It was also noted that there were limited data which suggested that repeated exposure to malachite green may promote the development of tumours which had been initiated by other agents.

(iv) Malachite green was clearly fetotoxic to rabbits, and there was some evidence to suggest that it may also be teratogenic. No NOAEL had been demonstrated.

(v) There were insufficient reliable toxicological data to permit a full safety assessment. It was not possible therefore to set an ADI for malachite green.

(vi) Only low concentrations of malachite green were found in only a small proportion (about 7%) of samples of farmed trout. The COT concluded that these occasional trace residues would probably not adversely affect the health of consumers. However, since there are insufficient data on the chemical nature and safety of malachite green, the COT considered that this material had not been proven to be safe for use in fish farming.

1.16 This advice was passed to the VPC for discussion in early 1994.

Mineral Hydrocarbons

1.17 The COT last considered the use of mineral hydrocarbons in food in 1988. These materials had previously been considered provisionally acceptable for use in food. However, after reviewing the results of new studies on two mineral oils, the Committee recommended that mineral hydrocarbons should be reclassified as substances for which the available data indicate definite or probable toxicity and which ought not to be permitted in food. The use of mineral hydrocarbons

in chewing gum was later considered as part of a general review of chewing gum bases in 1989. A decision was taken also to classify mineral hydrocarbons used in chewing gum bases as substances for which the available data indicate definite or probable toxicity. The Committee stated that it would need to see the results of 90-day studies on a range of mineral hydrocarbons representative of those used in food applications before it could reconsider their acceptability for use in food.

1.18 Following the advice, which was endorsed by the Food Advisory Committee (FAC), that mineral hydrocarbons were no longer acceptable for use in food, proposals amending the 1966 Mineral Hydrocarbons in Food Regulations were issued for public comment as required under the terms of the Food Safety Act. However, consultation on these proposals revealed legal problems which had not been anticipated and were difficult to resolve. At the same time the oil industry, through its European organization for environmental health protection (CONCAWE), indicated that it was sponsoring 90-day feeding studies on a range of mineral oils and waxes. In view of the legal difficulties and given that restrictions on the use of mineral hydrocarbons already existed in legislation and the fact that some of the uses of these materials had been discontinued or alternatives found, it was considered prudent to defer any further action for statutory restrictions on mineral hydrocarbons until the results of the industry sponsored 90-day studies had been evaluated.

1.19 These studies were completed in 1993 and were submitted for evaluation by the COT. The Committee's conclusions were given in the form of the following statement:

Introduction

(i) Recently, the results of several new studies on a range of mineral hydrocarbons were submitted to us. We understand that the substances tested in these studies were chosen as being representative of food-grade mineral hydrocarbons prepared from different crude oil sources, either naphthenic (N) or paraffinic (P), using two different routes of manufacture: an acid (A) or hydrogenation (H) process, and spanning the range of viscosities currently in use ie 10-100 centistokes (cSt). We have now carried out a review of the following studies:

(a) a 90-day study in Fischer 344 rats which investigated the effect of feeding six different mineral hydrocarbon oils and three mineral hydrocarbon waxes to rats at levels of 0.002, 0.02, 0.2 and 2% in the diet. In some of the treated and control groups, the 90-day dosing period was followed by a 28-day withdrawal period to determine the reversibility of any of the effects observed. The six oils tested were described as N15(H)[2], N70(A), N70(H), P15(H), N10(A), and P100(H). The three waxes tested were a low melting point paraffinic wax and two microcrystalline waxes: a high melting point wax and a high sulphur wax.

2. N15H – denotes a naphthenic (N) oil made by the hydrogenation (H) process and having a viscosity of 15 cSt.

(b) a 90-day study which investigated the effect of feeding an intermediate melting point wax and a 50 : 50 blend of the high melting point wax and the low melting point wax mentioned in (a) to Fischer **344** rats at dietary levels of 0.02, 0.2 and 2.0%. The low melting point wax mentioned in (a) was tested again in this study but only at the 2% dose level. Again, certain groups of treated and control animals were given a withdrawal period, in this instance 85 days, following the 90-day dosing period.

(c) a 90-day feeding study which investigated the effect of feeding to Fischer **344** rats a **P70(H)** mineral oil at levels of 0.002, 0.02, 0.2 and 2% in the diet. There was no reversibility phase in this study. This study was considered together with some older studies which had been submitted previously.

(d) a 90-day dose-ranging study which investigated the effects of feeding to Fischer **344** rats two paraffinic waxes, similar to the low melting point wax mentioned in (a) and (b), at levels of 0.002, 0.02, 0.2 and 240. For comparison, the waxes were also given to **Sprague** Dawley rats at a level of 2% in the diet. Only a short summary of this study was available.

Summary of results

(ii) Most of the mineral hydrocarbon oils and waxes tested in these new studies produced a range of toxic effects. The types of effects seen with the different oils and waxes were similar in nature but differed in severity ie a number of test materials did not give rise to significant effects at 2% in the diet whereas others produced effects at 0.002%. Female rats appeared to be more susceptible than male rats. With many of the test materials there was a good correlation of effects within groups and a clear dose response relationship.

(iii) With the more toxic materials ie the low melting point wax, intermediate melting point wax and the oils **N10(A), N15(H), P15(H),** the following were all evident: tissue accumulation of hydrocarbon material; increased organ weights, in particular liver and lymph node weights; altered serum enzyme levels eg **AlkP,** ALAT, **ASAT,** gamma-GT, and increased monocyte and neutrophil counts. The main histopathological findings were granulomata in the liver and histiocytosis in the lymph nodes. An inflammatory lesion at the base of the mitral valve in the heart was detected in rats dosed with the low melting point and intermediate melting point waxes; none of the oils tested produced this lesion.

(iv) Similar effects to those described above were also produced by the **N70(H)** and **N70(A)** oils. However, the incidence and severity of histiocytosis and granulomata induced by these oils were much lower and there were few significant changes in clinical chemistry and haematological parameters. The **P70(H)** oil produced no significant histological lesions, although accumulated hydrocarbon material was observed in the liver and lymph nodes. The **P100(H)** oil did not cause toxicity but there was evidence that hydrocarbon material from this oil may have accumulated in tissues during the 90-day treatment period and that the resultant levels did not

decrease during the reversal phase. No accumulation was observed with the high sulphur wax and the high melting point wax nor did these waxes produce any toxic effects.

(v) In the study referred to in paragraph (i)a, there was a decrease in the concentration of serum vitamin E in the oil dosed groups but not the wax dosed groups. In this respect male rats were more susceptible than females.

(vi) The results of those studies which included a withdrawal period indicated that most of the toxic effects were still evident following 28 days withdrawal of the test material and, with some of the waxes, after 85 days withdrawal. However, there was limited evidence that the severity of some of the effects had decreased during the reversal phase.

(vii) We are concerned that, with the exception of the high sulphur wax and the high melting point wax, hydrocarbon material from all other test materials accumulated in organs including the liver, mesenteric lymph nodes, kidney, spleen and perirenal fat. In general, the greatest accumulation was seen in the liver and lymph nodes. There was a strong association between the accumulation of hydrocarbon material and toxicity. The study referred to in paragraph (i)b reported the presence of birefringent material, which may be hydrocarbon material, in the mitral valve in the heart.

(viii) Although the histological lesions were largely foreign body reactions, we consider them to be undesirable toxic effects. The presence of liver granulomata accompanied by changes in haematology and clinical chemistry parameters is consistent with this being a chronic inflammatory reaction. There was some evidence that the low melting point wax was hepatotoxic. The lymph node histiocytosis was considered to be a non-inflammatory, non-specific effect. The mechanism and toxicological significance of the inflammatory lesion in the mitral valve are unclear; nonetheless, we are concerned about this effect which was not reported in previous studies.

Strain difference

(ix) The study described in (i)d was preliminary and therefore it is not possible to draw any definitive conclusions as to whether there is a strain difference in rats in their response to mineral hydrocarbons. Toxic effects, including increased organ weight and histopathological lesions, were evident in both strains of rats, although the incidence and severity of these effects were greater in Fischer **344** rats.

