Department of Health

1999 Annual Report of the Committees on Toxicity Mutagenicity Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

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Contents

About the Committees

This is the ninth 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 Committee's administrative secretary.

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

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

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

The report contains, at Annex 4, an alphabetical index to subjects and substances considered in previous reports. A second index, at Annex 5, contains details of the subjects on which the COT has given advice since 1988 as part of its consideration of the results of surveillance for chemicals in the UK diet. These considerations were published formerly in the Food Surveillance Papers which report this surveillance work, rather than in the Committee's annual reports.

The usual way in which committee reviews are conducted is that the relevant Secretariat critically assesses all the relevant data and prepares papers for the committee. These normally consist of appendices giving detailed summaries of the studies reviewed – methodology and results – and a covering paper in which the available data are briefly summarised, the most important points highlighted and recommendations presented for discussion by the Committee. Although original study reports are not routinely circulated to members, they are made available on request and are circulated if the study is particularly complex. Definitive summaries are necessary because documentation on any one chemical can amount to many hundreds of pages. The Committees cannot undertake to review information provided by individuals, industry or other organisations that has not been forwarded through, or discussed with, the appropriate Secretariat.

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

During 1999 the CMO announced changes to the procedures of the three Committees. These changes will result in greater openness of committee business and include the publication of agendas, finalised minutes, agreed conclusions and statements. It was also announced that some meetings could be open to members of interest groups, consumer organisations and professional societies to attend. The first of these meetings was held during February in Bath. More information on the changes can be found in a paper at Annex 7.

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

Preface

In keeping with the experience in previous years, the Committee has been fully engaged in the consideration of a wide range of toxicological problems. Whereas these are described in detail in the text, consideration of the topics points to a number of general matters which are important to the overall function of the Committee.

Together with its sister Committees, the COT has adopted new principles of working in order to increase openness, it is our intention to further increase the degree of openness, through a process of evolution. During the year we held an Open Meeting to discuss mathematical modelling in toxicology. This brought together a number of experts and provided a forum for discussion which will inform future methodology applied to toxicological assessment in man.

The section on the toxicology of breast implants illustrates how the Committee can act rapidly to provide an opinion. The rapidity of response is dependent upon the willingness of members of the Committee to act at short notice and also upon our being able to call upon a wide range of toxicological expertise from within the membership of the Committee.

During the year two Working Groups were meeting, one considering Food Intolerance and the other Organophosphates. The Report on Organophosphates was published in November 1999 and provided the basis for discussion of this topic by other Committees which advise Ministers in relation to policy so far as Organophosphates are concerned. It is the intention to publish the Food Intolerance report in the year 2000.

The report contains information on our consideration of the toxicology of a group of novel molecules (the Salatrims). This work underlines the importance of there being well-conducted human studies which inform the formulation of advice about the inclusion of novel foods within the human diet.

A further important general point was raised by our consideration of potatoes genetically modified to produce *Galanthus nivalis* lectins. During the assessment of genetically modified foods, considerable stress has rightly been laid upon the possible adverse effects of the genetic modification itself. The work on lectins described in the report underlines the fact that there exists the possibility of "downstream" effects of genetic modification upon the ability of an organism to produce toxic compounds. This Committee, together with others, are very much aware of the importance of this type of assessment of genetically modifies organisms.

As in previous years, we have been very well served by the Secretariat who have continued to ensure the smooth running of Committee proceedings and have provided working documents of the highest quality.

Professor H F Woods (Chairman)
BSc BM BCh DPhil FFPM FIFST HonFFOM FRCP (London & Edinburgh)

Alitame

1.1 Alitame is a novel intense sweetener derived from the amino acids L-aspartic acid and D-alanine. It is intended for use in a wide range of food products. A case of need for alitame was agreed by the Food Advisory Committee in 1988.

1.2 Alitame was initially considered by the Committee in 1989. A full submission of toxicological data was made, this included repeat dose studies of up to 2 years duration in rats and mice and 18 months duration in dogs, a 2-generation toxicity in rats, studies of foetotoxicity in rats and rabbits and *in vitro* genotoxicity studies. A tolerance study in human volunteers was also submitted. Alitame decomposes into a β-isomer both on storage and *in vivo* and additional toxicity data were provided on this compound.

1.3 Additional data were submitted to the COT during the period 1990-1994 and in 1998, both in response to requests made by the Committee and as a result of requests made to the company by other regulatory bodies. At the request of the company, consideration of alitame by the COT was suspended from 1994 until 1997 when consideration of the compound by the Joint FAO/WHO Expert Committee on Food Additives was completed.

1.4 After consideration of all the data submitted during the period 1989-1998 an Acceptable Daily Intake of 0.3 mg/kg bw per day was established. This was derived as follows: a significant elevation in liver weights was found in the dogs treated with 500 mg/kg bw per day alitame for 18 months. The Committee considered that there was a suggestion that liver weights were also elevated in male dogs receiving the next lowest dose (100 mg/kg bw per day). Whilst this elevation in liver weights was not statistically significant, the Committee considered that this finding was sufficiently unusual that it would be prudent to regard this as a LOAEL. Consequently, the NOAEL derived for alitame was 30 mg/kg bw per day, the lowest dose tested. Uncertainty factors of 10 for interspecies and 10 for intra-individual variability were then applied, resulting in an Acceptable Daily Intake of 0.3 mg/kg bw per day.

Breast Implants

1.5 At the request of the Department of Health and the Medical Devices Agency (MDA) an emergency consideration of data relating to the safety of breast implants containing soya bean oil was undertaken. This procedure is one that can be undertaken if necessary and with the agreement of the Chairman. It requires the identification of a limited number of members with particularly relevant expertise and the rapid circulation of the appropriate documentation to them. In such circumstances the outcome does not represent the views of the Committee but is the collated opinions of individual members. In this instance, the documentation was circulated to some members on Thursday, 4 March; their opinions were received by early afternoon on Saturday, 6 March; these were passed to the MDA. MDA and Department of Health advice on the implants, which referred to a voluntary withdrawal of the implants by the manufacturers, was made public on Monday, 8 March.