Discussion

(x) We have discussed the use of mineral hydrocarbons as direct and indirect food additives on several occasions and were concerned not only with the potential of these compounds to cause toxicity but by the fact that they are not adequately specified. These latest studies, together with the study we reviewed in 1988, confirm that with many of these materials

there is hydrocarbon accumulation in organs such as the liver and the mesenteric lymph nodes. This accumulation gives rise to reactions within these tissues and this is clearly of concern.

(xi) The oils and waxes tested in this series of studies are not defined chemicals but complex mixtures of hydrocarbons and other materials. The current statutory specifications provide only a general description of the materials, although additional information is now available on the mineral hydrocarbons discussed in this statement. This information is based largely on physical parameters such as viscosity and the molecular weight range of the hydrocarbon components. The absorption and subsequent toxicity of these materials appear to be linked to these physical properties in that those oils and waxes with lower molecular weight components and lower viscosities are more likely to be absorbed and to accumulate in tissues. However, there must be other factors which influence accumulation, as is evident from the results of studies described in paragraphs (i)a and (i)b on the high sulphur wax and the intermediate melting point wax. Examination of the carbon number distribution for these two materials shows that there is considerable overlap in terms of the molecular weight range of the hydrocarbons they contain. Thus both the intermediate melting point wax and the high sulphur wax contained approximately **25%** hydrocarbons with carbon numbers lower than **31**. However, the high sulphur wax did not give rise to accumulation nor cause toxic effects during the 90-day feeding period, whereas accumulation of hydrocarbon material was found with the intermediate melting point wax and it produced toxic effects at **0.02%** and above in the diet. Thus molecular weight cannot be the sole determinant of accumulation and toxicity.

(xii) There is some indirect evidence that the structure of the individual hydrocarbon components may have a role in the propensity of these materials to accumulate, since follow-up work on the study described in paragraph (i)a indicated that branched chain hydrocarbons were cleared more slowly from the liver than straight chain hydrocarbons. However, without a clear understanding of the factors which determine toxicity, acceptable limits of variability for individual products cannot be defined.

Mineral oils

(xiii) It is possible to derive no observed adverse effect levels (NOAELs) for some of the mineral hydrocarbon oils which were tested. However, given that the toxic effects produced by these oils appear to result from the accumulation of hydrocarbon material, we believe it is inappropriate to use a NOAEL from a short term toxicity study to set an acceptable daily intake (ADI) as accumulation may occur at lower doses following a longer period of exposure. It is also possible that conditions within the gut, such as fat content of the diet, could influence the degree of absorption of these

materials. We consider it inadvisable to permit, as food additives or processing aids, substances which have demonstrated the potential to accumulate and cause toxicity.

(xiv) Although no toxic effects were observed with the P100(H) oil, there is evidence that this oil may have given rise to hydrocarbon accumulation during the treatment period. The resultant tissue levels did not decrease during the reversal period, adding to the suggestion that this oil can accumulate. We would therefore need to see the results of a longer term study on this oil in order to be reassured that the ingestion of the oil over a longer time period would not give rise to accumulation to such an extent that it would cause toxicity.

Mineral waxes

(xv) The low and intermediate melting point waxes were among the most toxic of the hydrocarbon materials tested. It is not possible to identify a NOAEL for these waxes and we recommend that they remain unacceptable for use in food. There was no evidence of hydrocarbon accumulation in rats dosed with the high sulphur wax or the high melting point wax nor did these waxes cause toxicity even at the highest level administered. Therefore, we recommend a group temporary $ADI_{1993-1996}$ of 0-10mg/kg body weight/day for the high sulphur and high melting point wax.

Chewing gum bases

(xvi) We have again considered the specific case of the use of mineral hydrocarbon waxes in chewing gum base. After reviewing all the available data on the extraction of waxes from chewing gums *in vitro* and *in vivo*, we still consider that these waxes could be available for absorption from the gastrointestinal tract following chewing or swallowing of the gum. Therefore, we recommend that the temporary ADI set for the high melting point and high sulphur microcrystalline waxes should include the intake from the use of these waxes in chewing gum.

Further work

(xvii) In order to set a full ADI for the microcrystalline waxes, the chemical characterization of these waxes must be improved and the results of studies on the absorption of microcrystalline waxes in the presence of different levels of dietary fat, plus data on the nature of any accumulated material, must be submitted. Studies of the effect these waxes have on the uptake of fat soluble dietary components such as vitamins are also required. These data should be submitted by 1996.

(xviii) Although we cannot set an ADI for the P100(H) oil, further work on this material may enable an ADI to be set in the future. In order to reconsider

this oil we would need to see the results of a 12 month study in the rat at thedoses used in the 90-day study described in paragraph (i)a, in order to assess whether the oil would accumulate and cause toxic effects following long term exposure.

(xix) Before we could reconsider the other mineral hydrocarbon materials tested in these studies, a better understanding of those factors which are determinants of toxicity is required. It is evident from the results of the studies on the high sulphur wax that molecular weight is not the sole determinant of toxicity. In order to address this issue, further studies would need to be carried out on mineral hydrocarbon components covering a range of molecular weights and with branched and unbranched structures, in order to identify those fractions responsible for toxicity.

Recommendations

(xx) We therefore recommend that

(a) all mineral hydrocarbon oils are unacceptable for use in food.

(b) with the exception of the microcrystalline waxes described below, all other mineral hydrocarbon waxes are unacceptable for use in food.

(c) the high melting point and high sulphur microcrystalline waxes are temporarily acceptable for use in food, including chewing gum base, subject to a temporary group $ADI_{1993-1996}$ of 0-10mg/kg bw/day.

Perchloroethylene (Tetrachloroethylene)

1.20 Perchloroethylene is a chlorinated hydrocarbon solvent with widespread industrial application. It has been used as a dry-cleaning agent for over 30 years and its use for this purpose is likely to increase as an alternative solvent is phased out because of its ozone depleting potential. In recent years a number of epidemiological papers have been published which linked perchloroethylene exposure with an increased risk of spontaneous abortion (miscarriage). In 1992 the Health and Safety Executive (HSE) asked the COT for its expert opinion on whether there is a reproductive hazard associated with perchloroethylene and, if so, whether there is a risk of developmental toxicity arising from exposure in the workplace and for the general public and consumer. At the same time, MAFF asked the COT for advice on the health implications of residues of perchloroethylene which had been found in a survey of butter and lard from selected retail outlets in the UK[3].

1.21 The Committee reviewed the relevant animal toxicity data on perchloroethylene, as summarised in HSE Toxicity Review no. 17. It also set up a small subgroup to review the epidemiological papers, comprising those members with

3. This survey was announced in MAFF Food Safety Directorate Information Bulletin no. **41,** September **1993.**

particular expertise in reproductive toxicology and/or epidemiology, which coopted Dr E Roman, an epidemiologist from the International Cancer Research Foundation. It should be noted that the interpretation of epidemiology studies is usually difficult due to uncertainties about the level of exposure of the people studied to the chemical under consideration and the possibility of confounding factors which might have influenced the outcome. In this case, there were additional problems because of overlap between some of the individual studies ie the same people featured in different studies.

1.22 After a long review, which was completed in **1993,** the Committee concluded as follows:

(i) The available animal data did not support the view that perchloroethylene was a potential reproductive toxicant in humans.

(ii) The available epidemiological evidence, although based on studies that are methodologically weak, is consistent with the view that perchloroethylene may be a reproductive toxicant in humans.

(iii) The available animal data do not indicate a risk of teratogenicity and the general consensus view of the available epidemiological data is that perchloroethylene is not a human teratogen. However, the finding of an increased rate of unspecified malformations of the heart and circulatory system in babies of launderers and drycleaners in the data of the Office of Population and Census Statistics (OPCS) for **1980-1982** warrants further investigation. The available epidemiological evidence does indicate that there is an increased risk of low birth weight.

1.23 The COT further advised that, until the association between perchloroethylene and reproductive outcome in humans has been investigated further, it would be prudent to keep the exposure to perchloroethylene of women of child bearing potential working in the drycleaning and laundry industry to a minimum.

1.24 As a result of the COT's advice, the HSE is undertaking further studies to investigate the possible association between perchloroethylene and reproductive outcome.

1.25 As regards the advice required by MAFF, the COT concluded that even worst case intakes of perchloroethylene from contaminated butter and lard are likely to be more than **10,000** times lower than levels known to cause minor adverse effects in laboratory animals and more than **500** times lower than those suggested to cause minor adverse effects in man. Therefore the Committee concluded that the residue data gave no rise to concern.

Vitamin A

1.26 In **1990** the COT was made aware that liver on retail sale could contain large amounts of Vitamin A. High intakes of this vitamin are known to be teratogenic in laboratory animals and some chemically similar compounds are known to be teratogenic in humans. After discussion, the Committee advised that consumption of liver and liver products by pregnant women may pose a

teratogenic hazard to the fetus. As a result, the Chief Medical Officer (CMO) and Chief Nursing Officer (CNO) issued advice to health professionals in 1990 warning that women who are pregnant or might become pregnant should not consume liver, liver products nor high level Vitamin A supplements.

1.27 While there are a number of case reports of teratogenicity following chronic overprovision of Vitamin A from dietary supplements, the other major source of Vitamin A for pregnant women, there are no good data showing directly that Vitamin A in liver is harmful to the fetus. This raised the question of whether there may be differences in the amounts of the harmful derivatives of Vitamin A in the blood, and in the length of time they are present in the blood, after consumption of a liver meal compared to after consumption of supplements. Accordingly, the CMO and the COT requested in 1990 that a study be undertaken comparing the blood profiles of the metabolites when an identical amount of Vitamin A is ingested by young women either in the form of supplements, or in liver as part of a meal. The results of such a study became available in 1993 and prompted a re-examination of the COT's previous advice.

1.28 The main findings of this study are summarised below. The two derivatives of most interest are all-trans-retinoic acid (RA, tretinoin) and 13-cis retinoic acid (CRA, isotretinoin). Current opinion is that both are teratogenic to humans. CRA is prescribed as an anti-acne drug and is strongly contraindicated in women who are pregnant or those who may become pregnant because of its ability to produce fetal deformities.

1.29 The study showed that the increase in RA after a liver meal was sufficiently small to pose neglible risk of teratogenicity. However, plasma profiles of CRA were similar whether liver or supplements were ingested. As CRA is considered a teratogen, the COT decided that the results from this study do not allow a modification of the advice given in 1990. Accordingly, in November 1993, the CMO and CNO reiterated their advice to pregnant women and women who might become pregnant that they should not eat liver. It should be noted that there is no reason why children, men and other women should not continue eating liver as before.

Food Surveillance Papers

1.30 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 repqrts, 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.

1.31 During 1993 the COT considered a report from the Working Party on Naturally Occurring Toxicants in Food on miscellaneous naturally occurring toxicants in food (Food Surveillance Paper No 42. HMSO, London 1994). The

topics covered include diarrhoetic and paralytic shellfish poisoning, pyrrolizidine alkaloids in herbal products and other selected foods, other alkaloids in herbal products and ethyl carbamate. The COT's advice is included in this Food Surveillance Paper as an appendix.

Topics Still Under Consideration

1.32 The following topics, which were discussed by the COT at meetings held in 1993, are under review:

Poly 1,3-butylene glycol adipate

Chlorinated flour

Hydrocarbon propellants

Polychlorinated biphenyls

1993 Membership of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

Professor H F Woods BSc MB BCh MRCP D Phil FRCP(Lon) FFPM FRCP(Edin)

MEMBERS

P Aggett MB ChB FRCP MSc DCH
V Beral MB BS FRCP
Professor A D Dayan BSc MD FRCPath FFPM CBiol FIBiol
Professor G G Gibson BSc PhD
G Hawksworth BSc PhD
I Kimber BSc MSc PhD FIMLS CBiol MIBiol
D E Prentice MA VetMB MVSc MRCPath MRCVS
A G Renwick BSc PhD DSc
F M Sullivan BSc
A Thomas MB ChB MRCP PhD
Professor D Walker BVSc FRCVS CBiol FIBiol
Professor R Walker PhD FRSC CChem FIFST

SECRETARIAT

N R Lazarus MB BCh BSc PhD FRCPath (Medical)
Miss F D Pollitt MA DipRCPath (Scientific DH)
D Watson BSc PhD DMS CBiol MIBiol (Scientific MAFF)
Mrs D Davey (Administrative)

J M Battershill BSc MSc
A Browning MIScT CBiol MIBiol
Miss S O'Hagan BSc
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 1993

	Personal Intereet		Non Pereonal Intereet	
Member	Company	Interest	Company	Intereet
Prof H F Woods (Chairman)	Cadbury Schweppes Edinburgh Investment Trust Foreign & Colonial Investment Trust Hanson Smith & Nephew	Share Holder	Wide range of national & international food & chemical companies.	Dean of the University of Sheffield Medical School, Faculty of Medicine and Dentistry. Which has extensive activity in teaching and research in food science and technology and in topics related to and supported by, many companies in the food and chemical industry. Trustee of Hallamshire Therapeutic Research Trust Ltd, Harry Bottom Charitable Trust and Special Trustees for the former United Sheffield Hospitals.
Dr P J Aggett	Milupa Milupa UK	Occasional Fee Occasional Fee	SMA Nutrition Nestlé Nutricia Milupa	Research Support Research Support Research Support Research Support
Dr V Beral	NONE	NONE	NONE	NONE
Prof A D Dayan	Welcome Foundation Lilly Risk & Policy Analysts Schering Plough SmithKline Beecham British Petroleum British Gas British Steel Hanson National Power Powergen TI	Pension Consultant Consultant Consultant Consultant Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	Glaxo	Research Fellowship
Prof G G Gibson	NONE	NONE	Unilever plc	Research Studentship
Dr G Hawksworth	NONE	NONE	Zeneca Glaxo Group Research Servier Research & Dev Pfizer Central Research SmithKline Beecham Solvay Pharma	Research Support
Dr I Kimber	ZENECA Zeneca British Petroleum	Employee Share Holder Share Holder	Unilever plc	Grant for Research

	Personal Interest		Non Personal Interest	
Member	Company	Interest	Company	Interest
Dr D Prentice	Ciba Gensia Hoechst Veterinar Hoffman-La Roche Nestle Rhône-Poulenc Rorer Sandoz Syntax Synthelabo (LERS) TAP Pharmaceuticals Wyeth-Ayers	Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant Consultant	NONE	NONE
Dr A G Renwick	International Sweeteners Association SmithKline Beecham	Consultant Constultant	Bayer Ciba-Geigy Glaxo Hoffmann-La Roche Solvay-Duphar	Research Support Research Support Research Support Research Support Research Support
Mr F Sullivan	Rank Hovis McDougal Rothmans International Rhône-Poulenc Rorer-France Borax Ltd Wyeth- Ayerest	Consultant Consultant Occasional Fee Occasional Fee Occasional Fee	EC Community (DGV)	Grant
Dr A Thomas	NONE	NONE	NONE	NONE
Prof D Walker	SmithKline Beecham British American Tobacco BP	Consultant Consultant Share Holder	Robens Institute, Univ of Surrey	Retaining Fee
Prof R Walker	Proctor & Gamble IDV Ltd Food Safety Advisory Centre Coffee Science Info Centre Cadbury Beverages Europe	Consultant Consultant Consultant Consultant Consultant	Nestle	Research Studentship

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

Professor J M Parrv
(Chairman)
BSc PhD DSc

Preface

The correct functioning of our genetic material is necessary for the development and good health of each individual. When mutations occur in human genes they may lead to the formation of defective gene products and ultimately to birth defects or to somatic diseases such as cancer. The COM is primarily concerned with the evaluation of data concerning chemicals which may cause mutations, and the assessment of the relevance of these mutations to humans. The Committee spends the majority of its time considering specific substances referred to it by the regulatory authorities within the UK. The Annual Report reviews the chemicals referred to the COM and the conclusions reached by the Members during 1993.

During my 10 years as a member of the COM I have been able to observe and participate in the careful consideration by the Members of all the data provided on individual chemicals. The development of the analytical approach used by the Committee has been to the great credit of the previous Chairman, Professor Bryn Bridges, and the support provided by the Secretariat. It has been a great pleasure to be appointed as Professor Bridges' successor and to continue his work. Unfortunately, my first year as Chairman was saddened by the unexpected death of Dr Margaret Fox, a much respected member of the COM since 1989.