2-Chlorobenzylidene Malononitrile (CS) and CS Spray

1.6 At the request of the Department of Health, with the support of the Home Office, the advice of the Committee and its sister Committees was sought on CS spray in the context of its use as a chemical incapacitant because of potential public health concerns. The Committees produced the following joint statement:

(i) The advice of the Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COT, COM, and COC), on 2-chlorobenzylidene malononitrile (CS), specifically in the context of the use of CS spray as a chemical incapacitant, was sought by the Department of Health, with the support of the Home Office.

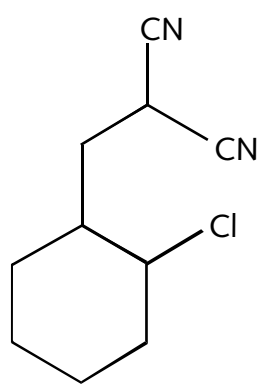
(ii) CS spray has now been used by some police forces in England and Wales since March 1, 1996 when it was introduced for a trial period which was followed by approval in September 1996. Although the use of CS as an aerosol or 'smoke' was reviewed following its use in Londonderry in 1969,[1,2] the spray itself has not been subjected to scrutiny by independent expert advisory committees. It was for this reason, and because of the potential public health concerns, that the Department of Health was of the opinion that such a referral was appropriate.

(iii) This statement incorporates the conclusions of each of the three Committees and is divided into sections concerned with a) the physical and chemical properties of the spray, b) the toxicological data on the compound CS, c) the toxicological data on the solvent, methyl isobutyl ketone (MIBK), and d) the toxicological data on CS spray. The COT considered toxicological data on CS itself and the solvent MIBK itself and then the very limited animal and human data on the combination, CS spray. Professor K.E. Donaldson of Napier University assisted the Committee in its deliberation and Dr V.S.G. Murray and her colleagues from the Chemical Incident Response Service (CIRS) and the National Poisons Information Service (NPIS), London Centre described cases of putative toxic effects of CS spray reported to their service.

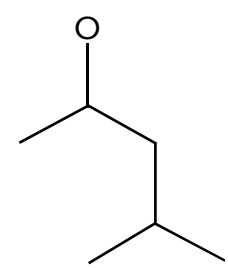
The nature of CS spray and its components

(iv) The CS spray used by police forces in the UK consists of a 5% (w/v) solution of CS in MIBK, comprising 1.5 grams (g) of CS dissolved in a total volume of 30 millilitres (ml), contained in a canister with nitrogen as a propellant. The chemical structures of CS and MIBK are given in the Figure below.

Figure



2-Chlorobenzylidene malononitrile (CS)



Methyl isobutyl ketone (MIBK)

Exposure to CS spray

(v) The COT noted that, although there are reports and studies which describe the effects of CS spray on humans, these do not provide data in respect of individuals who have been sprayed which allow any estimation of their exposure to the constituent chemicals. The guidelines for the use of CS spray that have been provided to police forces for the training of officers were available[3] but, in the absence of quantitative evidence relating to operational use, the Committee was unable to estimate exposure in the field.

(vi) However, to address this question the Police Scientific Development Branch (PSDB) of the Home Office carried out a study of 500 canisters that had been used operationally in the UK by police officers in the course of arrests of individuals. The Committee has been informed that it is standard

practice to replace a canister after it has been used once. By comparing the weights of the used canisters with the mean weight of unused canisters it was possible to estimate the quantities of CS spray that had been used during each incident. At the maximum flow rate permitted in the specifications, 10% of the canisters had been discharged for a total calculated period of more than 3 seconds and had weights corresponding to the release of between 12.0 and 23.7 g of the CS solution in MIBK. The peak of the exposure distribution corresponded to the release of 4 to 6 grams of the solution of CS in MIBK, of which 0.28 to 0.35 g is calculated to have been CS.[4]

(vii) The nature of the toxic effects of the spray will depend upon the extent to which exposure occurs to the eyes, to the skin and via inhalation or ingestion. The mass and size of the droplets of the CS solution in MIBK produced during spraying will determine how far the droplets can penetrate into the respiratory tract. In response to the COT's enquiries about the physical properties of the spray released from the canisters, the PSDB commissioned a study, undertaken by AEA Technology, to address the question of the size distribution of droplets produced under conditions simulating operational use.

(viii) The results of this study indicate that, when the CS spray is used at distances of 2 to 3 metres from a detector, the median diameter of the spray particles is between 417 to 441 micrometres (μm). There are, however, some particles with diameters of less than 100 μm and a few with diameters of less than 50 μm. When the spray was used at distances of less than 0.1 m, (a shorter distance than that recommended for operational use), the proportion of the smaller droplets decreased. When the spray was allowed to impinge on, and be scattered back from, a solid target the proportion of smaller diameter droplets increased. In a further study with 5 canisters of CS spray in which the diameter of the smaller particles was measured, none were found to have diameters of less than 28 μm.[5]

(ix) The Committee was of the view that, although the CS canisters release, for the most part, a coarse spray, there is a proportion of droplets with diameters of less than 100 μm which, in the event of full discharge of the can, could transport a maximum of 20 mg of the spray solution into the upper respiratory tract the smallest droplets of which (diameter 28 to 50 μm), could reach the large- and medium-sized airways of the lung. This proportion will be increased if the spray is scattered from any nearby surface. Since these are the airways that are affected in bronchial asthma, it is possible that an asthmatic attack could occur in susceptible individuals. It was also recognised that the increased rate and depth of respiration occurring in an individual under stress might, in addition, result in a greater dose of the CS spray being inhaled.

(x) In a separate study with CS canisters the vapour concentration of the solvent MIBK was measured at 6 positions placed either as close as could be achieved or up to 0.5 m from a target. The target was sprayed from a distance of 2.0 m for periods of 1 or 3 seconds or until the canister was empty. The resultant MIBK vapour concentration at each position was then measured at one second intervals for a period of 15 minutes. In these trials the Short Term Exposure Limit (STEL)* for MIBK of 100 ppm time-averaged over a 15 minute reference period[6] was exceeded on four out of eighteen occasions. However, static air conditions were used in these trials in order to achieve a greater reproducibility,[4] such conditions would reduce dispersion and increase average measured concentrations.