The reliable identification of mutagenic chemicals requires comprehensive testing. During 1993 the Committee has continued its long term interest in the strategy of testing chemicals for mutagenicity and has advised government officials involved in international negotiations on the harmonisation of such testing. The Committee's policy is to require the minimum number of tests needed to obtain a clear answer, with the minimum usage of animals, and with an emphasis on well designed studies and reproducible results. In the future I hope that the Committee will consider the relevance and validation of new assays and technologies as they become available. The Committee should be involved in the identification of the types of assays which are still needed to fully characterise human mutagenic hazards.

An important general area that the Committee has considered in the past year is aneuploidy, including biological mechanisms and a possible regulatory approach to setting safe limits for aneugenic chemicals. The Committee has

indicated the nature of the experimental data which might be used to demonstrate a threshold of action for aneugenic chemicals which act by inducing damage in the cell division spindle apparatus and this is included in this report.

JAMES PARRY

Bracken

2.1 In 1988 the COC considered the implications for human health of exposure to bracken. At that time MAFF had commissioned work to investigate whether mutagenic components in bracken could be passed into the milk of bracken-fed goats. Goats had been used in this study rather than .cows because of the reluctance of the latter to graze bracken, however the results of the study showed that bracken was equally unappetising to goats. MAFF received the results of the study at the end of 1992 and the COM were asked to comment on them.

2.2 Members agreed the following conclusions:

(i) Solvent extracts of bracken fern showed mutagenic activity in bacterial assays. There was evidence that most of the mutagenic activity appeared to be due to the compound ptaquiloside, but other potentially mutagenic compounds might be present. There were some data suggesting that this activity was expressed in mammalian cells ***in vitro***. There were limited data available which suggested this mutagenic potential might be expressed ***in vivo***.

(ii) Work carried out for MAFF on the possibility of bracken mutagens being transmitted to the milk of bracken-fed goats suggested that very little, if any, mutagenic activity is present in the milk of goats exposed to UK bracken for short periods of time (1 month).

(iii) In view of the fact that cattle and goats would not eat bracken if other food is available, the Committee did not recommend that any further work should be carried out at present on the risk of transmission of mutagenic compounds from bracken into milk for human consumption, provided that the milk was bulked and processed centrally.

Use of Chlorine to Treat Flour Used for Making Cakes

2.3 In 1990 the Committee had requested further mutagenicity data on this process because of the need to provide ,reassurance that mutagenic products were not formed due to reaction of the chlorination process with flour components. Four fractions produced by extraction of cakes baked with chlorinated flour were investigated and the results compared with those from similar fractions from cakes baked with unchlorinated flour.

2.4 The Committee agreed the following conclusions:

(i) Four fractions (lipids, water solubles, hydrolysed starch and insoluble) extracted from cake baked with chlorinated flour had been examined in a forward mutation assay in ***Salmonella typhimurium*** BA13 (the ARA assay), a DNA repair assay in ***Escherichia coli*** (pol A assay) and the mouse lymphoma assay. The results were compared with those obtained with similar fractions obtained from cake that had been baked with control flour.

(ii) Prior to these studies a small validation exercise had been carried out on the mutation assay in ***Salmonella typhimurium*** strain BA13 (the ARA

assay). This indicated that the method was able to detect a number of mutagens that had different mechanisms of mutagenic activity, including MX [3-chloro-4-dichloromethyl-5-hydroxy-2-2(5H)furanone - the principal component of the mutagenic activity, detected in bacteria, arising from the chlorination of organic material in water]. It was an appropriate assay to use to investigate mutagenic potential of material where contamination by amino acids would be difficult to remove. Since the ARA assay did not detect some mutagenic agents acting by other mechanisms, a DNA repair assay in bacteria (the pol A assay) was also included in the testing strategy.

(iii) Fractions 1, 2 and 4 (lipids, water solubles and insoluble) obtained from cakes baked with chlorinated flour gave no evidence of any mutagenic activity in a mutation assay in *Salmonella typhimurium* strain BA13. No meaningful assessment could be made of the results obtained with Fraction 3 (hydrolysed starch) due to the very high levels of glucose present, a known confounder of this assay.

(iv) All the fractions from cakes baked with chlorinated flour gave no evidence of induction of DNA damage in a DNA repair assay using *Escherichia coli* (the pol A assay).

(v) Fractions 2 and 4 (water solubles and insoluble) from cakes baked with chlorinated flour gave negative results in the mouse lymphoma assay, as did fractions 1 and 3 (lipids and hydrolysed starch) in the presence of rat S9. Equivocal results were obtained with fractions 1 and 3 in the absence of S9, with a significant increase in mutant frequency at the highest concentration tested (associated with a reduction in relative total growth to about 10%) observed in one experiment, which could not be confirmed by a repeat experiment. The Committee did not regard these results as having any biological significance.

(vi) These data provided adequate reassurance with regard to the lack of any mutagenic potential resulting from the use of chlorine to treat flour used to bake cakes and no further work was recommended in this area.

Sucralose

2.5 In 1989 the company manufacturing this proposed new sweetener had been asked for further data on the *in vivo* mutagenicity of its hydrolysis product. The company was now in a position to undertake further work and was proposing to conduct an *in vivo* unscheduled DNA synthesis (UDS) assay in the liver. Members' advice was requested on whether this proposal would address the outstanding concerns. After considering the proposed assay protocol, it was concluded that the liver UDS approach would be acceptable. An *in vitro* UDS assay in hepatocytes should be conducted initially, if this produced negative results, no further work would be necessary. If positive results were obtained, an *in vivo* liver UDS assay would be needed.

Olestra

2.6 Olestra, a fat substitute, was being proposed as a replacement for conventional triglycerides in the preparation of potato crisps. Additional mutagenicity studies to investigate olestra that had been subject to exaggerated heating conditions had been requested in **1990** and these additional data had now been provided.

2.7 The following conclusions were agreed:

(i) The mutagenic potential of a sample of olestra that had been repeatedly heated in the presence of potato chips (crisps) had been investigated in a battery of *in vitro* assays, namely the Ames test for gene mutation in bacteria, the mouse lymphoma **L5178Y** $TK^{+/-}$ assay, cytogenetics in Chinese hamster ovary (CHO) cells and UDS in primary rat hepatocytes. Negative results were consistently obtained. Negative results were also obtained using a sample of conventional triglyceride treated in a similar way.

(ii) Olestra itself and a sample repeatedly used to cook potato chips (crisps) showed no mutagenic potential in these limited conditions of use.

(iii) No further mutagenicity data were required at present. The need for an *in vivo* bone marrow assay would be reconsidered in the light of further data which had been requested by COT.

Captan

2.8 The COM's advice had been sought by the Scientific Sub-committee (now the Sub-committee on Pesticides) of the Advisory Committee on Pesticides (ACP), on the possible genotoxicity of captan. Captan is a fungicide, which has been shown to induce duodenal tumours in mice (see also COC consideration). The matter had been originally considered in **1989,** when the COM had concluded that:

> Captan had the potential to be a direct acting mutagen in *in vitro* systems, but conflicting results were obtained *in vivo.* Because captan was readily hydrolysed *in vivo* and rapidly removed from *in vivo* systems by reaction with thiol compounds, it was unlikely that any effects would be,seen *in vivo* below a high threshold dose. However because of the potential for widespread exposure to captan and the doubts about the complete absence of activity *in vivo* the Committee recommended further studies should be carried out to investigate covalent binding to DNA *in vivo.*

2.9 The results of these further studies became available in **1993.** The studies had been more complex than had been anticipated and the results were not readily interpretable, although they gave some added reassurance that a genotoxic mechanism was not involved. The Committee came to the following conclusions:

> Captan was a carcinogen, causing duodenal tumours in mice by an unknown mechanism. There was some evidence that the mechanism was non-genotoxic. The Committee concluded that the mechanism appeared

to operate only at high dose levels and that there was a threshold for the effect. No effects would be expected to occur at low dose levels. No further work on the mutagenic potential of captan was recommended.

Thresholds for Aneuploidy Inducing Chemicals

2.10 The Committee concluded that it was reasonable to assume that aneuploidy inducing chemicals (particularly those which function by damaging the cell division spindle) have a threshold of action but noted that it was not generally possible to clearly demonstrate this, or to identify threshold levels from the currently available experimental data. Following 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 act by inducing damage in the cell division spindle apparatus.

2.11 In view of the considerable problems involved in compound distribution, it was unlikely that a mechanistic threshold of action could be unambiguously demonstrated with the currently available *in vivo* methodologies, which were generally limited to the bone marrow. The Committee was of the view that 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.

2.12 From current knowledge the most appropriate chemicals to study the induction of aneuploidy and to evaluate potential thresholds would be a series of spindle damaging chemicals (preferably 3 for a validation study) which interact directly, rather than after metabolic activation, with the spindle apparatus, leading to induced aneuploidy by diverse mechanisms. A suitable (but not exclusive) set of such chemicals would be:

(i) Colchicine or the related compound colcemid, which prevents tubulin assembly into filaments but does not inhibit depolymerisation.

(ii) Vinblastine, which inhibits the assembly of microtubules and disassembles preformed microtubules leading to the precipitation of tubulin and the formation of tubulin crystals in the cell.

(iii) Chloral hydrate, which inhibits pole to pole microtubule formation and thus inhibits elongation of the spindle.

2.13 Experiments designed to demonstrate a threshold of action of a spindle damaging agent would need to be undertaken with sufficient statistical power and appropriate statistical analysis used to clearly demonstrate a concentration range over which no increases in aneuploidy induction occur, followed by a concentration range over which statistically significant and dose-related increases occur.

2.14 In view of the numbers of cells which would require analysis to achieve an adequate statistical power the experiments would probably require the analysis of chromosome numbers in both metaphase and interphase cells. In the first instance it would be necessary to demonstrate that analyses at metaphase and interphase provide comparable data.

2.15 The Committee considered that chromosome painting of selected chromosomes by fluorescence *in situ* hybridisation (FISH) was the most appropriate analytical technique of the currently available methods. For metaphase analysis, whole chromosome probes for selected chromosomes would be suitable and are commercially available. For interphase analysis, centromeric probes should be utilised. In both interphase and metaphase cells both induced aneuploidy and polyploidy should be evaluated.

2.16 Chromosomes selected for analysis by FISH might be chosen on a number of criteria which include:

(i) probe availability

(ii) chromosome size

(iii) frequency of aneuploidy in newborn and abortuses

(iv) presence of genes of relevance in human cancers.

2.17 A suitable range of chromosomes for FISH analysis selected on the basis of the above would be:

(i) chromosomes 1 and 11: larger metacentrics, 11 associated with Wilms' tumour and aniridia.

(ii) X chromosome: frequently associated with live aneuploidies.

(iii) chromosomes 17 and 18: 17 is associated with the p53 gene and breast cancer predisposition, 18 is associated with live aneuploidies.

(iv) chromosome 8: trisomy is associated with a number of tumour types.

2.18 The availability of data from experiments following the general principles outlined above would provide the Committee with the information necessary to make a definite judgement as to whether spindle inhibiting chemicals have thresholds of action. New candidate chemicals for which a threshold of action is postulated could then be evaluated with adequate rigour. The general procedures outlined should be modified when appropriate to take account of chemicals which may require metabolic activity to produce metabolites capable of damaging the cell division spindle. Also the possibility of sequestering should be considered and controlled for by varying the serum concentration during experiments. These studies would allow an estimate to be made of the maximum concentration *in vitro* at which no aneugenic effects would be expected to occur, which could be used with toxicokinetic data for the individual compound to assess the risk that aneuploidy would be induced at human exposure levels.

2.19 The procedures outlined above would provide data allowing the evaluation of chemicals inducing aneuploidy by damaging the cell division spindle. However, there are a variety of other cellular targets, damage to which

may theoretically lead to the induction of aneuploidy. In particular agents which produce specific interaction with meiotic cells by, for example, modifying chromosome pairing and/or recombination could not be evaluated by the above methods. In such cases data would be required from germ cells. However to date there is no convincing evidence for compounds that act specifically on meiotic cells.

Diethylstilboestrol

2.20 During discussions on aneuploidy, the Committee reviewed the available data on diethylstilboestrol (DES), a recognised aneugen and human carcinogen. The review was concerned specifically with the aneugenic and other genetic effects of DES and not the carcinogenic effects.

(i) DES showed a specific range of genetic effects ***in vitro*** depending on concentration, with aneugenic effects predominating at low doses and gene mutation and clastogenicity only at relatively high doses.

(ii) Gene mutation had been reported in mammalian cells, but negative results had been consistently obtained in bacteria. There was some evidence that DES was clastogenic ***in vitro*** at relatively high concentrations. Inconsistent results were obtained in studies for sister chromatic exchange (SCE) induction ***in vitro*** and ***in vivo***. Negative results were usually obtained in UDS assays ***in vitro*** and in covalent binding studies, and there was no convincing evidence for DNA adduct formation. However, the compound did induce DNA strand breaks at high concentrations ***in vitro***.

(iii) DES consistently induced cell transformation in mammalian cells in the absence of gene mutation or structural chromosome aberration, but transformation was closely paralleled by aneugenic effects. Transformation was seen over all concentrations investigated in the range **0.001–1** μg/ml and was believed to be related to the aneugenic effects of DES. A threshold had not been identified.

(iv) The ability of DES to induce aneuploidy ***in vitro*** has been extensively studied in human fibroblasts and Syrian hamster embryo (SHE) cells. Positive results were obtained at 5 μg/ml and above, with a threshold in one study of 3 μg/ml. The mechanism of the aneugenic effects of DES was believed to be related to the inhibition of microtubulin assembly, although meiotic cells might also be sensitive to hormonal status, leading to aneuploidy through a different mechanism.

(v) It was not possible from the data available to draw any definite conclusions regarding thresholds for the aneugenic effects of DES. In one study no aneugenic effects were seen at **1** and 3 μg/ml in SHE cells, but transformation has been induced in such cells at concentrations as low as **0.001** μg/ml with no threshold being identified.

Test Methods

2.21 The Committee continued to provide advice on test methods, particularly in the context of the test methods recommended by the Organisation for Economic Cooperation and Development (OECD), which are currently being updated. Consideration was given to proposals from an OECD consultation meeting which was held in London in December 1992 and from an international Workshop on standardisation of genotoxicity test procedures held in Melbourne in February 1993. Advice was also provided for input into the discussions by the Genotoxicity Working Party of the International Conference on Harmonisation of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH).

2.22 The Committee affirmed its view that the results of ***in vitro*** studies should be verified in an independent confirmatory (but not necessarily identical) experiment, this second test providing an opportunity to optimise the conditions for detecting genotoxicity. The need for replicate cultures in mammalian cell assays in order to provide scientific reliability and enable appropriate statistical analyses was also noted.

Joint COC/COM Symposium on p53 Tumour Suppressor Gene

2.23 A joint scientific meeting was held on 9 June 1993. The **p53** gene attracts considerable current interest since mutations of this gene are the most common cancer-related genetic change known to occur in human tumours at the gene level. Point mutations, allelic loss, rearrangements and deletions of the **p53** gene play a role in many human malignancies. Studies of **p53** mutational spectra in various tumours may provide information about the causes, including individual chemical carcinogens, of the mutations which gave rise to human cancers. The position of the mutations in the **p53** gene indicate regions of the **p53** protein which are essential for its tumour suppressor activity and study of the gene is increasing understanding of malignancy at the cellular level.

2.24 The following presentations were given:

General overview of the role of the **p53** gene - Professor D Lane, Cancer Research Campaign Laboratories, University of Dundee

Mutation spectra within the **p53** gene - Dr M Stratton, Institute of Cancer Research, Sutton

Methodology for examining **p53** mutations - Mr W Warren, Institute of Cancer Research, Sutton

Mutations of the **p53** gene and skin cancer - Dr A Balmain, **Beatson** Institute, Glasgow

Mutations of the **p53** gene and breast cancer - Dr J Prosser, Medical Research Council Human Genetics Unit, Edinburgh

2.25 A discussion followed and some general conclusions were drawn. The p53 gene is of central importance in the development of human cancer. Genetic instability, consequent upon p53 mutation and loss of gene function, appears to be a driving force in the evolution of malignant tumours. Such evolution is clonal and depends on cells with a long lifespan, which (in turn) may be related to a loss of p53 activity, as the normal p53 gene is involved in apoptosis. It may eventually be possible to use p53 mutational spectra as "fingerprints" for identifying carcinogens. Mutational effects may, however, vary in different tissues, for reasons which are not yet understood and mutational spectra may be difficult to interpret when exposure involves mixtures or hitherto unrecognised carcinogens.