(xi) Because of the nature of this trial, and the differences in circumstances from operational use where static air conditions would be unlikely, the Committee felt that these results probably did not represent a cause for concern, provided that the spray is used in accordance with the operational guidelines.

* An occupational exposure limit defining a level of exposure over a 15 minute reference period which should never be exceeded.[6] Such values are typically set to protect workers against effects that occur rapidly after exposure eg irritation of the eyes, nose and throat.

Toxicity of CS

(xii) Most of the data on the toxicity of CS derive from studies which have used either CS aerosols or pyrotechnically-generated 'smokes'. In both cases respirable particles were produced. Data have been obtained on the size of droplets resulting from the use of CS dissolved in an organic solvent and delivered in the form of a spray; these are discussed in paragraphs (vii) to (ix) above.

Metabolism

(xiii) Studies of the metabolism of CS have been conducted on the compound itself and not in the form in which it would be used as an incapacitating agent by police officers. It is readily hydrolysed in aqueous mixtures[7,8], and reacts readily with plasma proteins and glutathione *in vitro* and *in vivo*.[9,10] It undergoes rapid metabolism and chemical breakdown *in vitro* and *in vivo*, initially to 2-chlorobenzaldehyde and malononitrile, each of these then undergo further rapid reactions. The half lives (t½) of CS and the metabolites, 2-chlorobenzaldehyde and 2-chlorobenzylmalononitrile in one *in vivo* experiment involving the administration of compounds by intra-arterial injection into cats were 5.4, 4.5 and 9.5 seconds respectively.[11] After oral administration CS is metabolised and eliminated largely (*circa* 70%) via the urine as 2-chlorohippuric acid and 2-chlorobenzoic acid.[12] Other metabolites have been identified but there is no evidence of dechlorination. It was noted however that the available data were not as comprehensive as would have been obtained if modern techniques had been used. In addition, no data were available on the kinetics of CS administered as a solution in MIBK.

Experimental studies in animals

(xiv) The acute toxicity of CS following exposure via inhalation is characterised by sensory irritancy followed by prompt recovery. Acute studies in rodents and guinea pigs using pyrotechnically-generated CS smokes indicated that short term exposure (10 to 20 minutes) to concentrations of CS of around 4 grams/metre^3 (g/m^3), or longer exposure (several hours) to levels of around 30 to 40 mg/m^3, resulted in deaths. Death was due to severe lung damage (comprising haemorrhages and oedema).[13] Animals that survived showed no pathological abnormalities when examined 14 days later.

(xv) Studies to investigate skin irritancy in rats, rabbits and guinea pigs indicated that a 12.5% (w/v) solution of CS in corn oil or acetone applied for 6 hours without occlusion produced mild skin irritation.[7] No conclusions can be drawn with regard to its potential to induce skin sensitisation from the two animal studies available due to limitations in the methodology used.[14,15] There are, however, some data in humans to indicate that CS can provoke skin sensitisation (see paragraph xxx).

(xvi) The eye irritancy of CS has been shown to be dependent upon the solvent used. A 5% (w/v) solution in PEG-300 (polyethylene glycol) produced severe irritant effects in the rabbit (mild or moderate keratitis lasting for 2 weeks or more after a single application) whereas a 10% (w/v) solution in trichloroethane produced some conjunctivitis but no corneal damage and no effects were seen after 7 days.[16,17] Results of eye irritancy studies in rabbits using a 7% (w/v) solution in MIBK are given in paragraph xliv; signs of severe irritation were seen initially with recovery after 8 days.

(xvii) Repeated dose inhalation studies involving exposure for 1 hour a day for 120 days, indicated a NOAEL* of about 30 mg CS/m^3 in a range of species (mice, rats, guinea-pigs).[18] At around 200 mg/m^3 in mice and guinea pigs, deaths of 23% and 48% respectively of the exposed animals occurred in the first month of the experiment.

Mutagenicity

(xviii) The mutagenicity data on CS were considered by the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM). Their conclusions are given in the following paragraphs.

In vitro *studies*

(xix) The mutagenicity of CS has been extensively studied in vitro. Negative results were obtained in Salmonella assays, but there were reservations regarding the suitability of the standard protocols used in these tests with respect to CS in view of its very short half life.[19–23] Positive results were noted in assays in V79 cells for gene mutation and also in the mouse lymphoma assay.[20,24,25] Positive results were documented also in metaphase analysis for clastogenicity in V79 and CHO cells.[20,26] In addition, CS has been shown to induce SCEs (Sister Chromatid Exchanges) in CHO cells.[20] These data indicate that CS has clastogenic potential.

(xx) There is evidence from *in vitro* studies to indicate that CS has aneugenic effects. It has been shown to interfere with the spindle machinery and cell division in mammalian cells resulting in C-mitosis and metaphase block.[27–32] CS has also been shown to induce micronuclei in mammalian cells *in vitro*.[25] These data suggest that CS has aneugenic potential.

(xxi) The clastogenic effects seen appear to be due to CS itself, or an unknown short-lived intermediate.[26] The mechanism of aneugenicity appears to differ from the clastogenicity with 2-chlorobenzaldehyde being the important metabolite regarding aneugenicity but not in respect of clastogenicity.[29]

In vivo *studies*

(xxii) Negative results were consistently obtained in bone marrow or peripheral blood assays for micronuclei induction using high dose levels and both the oral and intraperitoneal routes.[23,33] (These assays are capable of detecting clastogens and aneugens if the active metabolite reaches the bone marrow.) It was noted that no data were available to indicate if adequate amounts of CS or short lived reactive metabolites reached the target organ. Data from DNA binding studies in the liver and kidney did not help in this regard as no relevant analysis of tissues of initial contact (ie skin or nasal mucosa) were undertaken.[21] Studies using Drosophila (fruit flies) did not provide any meaningful data as the experimental design was unlikely to result in exposure of Drosophila to biologically active CS.[23] It was felt prudent for complete reassurance on the lack of mutagenic activity of CS *in vivo* to have data from a study to investigate genotoxicity to measure potential mutagenicity at a site of contact, for example in the nasal mucosa. However, some members of COM recognised that the design of such an animal study would be difficult both from practical and ethical standpoints and were of the opinion that these studies were not necessary.