Topics Still Under Consideration

2.26 The following topics, which were discussed by the COM at meetings held in 1993, are under review:

Dithiocarbamates in latex products

Polycyclic aromatic hydrocarbons

1993 Membership of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

Professor B A Bridges BSc FIBiol (Until March 1993)
Professor J M Parry BSc PhD DSc (From April 1993)

MEMBERS

Professor J Ashby BSc PhD CChem FRCS
R L Carter MA DM DSc FRCPath
J Cole BSc DPhil
C Cooper BSc PhD DSc
Professor D S Davies BSc PhD CChem FRCS MRCPath
Professor H J Evans PhD FRSE CBiol FIBiol FRCPE FRCCSE
M Fox BSc PhD
Professor R F Newbold BSc PhD
S Venitt BSc PhD
R M Winter BSc MB BS FRCP

SECRETARIAT

T Marrs MD MSc FRCPath FIBiol DipRCPath (Medical)
R J Fielder BSc PhD DipRCPath (Scientific)
K N Mistry (Administrative)

Mrs A McDonald BSc MSc

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

DECLARATION OF INTEREST DURING THE PERIOD OF THIS REPORT 1993

	Personal Interest		Non Personal Interest	
Member	Company	Interest	Company	Interest
Prof B A Bridges (Chairman) to **31.3.93**	Rank Xerox	Consultant	Unilever Boots	Student Support Student Support
Prof J M Parry (Chairman) from **31.3.93**	Glaxo Research Fisons Pharmaceuticals SmithKline Beecham BAT British Petroleum Compass Catering British Telecom South Wales Electricity JIB Insurance	Consultancy & Grant Consultant Consultant Consultant Share Holder Share Holder Share Holder Share Holder Share Holder	Amersham International Pfizer SmithKline Beecham	Grant Studentship Studentship
Prof J Ashby	ZENECA	Employee	NONE	NONE
Dr R L Carter	ICI plc	Share Holder	NONE	NONE
Dr J Cole	NONE	NONE	NONE	NONE
Dr C Cooper	NONE	NONE	NONE	NONE
Prof D S Davies	ICI plc ML Laboratories plc Clinical & Biochemical Pharmacology Consultancy Ltd	Share Holder Non Executive Director Director	Astra Boots Glaxo Hoechst ML Laboratories Plc Pfizer Roche Products Wellcome Foundation Daiichi Pharmaceuticals SmithKline Beecham Lilly Fisons Kali Chemi Pharmacia Stirling Zeneca	Fellowship Research Research & Studentship Research Fellowship, Studentship & Research Research Research Research Fellowship Travel grant Research Research Research Research Research Research
Prof H J Evans	Merck, Sharpe & Dohme (Astra Sweden) ICI plc Fisons Croda British Petroleum British Telecom Southern Electric Scottish Power Y Water W Water	Consultant Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	Boehronger Mannheim Pharmacia ICI plc Wellcome	Capital Equipment Fellowships Studentships Running Expenses

	Personal Interest		Non Personal Interest	
Member	**Company**	**Interest**	**Company**	**Interest**
Dr M Fox	Sun Alliance Barclays Bank Burmah Oils Hanson plc Midland Bank Royal Insurance British Oxygen Co Allied Lyons BP Greenhall Whitley General Accident Bass plc BAT Joseph Holt Nat West Burton Wood Wiggins Teape	Share Holder of these Companies	NONE	NONE
Prof R F Newbold	NONE	NONE	NONE	NONE
Dr S Venitt	Abbey National plc	Share Holder	NONE	NONE
Dr R M Winter	NONE	NONE	NONE	NONE

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

Dr R L Carter
(Chairman)
MA DM DSc FRCPath

Preface

The COC evaluates chemicals for human carcinogenic potential at the request of the Department of Health and other Government departments. This evaluation draws on many sources of information, including epidemiology, structural chemistry, metabolic studies and short term mutagenicity tests, as well as long term animal testing. Several of the assessments undertaken during 1993 have been based primarily on evidence derived from epidemiological studies of human populations, including the possible carcinogenic effects of passive smoking, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (commonly known as dioxin) and occupational exposure to certain pesticides. Consideration of the observed effects in humans is extremely useful when evaluating putative human carcinogens, since the problems of extrapolating from experimental animals are avoided and some estimate of the size of the risk can be made. There are, however, important limitations and sources of potential error in such epidemiological studies, and the wide expertise of the whole Committee has been needed in considering possible mechanisms of causality and overall biological plausibility of the suggested associations.

A useful joint scientific meeting was held with the COM on mutations of the p53 gene, the most frequent genetic abnormality known to occur in human cancers at the gene level. Knowledge of such molecular aspects of carcinogenesis continues to increase and this information will undoubtedly become increasingly relevant to the COC's assessments of human carcinogenic risk.

RICHARD CARTER

Fumonisins

3.1 Fumonisins (fumonisin B_1 and fumonisin B_2) are mycotoxins produced by ***Fusarium*** moulds, which contaminate cereals, particularly maize, and also nuts. The level of contamination of the UK diet with these mycotoxins is largely unknown, although it appears to be low. The COC view on the carcinogenic hazard presented by fumonisins was sought in order to inform decisions on surveillance programmes for mycotoxins.

3.2 The Committee concluded that the available data did not provide adequate evidence to determine the carcinogenicity of individual fumonisins, but ***Fusarium*** contamination of food was associated with carcinogenic effects in laboratory animals.

Perchloroethylene

3.3 The COT were reviewing the toxicity of perchloroethylene and sought the COC's opinion on whether perchloroethylene should be regarded as a carcinogenic risk for humans.

3.4 The Committee agreed on the following conclusions:

(i) Perchloroethylene was carcinogenic in rodents, inducing an increased incidence of hepatocellular adenomas and carcinomas in mice and renal adenomas and carcinomas in rats. There was adequate evidence of the mechanism of tumour induction in the mouse liver to indicate that this effect was species-specific and not relevant to humans. Regarding the renal neoplasms in the rat there were marked differences between rats and humans in the enzymes believed to be involved in the induction of such tumours in the rat kidney; even allowing for possible human variability in the enzyme glutathione transferase, the Committee concluded that the carcinogenic effects seen in rat kidney were unlikely to occur in humans.

(ii) In an experiment using **F344** rats an increase in mononuclear cell leukaemia was also reported. The Committee considered it unlikely that this effect was treatment related and concluded that, in general, this form of rodent leukaemia had had no predictive implications for humans.

(iii) The Committee considered a number of epidemiological studies based on occupational groups; none dealt exclusively with workers exposed to perchloroethylene, and the exposures were to a mixture of solvents and were poorly defined. The available data were inadequate to allow any definite conclusion to be reached on the relationship between occupational exposure to perchlorethylene and cancer in humans.

(iv) The Committee endorsed the COT's conclusion that data on residues of perchloroethylene in butter and lard in the MAFF survey did not give rise to concern with regard to human health effects, although adventitious contamination of this type was always undesirable.

2,3,7,8-Tetrachlorodibenzo-p-Dioxin [TCDD] - Commonly Known as Dioxin

3.5 Various aspects of the toxicology of TCDD have been considered on several occasions by the COT, COM and COC. In **1988** the COC advised that TCDD had been shown to be carcinogenic in rodents at unusually low oral doses. It was not, however, genotoxic and limited evidence suggested that the compound acted as a tumour promoter and that a threshold might exist for its carcinogenic effects.

3.6 In **1993** the Department requested further advice from the COC on the status of TCDD as a possible human carcinogen, based on the Committee's opinion of **3** recent epidemiological studies. No additional animal or mechanistic data were considered[4].

3.7 The Committee came to the following conclusions:

(i) TCDD and other chlorinated, dioxins are widely distributed in the environment and the development of new analytical techniques will make them detectable at ever lower concentrations. The Committee were asked to consider epidemiological investigations of occupational exposure to relatively high levels of TCDD.

(ii) The design and conduct of three new epidemiological investigations were broadly satisfactory, although there were some methodological limitations. Cohort size was relatively small, except in the Fingerhut et al study. All three studies suffered from the common problems of mixed exposures to other chemicals, such as benzene which could have been widely used in the plants studied, and the fact that exposure to dioxin was due to their presence as low level contaminants of other chemicals (such as phenoxy herbicides) to which the cohort had much greater exposure. The Committee had noted the increases in total cancers and cancers developing in certain anatomical sites shown in these reports, but drew attention to inconsistencies between them as to the magnitude of the increase and the anatomical sites where the increased incidence of tumours was detected. Chemical carcinogens usually demonstrate organ specificity, but occupational exposure to TCDD could not be consistently linked to cancer at any specific anatomical site. Some consistency was demonstrated for an increase in bronchial cancers, but this finding might have been due to the confounding effect of smoking, which was partially controlled for in only one of the studies. In some earlier studies it had been reported that the incidence of soft tissue sarcomas was increased in exposed populations, but this observation was not substantiated by two of the three new studies. Despite these difficulties in interpretation, the Committee concluded that the three studies showed a consistent increase in total cancers in individuals who had been occupationally exposed to very high levels of TCDD compared to background environmental levels.

[4] Studies considered: Fingerhut MA, Halperin WE, Marlow DA et al (1991) Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. N Engl J Med 324: 212–218; Manz A, Berger J, Dwyer JH et al (1991) Cancer mortality among workers in chemical plant contaminated with dioxin. Lancet 338: 959–964; Zober A, Messerer P, Huber P (1990) Thirty-four-year mortality follow-up of BASF employees exposed to 2,3,7,8-TCDD after the 1953 accident. Int Arch Occup Environ Health 62: 139-157.

(iii) These new investigations thus strengthen the possibility of an epidemiological link between occupational exposure to TCDD and an increase in total cancers in humans, although there was no consistent association with cancer at any specific anatomical site(s). There was insufficient evidence for a clear causal link, but it would be prudent at present to regard TCDD as a possible human carcinogen.

Bromate

3.8 Bromate is a major by-product of ozone disinfection of drinking water, formed when waters containing bromide are ozonated. Bromate is found in UK drinking water at levels generally below 100ug/litre. Discussions were taking place within the UK and the European Union about the necessity for a statutory limit for bromate in drinking water. The Committee was asked to comment on the carcinogenicity and genotoxicity of bromate, which was both an *in vivo* mutagen and a carcinogen, inducing renal adenomas and carcinomas and peritoneal mesotheliomas in rats.

3.9 The Committee confirmed that bromate was genotoxic and carcinogenic, and that there was no evidence of any threshold for these effects. It was recommended that bromate levels in drinking water should be kept as low as possible.

Enrofloxacin

3.10 The Committee had considered enrofloxacin, a quinolone antibiotic with potential veterinary use, in 1992 [see Annual Report 1992]. At that time the Committee were unable to reach any decision on the carcinogenicity of the compound in rats because of inadequately documented histopathology.

3.11 In 1993 the Committee was provided with an independent review of the pathology slides from the original study, which indicated that the effects originally reported were not associated with treatment. Two further investigations had also been carried out in the same strain of rat. No neoplastic changes were detected in either of these studies. The Committee concluded that enrofloxacin was not carcinogenic in the rat and that there was no further concern over its carcinogenicity in laboratory rodents.

Captan

3.12 Captan is a fungicide, which was evaluated by the COC at the request of the Scientific Sub-committee (now the Sub-committee on Pesticides) of the Advisory Committee on Pesticides (ACP). Several oral carcinogenicity studies had been carried out in rats and mice. It was generally accepted that captan was not carcinogenic in rats, but four studies in mice had shown that diets containing

captan induced adenomas and carcinomas of the duodenum. The tumours were treatment, and to some extent dose, related. Various mechanisms of tumour induction had been proposed. The possible genotoxicity of captan had been examined by the COM (see above), who concluded that there was some evidence that the mechanism was nongenotoxic and operated only at high dose levels. No effects would be expected to occur at low dose levels.

3.13 It had been suggested that the duodenal tumours might be caused by non-genotoxic mechanisms involving a progression from captan-induced mucosal hyperplasia to adenomas to carcinomas. The presence of preexisting mucosal hyperplasia was questionable, but there were difficulties in identifying these changes by standard morphological methods. There was no evidence of sustained chronic local inflammation.

3.14 All the evidence from carcinogenicity tests was consistent with the hypothesis that captan induced tumours in a single tissue of a single species, but the COC was not reassured that there was a safe level of human exposure to captan. The mechanism was unclear, although it appeared to be an effect which would be expected to have a threshold. The ACP might wish to ask for further studies to allow the setting of a scientifically based threshold for carcinogenicity. The COC recommended the following possible approaches:

(i) Study of bioavailability of captan in the duodenal lumen at low doses (including the effect of pH).

(ii) Investigation of captan-induced mucosal proliferation in the small intestine using sensitive techniques.

Non-Hodgkin's Lymphoma and Occupational Exposure to Certain Pesticides

3.15 The incidence of non-Hodgkin's lymphoma (NHL) in the US and Europe has increased by approximately 2% per annum over the past 20 years. One suggested cause for this increase was exposure to certain pesticides. The Committee was asked to consider the epidemiological studies which examined this proposed link and advise the Department whether there was cause for concern.

3.16 Members concluded that the evidence available suggested a possible link between NHL and farming in general rather than specific exposure to pesticides. The Committee's overall conclusions were as follows:

(i) The Committee considered a large number of epidemiological investigations of non-Hodgkin's lymphoma carried out in North America, Europe and New Zealand, with reference to a link with herbicide or insecticide use. These studies showed inconsistent evidence of a link between non-Hodgkin's lymphoma and occupations in farming. There was insufficient information to suggest the cause(s) of this link. The Committee noted that manufacturers and formulators of herbicides, who had been exposed to higher levels than farm workers, did not show an

increased incidence of non-Hodgkin's lymphoma, suggesting that any link between farming and non-Hodgkin's lymphoma was not due to herbicides.

(ii) The Committee did not recommend that further epidemiological investigations should be carried out in the UK, unless populations with instances of validated tissue diagnoses of non-Hodgkin's lymphoma and reliable data on exposures could be identified. Further well-designed international epidemiological studies could help to clarify the issue.

Health Effects of Passive Smoking - Lung Cancer

3.17 Several studies have been published since the early 1980s demonstrating a small increase in the incidence of lung cancer among non-smoking wives of smokers. The US Environmental Protection Agency issued a report on the respiratory health effects of passive smoking in December 1992, which classified environmental tobacco smoke (ETS) as a human carcinogen. The Department of Health accordingly sought the Committee's views on the association of ETS with lung cancer.

3.18 The Committee came to the following conclusions:

(i) The consensus from the published literature was that there was a small, statistically significant risk of lung cancer among non-smokers exposed over a substantial part of their lifetime to environmental tobacco smoke. The Committee agreed with the conclusion of the Independent Scientific Committee on Smoking and Health [Fourth Report, HMSO, 1988] that the relative risk among such people was in the range 1.1 to 1.3, accounting for several hundred lung cancer deaths per annum in the UK.

(ii) Additional epidemiological studies were not recommended, but there should be further research on levels of exposure to ETS, particularly in the workplace and public areas, to enable comparison with domestic levels on which the epidemiological findings were based. Research into more reliable biomarkers for exposure to ETS was recommended.

(iii) Continuing efforts should be made to ensure that in indoor environments frequented by the public, including public transport, the workplace and leisure environments, exposure to environmental tobacco smoke was kept to a minimum.

Presentations

3.19 Two presentations were made to the COC during the course of 1993:

Presentation on the work of the Small Area Health Statistics Unit (SAHSU)

Dr Paul Elliott, director of SAHSU, described the work of the unit which was set up after the Black Report in 1984 on the investigation of the possible increased incidence of cancer in West Cumbria. That report

recommended that there should be an organisation "to coordinate centrally the monitoring of small area statistics around major installations producing discharges that might present a carcinogenic or mutagenic hazard to the public. In this way early warning of any untoward health effect could be obtained".

SAI-ISU was established in 1987 at the London School of Hygiene and Tropical Medicine. Descriptions of its work can be found in various publications: Elliott P, Westlake AJ, Hills M et al (1992) The Small Area Health Statistics Unit. J Epidemiol Community Health 46: 345-349; Smales E, Fielder R, Wadge A (1992) The Small Area Health Statistics Unit. Health Trends 24: 80-81.