* No Observable Adverse Effect Level

Carcinogenicity

(xxiii) The carcinogenicity data on CS were considered by the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC). Their conclusions are given in the following paragraph.

(xxiv) The US National Toxicology Program carcinogenicity studies provide no evidence that CS had any carcinogenic effects in adequately conducted inhalation bioassays in rats or in mice following 2 year exposure at up to 0.75 mg/m^3 and 1.5 mg/m^3 respectively.[20] These data provide reassurance that CS does not have mutagenic activity *in vivo* at site of contact tissues, a concern raised by the COM. No further work relating to CS is therefore needed in this area.

Reproductive toxicity

(xxv) Developmental toxicity (teratogenicity) studies using the inhalation route and an aerosol of CS (1-2 µm mass median diameter) did not indicate any teratogenic or foetotoxic effects in rats or rabbits exposed to 60 mg/m^3 CS (5 minutes per day) on days 6 to 15 of pregnancy.[34] Similar results were obtained when CS was given by the intraperitoneal route at 20 mg/kg as a single dose on day 6, 8, 9, 10, 12 or 14 of pregnancy.

(xxvi) There were no data available relating to single or multigeneration reproductive toxicity studies.

Effects in humans

(xxvii) Most of the data available relates to studies involving CS smoke or aerosol and exposure via inhalation. Aerosols were generated by thermal dispersion (particle size about 0.5 µm) or from a solution in methylene chloride (particle size about 1 µm). Studies on volunteers indicate that exposure to about 0.5 to 1 mg/m^3 CS for 90 minutes in an exposure chamber produced profuse tears (lachrymation), involuntary repeated closure of eyes (blepharospasm), a burning sensation in the mouth, nasal irritation and symptoms of tightness in the chest; in some cases difficulty in breathing was experienced, particularly upon initial exposure.[35,36] Subjects were able to tolerate exposure at these levels throughout the 90 minute duration of this experiment. In general exposures of about 2.5 mg/m^3 could be tolerated only for a few minutes. These data relate to subjects not previously exposed to CS. There is evidence for the development of some tolerance if exposures are built up slowly with 7/8 (88%) subjects then being able to tolerate 2.5 mg/m^3 for 60 minutes.[36] Once exposure ceased all symptoms and signs, apart from headache, disappeared within a few minutes. No biologically significant effects were seen on respiratory function, blood chemistry nor in the pattern of electrocardiograms (ECG). However, the observation of effects on the ECG would be very dependent on the time after exposure at which they were measured and it is not clear from the published paper how long a delay occurred after exposure had ceased.[36] Dermal exposure of volunteers, by body drenching whilst only lightly clothed, with very dilute aqueous solutions of CS (up to 0.0005% w/v) resulted in marked transient skin and eye irritation.[37] During this period a rise in both systolic (30 to 59 mm Hg) and diastolic (15 to 30 mm Hg) blood pressures was noted which took 2 to 25 minutes to fall to within 10 mm Hg of the controls. This was not dose-related and was not exacerbated by exercise.

(xxviii) Data from volunteer studies and experience in use, both in the manufacture of CS and its use in riot control, indicate that CS itself is a skin irritant. Volunteers whose forearm skin was exposed to dry powder experienced a mild, transient skin reaction.[38] The effects were more pronounced if the powder was moistened, when erythema lasted for between 1 to 2 days. Studies have also been carried

out on volunteers whose forearm skin was exposed to high concentrations of CS under simulated tropical conditions.[39] Marked irritant effects could be produced although there was much variability in response depending on the individual and on local conditions (heat and moisture). A high incidence of dermatitis on the arms and neck has been reported at the industrial site in the USA that manufactured CS in the past.[39] Occupational hygiene standards at this plant were poor, with airborne CS concentrations of levels up to 12 mg/m^3 (much greater than the Threshold Limit Value TLV* at the time of 0.4 mg/m^3).

(xxix) Skin problems were common in individuals exposed to CS from grenades when these were used in Hong Kong during rioting at a Vietnamese detention camp, under circumstances where the rioters were not able to disperse.[40,41] A subsequent review of case records of 184 patients with symptoms consistent with CS exposure revealed a high incidence (52%) of skin problems including contact dermatitis and minor burns, most of which resolved within 2 weeks. Some of the skin injuries were caused by contact with hot canisters or grenades.

(xxx) There is some evidence that CS can also produce skin sensitisation. At the CS manufacturing site referred to in paragraph xxviii, 8% (2/25) of the individuals who were patch tested with CS showed skin reactions consistent with allergic contact dermatitis.[39] There are also case reports of CS-induced allergic contact dermatitis in four individuals who were also exposed to CS from tear gas grenades.[42–44] There is, however, no information on the sensitisation potential of CS in solvent formulations.

(xxxi) Data on eye irritancy are available from studies in volunteers. Exposure of young male volunteers to 0.1 or 0.25% CS as a slurry in 0.5% polysorbate, either directly (0.25 ml drop) or as a spray (hand-held disperser from 15 feet), resulted in a severe pain response for a few minutes, profuse tears and redness of the conjuctiva for about 10 minutes.[45] Comparable effects were seen in volunteers exposed to up to 1% CS in an organic solvent (trioctyl phosphate) using identical methodology.[46] There was complete recovery after about 30 minutes. Similar effects were seen in volunteers exposed to CS powder (0.8 mm mass median diameter) at up to 6.7 mg/m^3 for 10 minutes.[47] There were no effects on visual acuity several minutes after exposure ceased. Data from experience in use indicates similar effects with transient pain, profuse tears and conjunctival reddening. There is no evidence from these studies of any permanent damage.

(xxxii) The question as to whether subjects being treated with neuroleptic drugs are likely to be more sensitive to CS spray has been raised.[48] There are no experimental data to allow any conclusions to be drawn on this aspect of the toxicity of CS.