New implications of results from conventional rodent carcinogenicity bioassays - a presentation by Professor John Ashby

Professor Ashby gave a talk on the outcomes of an exercise in predicting the results of carcinogenicity bioassays which were currently being conducted. These predictions were based on information concerning *in vitro* mutagenicity, toxicity, dose route and level, and chemical structure.

After examining the available information on compounds which had been previously tested for carcinogenicity in two rodent species in the USA's National Toxicology Program and incorporating information on structural alerts and genotoxicity testing, Professor Ashby was able to place carcinogens in two groups: the multi-site, trans-species genotoxic carcinogens and the non-genotoxic carcinogens where expression depended on species, tissue and dose route. This background information was used in the predictive exercise. Predictions based on expert consideration of all the available data were better than those based on specific aspects of the compound, such as chemical structure.

Topics Still Under Consideration

3.20 The following topic, which was discussed by the COC at meetings held in 1993, is under review:

Dithiocarbamates in latex products.

1993 Membership of the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

R L Carter MA DM DSc FRCPath

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

DECLARATION OF INTEREST DURING THE PERIOD OF THIS REPORT 1993

	Personal Interest		Non Personal Interest	
Member	Company	Interest	Company	Interest
Dr R L Carter (Chairman)	ICI plc	Share Holder	NONE	NONE
Prof P G Blain	NONE	NONE	ICI plc Unilever plc	Research Studentship Research Studentship
Prof R A Cartwright	Central Electricity Generating Board National Grid Co Medico-Legal Consultancies	Consultant Consultant Consultant	NONE	NONE
Prof C Chilvers	Zeneca ICI plc Tomkins British Gas	Share Holder Share Holder Share Holder Share Holder	Merck, Sharp & Dohme	Research Support
Dr C Cooper	NONE	NONE	NONE	NONE
Prof A D Dayan	Wellcome Foundation Lilly Risk & Policy Analysts Schering Plough SmithKline Beecham British Petroleum British Gas British Steel Hanson National Power Powergen TI	Pension Consultant Consultant Consultant Consultant Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	Glaxo	Research Fellowship
Dr P B Fanner	NONE	NONE	Ciba-Geigy DOW BAT Zeneca	Research Collaboration Research Studentship Research Studentship Collaborative – Studentship
Prof R F Newbold	NONE	NONE	NONE	NONE
Prof J M Parry	Glaxo Group Research Fisons Pharmaceuticals SmithKline Beecham BAT British Petroleum Compass Catering British Telecom South Wales Electricity JIB Insurance	Consultancy & Grant Consultant Consultant Consultant Share Holder Share Holder Share Holder Share Holder Share Holder	Amersham International Pfizer SmithKline Beecham	Grant Studentship Studentship
Dr 1 F H Purchase	ZENECA	Employee	NONE	NONE
Dr A G Renwick	International Sweeteners Asociation SmithKline Beecham	Consultant Consultant	Bayer Ciba-Geigy Glaxo Hoffmann-La Roche Solvay-Duphar	Research Support Research Support Research Support Research Support Research Support
Dr S Venitt	Abbey National plc	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
Department of Energy
Health and Safety Executive
Medicines Control Agency: Section 4 Committees and the Licensing Authority
Committee on the Medical Aspects of Food Policy
Home Office
Scottish Home and Health Department
Department of Agriculture and Fisheries for Scotland
Welsh Office
Department of Health and Social Services for Northern Ireland
Other Government Departments

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

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

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

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

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

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

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

Glossary of Terms

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

ADENOMA See 'tumour'

ADI Acceptable daily intake, defined as 'An estimate of the amount of a food additive, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk'.

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

ALLELES Alternative forms of a gene found at the same locus on homologous chromosomes.

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

ANEUGENIC Inducing aneuploidy (qv).

ANEUPLOZDY 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.

ANIRIDIA Congenital absence of the iris of the eye.

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

BIOAVAILABILITY A term referring to the proportion of a substance which reaches the systemic circulation unchanged after a particular route of administration.

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

BRONCHIAL Relating to the air passages conducting air from the trachea (windpipe) to the lungs.

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

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

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

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

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.

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

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

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.

CLONAL Developing from the division of a single cell, the member cells of the clone having the same genetic constitution.

COHORT A defined population.

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.

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

DEPOLYMERISATION The breakdown of a polymer (compound formed from the combination of two or more identical molecules) to the original molecules.

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

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

DUODENAL Relating to the first of the three parts of the small intestine.

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

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

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

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

GERM CELL Reproductive cell eg spermatid.

GRANULOMA A tumour or new growth made up of granulation tissue and caused by various forms of chronic inflammation.

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

HEPATOTOXIC Causing damage to the liver.

HISTIOCYTOSIS Focal collections of macrophages (histiocytes).

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

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

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

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

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

LEUKAEMIA A group of neoplastic disorders affecting blood-forming elements in the bone marrow, characterised by uncontrolled proliferation and disordered differentiation or maturation. Examples include the lymphocytic leukaemias which develop from lymphoid cells and the myeloid leukaemias which are derived from myeloid cells.

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

LYMPHOCYTE Type of white blood cell.

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

LYMPHOMA Malignant tumours arising from lymphoid tissues. 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'

MEIOTIC Relating to meiosis, the type of cell division occurring in germ cells, by which gametes containing the haploid chromosome number (single set of unpaired chromosomes) are produced from diploid cells (containing pairs of equivalent chromosomes).

METABOLIC ACTIVATION Conversion by enzymes of a chemical from one state to another, for example by 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.

METACENTRIC Describing a chromosome with a centrally placed centromere (qv).

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.

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.

MONOCYTE A type of white blood cell.

MONONUCLEAR CELL LEUKAEMIA A type of leukaemia (qv) occurring spontaneously in ageing F344 rats.

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.

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'.

NDUTROPHIL A type of leucocyte or white blood cell.

NON-GENOTOXIC See 'carcinogens'.

NON-HODGKIN'S LYMPHOMA (NHL) A group of malignant lymphomas (qv), which do not have the histopathological features of Hodgkin's disease.

PARENTERAL The word applied to the administration of substances to an animal or to man by any route other than by the 'mouth or by the bowel.

PERITONEAL MESOTHELIOMA A rare tumour, usually malignant (see "tumour") which develops from the thin, flattened (mesothial) cells which line the abdominal cavity.

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.

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.

POLYSACCHARIDE A type of carbohydrate.

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

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

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

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

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

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 defects induced during development between conception and birth and their causes.

THIOL COMPOUNDS Organic compounds containing sulphur as an-SH group.

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

TRANSFORMATION The process by which a normal cell acquires the capacity for neoplastic growth. Complete transformation occurs in several stages both *in* vitro and *in* vivo. One step which has been identified *in* vitro is 'immortalisation'

by which 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.

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

TRIGLYCERIDES Esters formed from one molecule of glycerol and three fatty acid molecules, found in fat.

TUBULIN The principle protein component of microtubules (qv).

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 recognisable features of their tissue of origin but these characteristics are progressively lost in moderately and poorly differentiated malignancies: undifferentiated or anaplastic tumours are composed of cells which resemble no known normal tissue. Most malignant tumours grow rapidly, spread progressively through adjacent tissues and metastasise to distant sites. Tumours are conventionally classified according to the anatomical site of the primary tumour and its microscopical appearance, rather than by cause. Some common examples of nomenclature are as follows:

> Tumours arising from epithelia (qv): benign - adenomas, papillomas; malignant - adenocarcinomas, papillary carcinomas.
>
> Tumours arising from connective tissues such as fat, cartilage or bone: benign - lipomas, chondromas, osteomas; malignant - fibrosarcomas, liposarcomas, chondrosarcomas, osteosarcomas. Tumours arising from lymphoid tissues are malignant and are called lymphomas (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 'nongenotoxic carcinogen' and 'promoter' are synonymous.

TUMOUR SUPPRESSOR GENE (Synonym – anti-oncogene, recessive oncogene) A gene whose continued expression is thought to be essential for normal growth and differentiation of cells. Many tumour suppressor genes probably exist, deletion or suppression of which appears to be a critical event in tumour development.

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.

WILMS' TUMOUR (nephroblastoma) A malignant tumour of the kidney found only in children.

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