(xxxiii) The only data on the effects of repeated exposure to CS derived from case reports of workers occupationally exposed.[2] These do not indicate any effects other than local irritant effects seen after acute exposure, but no conclusions can be drawn from these very limited data.

Toxicity of MIBK

(xxxiv) MIBK is readily absorbed and widely distributed in various tissues of rats and mice following oral or inhalation exposure.[49,50] The major metabolites in rodents are 4-hydroxy-4-methyl-2-pentanone (4-OHMIBK) and 4-methyl-2-pentanol (4-MPOL) which may be further conjugated, or metabolised and eliminated as carbon dioxide, or incorporated into tissues.[50,51] Data on elimination of MIBK are incomplete. Studies in humans suggest that absorbed MIBK is rapidly cleared from blood and that very little unchanged MIBK is eliminated in the urine.[52]

* Occupational exposure limit, for an 8 hour time weighted exposure, recommended by the American Conference of Government Industrial Hygienists in the USA.

(xxxv) Studies using hens have indicated that MIBK has the potential to induce microsomal metabolism carried out by cytochrome P450 enzymes in the liver after repeated exposure for 3 months. MIBK would thereby potentiate the effects of other chemicals (including drugs) that undergo activation via cytochrome P450-mediated metabolism.[53] These data, however, derived from studies involving prolonged, repeated exposure and are not relevant to single exposure, as is the case in the use of CS spray in the field.

(xxxvi) MIBK is of low acute toxicity in rats or mice both by inhalation (4 hour LC_{50}* circa 12 g/m^3) or by ingestion (oral LD_{50}** 2 to 5 g/kg b.w.).[54–56] Studies in rabbits to investigate skin irritancy using an occlusive dressing and 10 to 24 hour exposure resulted in minor transient effects, indicating that MIBK has a low skin irritant potential.[57,58] Repeated exposure produced drying and flaking of the skin due to the defatting action of MIBK. Eye irritancy studies in the rabbit using neat MIBK (0.1 ml) resulted in transient effects.[54] These results indicate that MIBK has low eye irritant potential.

(xxxvi) Repeated dose (90-day) studies by inhalation showed effects on the liver and kidneys.[59] In mice the only effect seen, apart from lachrymation, at the top dose (4100 mg/m^3) was a small increase (11%) in liver weight, not accompanied by histopathological abnormalities. A similar effect was seen in the liver of rats. In addition, nephrotoxicity was seen in the proximal tubules of the rat kidney at concentrations of 1025 and 4100 mg/m^3 of MIBK. Nephrotoxicity was limited to the male rat and was associated with hyaline droplet deposition. It may have been due to binding to alpha-2 urinary microglobulin, a male rat specific protein; this mechanism is believed to be specific to the male rat.[60] The NOAEL was 205 mg/m^3 in the rat and 1025 mg/m^3 in the mouse.

(xxxvii) In an unpublished 90-day oral study in the rat, histological evidence of kidney damage was reported at doses of 250 mg/kg and above, both in male and female animals. There was increased liver weight, not accompanied by histopathological damage, at 100 mg/kg.[61] The NOAEL for this study was estimated to be 50 mg/kg.

(xxxviii) There is no evidence that MIBK or its major metabolite 4-OHMIBK have any genotoxic properties. Negative results were obtained with MIBK in the Salmonella assay, a metaphase analysis for clastogenicity in hepatocytes, a mouse lymphoma assay, an unscheduled DNA synthesis (UDS) assay in hepatocytes and also *in vivo* in a bone marrow micronucleus assay.[62,63] Negative results were obtained for the metabolite in the Salmonella assay and metaphase analysis in hepatocytes.[62]

(xxxix) The developmental toxicity (teratogenicity) of MIBK in rats and mice has been assessed following exposure between 300 and 3000 ppm by inhalation on gestation days 6-15.[64] Maternal toxicity and foetotoxicity were observed in both species at 3000 ppm, but not at 1000 ppm. Significant reductions in foetal weight and ossification in the rat at 300 ppm were probably related to litter sizes and were not treatment-related. Contrary to the statement in some reports (which have relied on secondary sources and not the original article), there was no evidence of teratogenicity in either species, even at the maternally toxic exposure concentration of 3000 ppm.

(xl) It was noted that no carcinogenicity bioassays nor any single or multigeneration reproductive toxicity studies have been carried out on MIBK.

(xli) The characteristic effects noted in humans relate to local irritant effects and non-specific central nervous system (CNS) effects (eg headache, nausea) at occupational exposures of about 100 ppm and above.[52,65,66] The odour threshold is low (0.4 ppm) and the irritant effects can be detected at about 2 ppm.[67]

* Lethal concentration estimated to result in deaths of 50% of the exposed animals.

** Lethal dose estimated to result in deaths of 50% of the exposed animals.

Data on the combination of CS and MIBK

(xlii) The Committee noted the sparsity of data on the combination of CS dissolved in MIBK. There are no data available on the metabolism, kinetics, acute toxicity, or skin irritancy of CS when administered in MIBK as solvent.

(xliii) The only experimental data specifically on this combination consist of a study on the eye irritancy of 7% CS in MIBK (w/v) in rabbits.[68] This indicated that spraying 7% CS directly into the eyes of rabbits from close range produced severe irritant effects, including a degree of corneal opacity, which had cleared by day 8 and was not followed by irreversible damage.

(xliv) Information was available from experience arising from the use of the spray from studies carried out by NPIS London Centre and the CIRS.[69,70] These indicated that there were cases of dermatitis following the use of CS spray: the effects produced were noted 6 hours after the exposure. No longer term follow-up studies have been carried out. There are also case reports of marked dermatitis following the use of CS spray in France. The report describes eleven subjects of whom five had multiple exposures to CS. The authors considered that a direct irritant effect was responsible, although an allergic dermatitis could not be ruled out. It is not clear from the published information whether exposure was to CS in MIBK or to another formulation. In addition, no information is available on the ethnicity of the exposed individual who developed dermatitis.[71]

(xlv) Literature searches did not reveal reports of serious eye damage caused by CS spray. Furthermore such cases were not identified as a consequence of exposure to CS spray in the data provided by NPIS London Centre.

Conclusions

(xlvi) The Committee noted that there are considerable data available to assess the toxicity of CS itself, and to a lesser extent, the solvent MIBK itself. CS is a potent sensory irritant, particularly to the skin and the eyes. It is rapidly hydrolysed and therefore tissue exposure to CS itself is transient. Experience in use indicates that it is a skin irritant and there are some reports of skin sensitisation occurring. There are no concerns relating to the mutagenicity, carcinogenicity or teratogenicity of CS itself. The toxicity of the solvent MIBK is characterised by transient local irritant effects and central nervous system (CNS) effects (particularly headache, nausea) resulting from occupational exposures of about 100 ppm and above. Negative results were obtained in mutagenicity tests and there was no evidence of teratogenicity in developmental toxicity studies. There is no information from carcinogenicity or multigeneration reproductive toxicity assays.

(xlvii) There are very few data on the formulated material. A 7% (w/v) solution of CS in MIBK produced severe irritant effects in rabbit eyes followed by recovery in 8 days. This is consistent with the absence of evidence of serious permanent eye damage in humans. Experience in use indicates that it has skin irritant properties, and can cause dermatitis.

(xlviii) The Committee's conclusions regarding the health effects of CS spray were based on consideration of the toxicity data on CS and MIBK. As noted above there was very little information on the formulated product. The Committee's advice applies to all individuals exposed to CS spray during its use as a chemical incapacitant.

(xlix) The Committee considered that the available data did not, in general, raise concerns regarding the health effects of CS spray itself. Local irritant effects are short term and there exists the possibility of skin sensitisation occurring in some individuals. It must be noted that no comprehensive

investigation of the effects of CS spray in humans was available, nor has there been any systematic follow-up of individuals who have been sprayed with CS spray. The Committee has concerns regarding exposure to CS spray in susceptible groups. These are:

(a) Individuals with bronchial asthma or chronic obstructive airways disease whose condition could be aggravated by the irritant effects of CS spray on the respiratory tract.

(b) Individuals suffering from hypertension or other cardiovascular disease because of the transient effects of CS spray in increasing blood pressure.

(c) It was not possible, on the basis of the available data, to comment on whether individuals being treated with neuroleptic drugs are more likely to be sensitive to the effects of CS spray.

(l) The Committee noted that adherence to the operational guidelines for the use of CS spray was of particular importance since at the time of exposure it would be exceedingly unlikely that the medical status of those exposed would be known. These concerns, and the uncertainties noted earlier, lead to the conclusion that particular care needs to be taken to follow the recommended aftercare guidelines for all persons exposed to CS.

(li) The Committee considered that further information needs to be obtained on the effects of CS spray in humans. In this regard it was noted that systematic studies in volunteers to investigate the toxicity of CS spray may present insurmountable difficulties. The Committee thus recommended that follow-up studies be carried out on individuals treated for the immediate effects of CS spray in order to obtain data on whether delayed effects occur. Information should also be collected in these studies relating to the previous medical history of the individuals involved, particularly with regard to respiratory or cardiovascular disease, or treatment with neuroleptic drugs.

Epoxidised Soya Bean Oil

1.7 Epoxidised soya bean oil (ESBO) is a mixture of substances that is produced by the controlled epoxidation of soya bean oil in which the carbon-carbon double bonds are largely converted to epoxy groups. It is used for plasticising gaskets in the lids of most glass containers used in this country for baby foods. The Committee was asked to give consideration to the results of a MAFF/DH Joint Food Safety and Standards Group (JFSSG) survey of ESBO migration from plasticised gaskets in the lids of glass containers used for baby foods.

1.8 The Committee had reviewed the available toxicology data on ESBO in 1989 when it considered a report on plasticiser levels in food contact materials prepared by the Working Group on Chemical Contaminants from Food Contact Materials of the Steering Group on Chemical Aspects of Food Surveillance. A further review was undertaken during 1994 when the Committee recommended a tolerable daily intake (TDI) of 1 mg/kg bw per day. This was derived by applying a safety factor of 100 to the NOAEL of 100 mg/kg bw per day determined by a two-year feeding study in rats.

1.9 After consideration of the latest survey data, the Committee concluded that it would be prudent that action should be taken to reduce the levels of ESBO in these products in order to restore the safety margin incorporated into the TDI. The Food Advisory Committee (FAC) welcomed the action already being taken by industry with this objective in mind and noted that the design of the packaging and the use of ESBO in it have provided confidence that the products are safe. The FAC concluded that action by industry must continue and be done in ways that continue to ensure the highest level of safety of the products.

Food Intolerance

1.10 The Working Group on Food Intolerance prepared a first draft report which was discussed by the Committee during the year. The final report is in preparation.

French Maritime Pine Bark Extract

1.11 Consideration was given to additional, more detailed toxicological data on a commercial extract of pine bark that is marketed as a dietary supplement. This matter had been referred to the Committee by the Advisory Committee on Novel Foods and Processes and a statement was prepared in 1998. In 1999 the Committee prepared the following statement:

(i) In 1998 we were asked to consider a submission which had initially been made to the Advisory Committee on Novel Foods and Processes (ACNFP) seeking food safety clearance of an extract derived from the bark of the pine tree (*Pinus pinaster*). The French maritime pine bark extract is sold in the form of a dietary supplement. The ACNFP had advised that since the extract has been on sale in the UK and other European countries for a number of years, it would not be considered a novel food. However, the ACNFP did have some concerns regarding the toxicological data included in the submission and it was therefore referred to this Committee.

(ii) In our consideration of the submission we identified problems with the data received, as some of the information was in the form of brief summaries of old, unpublished studies. In addition, it was not clear that the studies had used material which corresponded to that currently marketed. We therefore indicated that an adequate specification of the extract should be provided before any further work was undertaken. The deficiencies in the reporting or execution of the toxicological studies led us to recommend that some studies should be repeated or appropriate new studies be performed in order to clarify aspects of the toxicity of the extract. In particular we had concerns about a report, in an abstract, of lesions occurring in the brain of dogs after a 6-month feeding study, about potential teratogenic/foetotoxic and endocrine/reproductive effects and also the possible mutagenicity and allergenicity of the extract. Our conclusions were recorded in the form of a statement.[72]

Consideration of new data

(iii) We have since received additional documentation which comprises:

a) more detailed specifications of the product;[73]

b) history of the production and nomenclature of the extract;[74]

c) a new mutagenicity study;[75]

d) a copy and certified translation of the report of the original 6-month feeding study in dogs;[76]

e) an expert opinion of the report of the original 6-month feeding study in dogs;[77] and

f) a statement as to the absence of protein in the product.[78]

Specifications

(iv) We are satisfied that, within the limitations of the analytical methodology, the specifications and analytical data submitted provide adequate assurance of the consistency of batches of French maritime pine bark extract prepared by current production methods.[73] Although there is an extensive history of production of the extract, the analytical data are not available to demonstrate that the extract used in early studies is similar to that currently produced.[74]

Mutagenicity

(v) We have been informed that the recently completed mutagenicity assay (Ames test) has been reviewed by the Secretariat of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment who concluded that the extract was non-mutagenic in the assay. Having seen the report of this study,[75] we endorse this conclusion.

The 6-month toxicity study in dogs

(vi) When we first considered the toxicity data available for the French maritime pine bark extract the only information on this study, carried out in 1975, was a short abstract. A statement about the development of lesions in the brains of dogs raised concerns that were not resolved by the other available toxicity data. Unfortunately, the full report of the study did not remove these concerns.[76] Although the lesions of the brain and spinal cord occurred only in the group of animals receiving the highest dose, we note that the study pathologists had not been able to determine whether the lesions were treatment-related or were artefactual, possibly having resulted from some difference in the post mortem processing of the tissues. Lack of detail in the report meant that we were unable to make any judgement on this issue.

(vii) Other aspects of this study were of concern. The dosing schedule at the start of the treatment had involved the oral administration of 0, 60, 150 and 500 milligram of the extract per kilogram of body weight (mg/kg b.w.) on only six days of each week. Six weeks before the end of the study the top dose was increased to 1000 mg/kg b.w. administered continuously, which we assume to be on seven days per week. This variation to the dosing schedule of the top dose group makes comparisons with the two lower dose groups difficult to interpret.

(viii) It has been suggested to us that the administration of six doses per week would have been considered reasonable at the period when the study was carried out.[77] This is not our opinion, as at that time it was recognised that the use of such a schedule could offer a period of recovery from any adverse effects of the test substance. Since the report does not provide information to indicate at what stage of the weekly dosing schedule animals were killed or samples were taken for haematology and clinical chemistry it is possible that some of the changes recorded during the study and at its termination may have been minimised by an intervening period of recovery.

(ix) In addition to the lesions of the nervous system there were effects on the heart rate and body weight gain in the top dose group. These were restricted to that group. However, some changes, e.g. in organ weights, in leucocyte counts and in blood triglyceride and glucose concentrations were reported as occurring in all the treated groups, although there was evidence of a sex-specificity of changes in some organ weights.

(x) The deficiencies in the study mean that we reiterate our opinion that this study needs to be repeated in order either to dismiss or to substantiate the concerns that it has raised.

Allergenicity

(xi) We considered a statement which indicated that no nitrogen had been detected in three batches of the French maritime pine bark extract by elemental analysis and that no protein had been detected by an electrophoretic technique (SDS-PAGE). Without information on the procedures involved and the Limits of Detection for the samples the statement does not provide any reassurance as to the absence of allergenic protein in the extract.[78]

Reproductive toxicity

(xii) Neither new data nor original full study reports have been submitted to address our concerns about possible teratogenicity and endocrine and/or reproductive effects of the French maritime pine bark extract. We have been informed that a consultation with industry is in progress about the labelling of the product in order to indicate that it is a preparation for adults but should not be taken during pregnancy or breastfeeding. We have been asked whether it would be sufficient to advise that it not be taken by children younger than 8 years of age. In the absence of data that provide reassurance in these matters we would not recommend that the suggested age be lowered from that of adulthood.

Conclusions

(a) We accept that, within the limits of the techniques used, the analytical data submitted on batch-to-batch variation of the French maritime pine bark extract provide evidence of consistency of the currently manufactured product.

(b) The full report of the 6-month dog study has not removed our concerns about the possible adverse effects resulting from the administration of French maritime pine bark extract. We do not consider that this study is adequate for the determination of a No Observed Adverse Effect Level. Therefore, we reiterate our view that it is necessary to repeat this study to present-day testing guidelines.

(c) On the basis of the studies originally and recently submitted we consider that the extract is non-mutagenic.

(d) We would wish to see the experimental details, including the Limits of Detection, of the studies undertaken to detect any protein in the extract before accepting that allergenic proteins are absent.

(e) We repeat our recommendation that the original reports of the high dose embryo-foetal studies be provided and that *in vitro* and/or short-term *in vivo* studies be undertaken to address the question of possible endocrine or reproductive effects of the extract. In the interim, we support the suggested labelling of the product to indicate that it should be taken only by adults but not by pregnant women or nursing mothers.

Hemicellulase Enzyme in Bread-Making

1.12 The Committee carried out a postal consultation on the toxicological aspects of a submission to the Food Advisory Committee requesting clearance for the use of a hemicellulase enzyme in bread-making. It was agreed that the enzyme could be given temporary clearance for one year subject to the company's provision of an assurance that the frequency of testing for mycotoxins and antibacterial activity would be increased to one batch in four and of

evidence to support the company's claim that no residual enzyme activity would be expected in the bread after the baking process.

Hypospadias and Maternal Nutrition

1.13 The Committee was asked to review an unpublished paper describing an investigation of the possible role of maternal nutrition in the origin of hypospadias in male children. The study had involved a cohort of mothers and their children participating in the Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC). It was claimed that there was an association between hypospadias and the mother eating a vegetarian diet. The incidence of hypospadias in the study population was higher than that recorded in the 1992 figures from the Office of National Statistics, however the Committee agreed that it was not possible to be certain that the increase represented a true increase in the incidence of this malformation.

1.14 The limited number of cases of hypospadias, as well as the classification of the degree of severity of these, in the group of vegetarian mothers were of concern to members. It was noted that, since random fluctuation in incidence could not be ruled out, it was important to validate the classification.

1.15 The biological plausibility of the hypothesis that the concentration of phytoestrogens in the maternal diet was a risk factor for hypospadias was discussed. It was noted that the mother's self-definition of 'vegetarianism' varied between individuals and that the information on the consumption of phytoestrogens was not detailed enough.

1.16 The Committee concluded that the paper was based on a study with a good prospective longitudinal design but that there were considerable concerns about the possible confounding factors. There was concern that the link to oestrogen exposure and alternative mechanisms had not been considered. In addition, there were reservations about the ascertainment of the hypospadias and about the objective interpretation of the data.

Iodine in Milk

1.17 The results of a survey of the concentrations of iodine in retail samples of milk was considered by the Committee. This survey had been carried out by the Joint Food Safety and Standards Group in response to the points raised by the Committee in 1997. The following statement was produced:

(i) We have considered a draft Food Surveillance Information Sheet[79] describing the results of a survey carried out for the MAFF/DH Joint Food Safety and Standards Group on the concentration of iodine in cows' milk.

Results of the survey

(ii) Representative purchases of whole (93 samples), semi-skimmed (107 samples) and skimmed (20 samples) pasteurised cows' milk on sale in the UK were made during the summer of 1998 and the winter of 1998/1999 from Belfast, Edinburgh, Swansea, Sunderland, Leicester and London and were analysed for total iodine. We welcome this latest survey of iodine in cows' milk. The overall mean iodine concentration in cows' milk was 311 mg/kg. We note that mean iodine concentrations were lower in summer (200 mg/kg) compared to winter (430 mg/kg). The higher concentrations in winter may reflect greater use of supplemented compound feedstuffs during this period. We also note that the fat content of the milk did not influence these values.

(iii) Since 1989 we have reviewed iodine concentrations in cows' milk on a number of occasions, most recently in September 1997.[80] In our 1997 review we noted that iodine concentrations in cows' milk were higher than in previous surveys, though the reasons for this were unclear. We consid-

ered that these higher concentrations of iodine were unlikely to pose a risk to health but we recommended that the monitoring of iodine in cows' milk should continue. Possible sources of iodine in cows' milk include iodine in the animal diets and the iodophors used as sterilants of cow teats and milking vessels. Therefore, we encouraged investigation of the bioavailability of iodine in iodophors and the different chemical forms of iodine in cows' milk. This would help to identify potential sources of iodine in cows' milk. Additionally, we requested study of the different chemical forms of iodine in infant formulas. We have been informed that research is underway to investigate the iodophors and the different chemical forms of iodine in cows' milk and infant formulas.

(iv) We note that the concentrations of iodine in cows' milk reported in this latest survey are similar to those we considered in 1997 but are higher than values reported in the earlier surveys.[81–83]

Dietary iodine intake

(v) Iodine is an important nutrient element and is required for the synthesis of thyroid hormones. An inadequate intake can lead to a broad spectrum of iodine deficiency diseases. For protection against iodine deficiency the Department of Health's Committee on Medical Aspects of Food Policy has recommended a Reference Nutrient Intake (RNI) for adults of 140 mg/day. The RNI for children ranges from 50 mg/day (for children aged 0-3 months) to 140 mg/day (for children aged between 15-18 years).[84] However, a high intake of iodine can result in functional impairment of, and tissue damage to, the thyroid gland and death has been caused by extremely high intakes. In our previous review.[80] we took reassurance from a study in which 1-11 year old children were given doses of iodine up to 1000 mg/day over a period of 4 months without signs of toxicity.[85] In order to protect individuals against the toxic effects of excessive iodine intake the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) has recommended a Provisional Maximum Tolerable Daily Intake (PMTDI) of 0.017 milligrams per kilogram body weight (mg/kg b.w.).[86] This is applicable to individuals over the age of 12 weeks and is equivalent to 1000 micrograms (mg)/day for a 60 kg adult (the equivalent values for children are reported in Table 1).

(vi) There are few data on which to base estimates of the potential intake of iodine by different age groups. Iodine intakes from milk have been estimated for adults and for children aged 1½ – 2½, 2½ – 3½, and 3½ – 4½ years, using mean iodine concentrations in milk from this survey[79] and consumption data from the National Dietary and Nutritional Survey.[87,88] No intakes were estimated for schoolchildren as the available consumption data for this age group are limited and outdated. It is understood that new data to address this point will be available soon. To estimate the current total dietary intake, incorporating the latest concentrations of iodine in milk, the mean population dietary intake estimated from the 1995 Total Diet Study[89] minus the contribution from milk was added to the intakes from milk alone estimated from this survey. These intake estimates from milk alone and from the total diet are presented in Table 1. We note that the estimate of mean population dietary intake is based on household, rather than individual, consumption. Therefore the estimate of the non-milk intakes will be biased by the intakes of adults in the household; in consequence the value added for non-milk sources (123 mg/day – see Table 1) is likely to be an overestimate for children.

(vii) Calculations for adults suggest that the average and high level (97.5th percentile) consumer of milk would ingest 194 and 301 mg/day respectively from the total diet (including milk), which are both within the guideline intake calculated from the JECFA PMTDI of 0.017 mg/kg b.w. As detailed in Table 1 the estimated total dietary intakes for average level consumers of milk in the 1½ – 2½, 2½ – 3½, and 3½ – 4½ age ranges are 221, 215, and 204 mg/day respectively, which are

close to the guideline intake. For high level consumers of milk in the 1½ – 2½, 2½ – 3½, and 3½ – 4½ age ranges the estimated total dietary intakes are 362, 379, and 330 mg/day respectively, all of which exceed the relevant guideline intake. For high level consumers of milk in the 1½ – 2½ and 2½ – 3½ age ranges, though not the 3½ – 4½ age range, the estimated intake from milk alone exceeds the relevant guideline intake. As shown in Table 1 exceedence of the guideline intake occurs mainly in the 1½ – 2½ and 2½ – 3½ age ranges and after children reach the age of 3½ exceedence is restricted to high level consumers only.

Recommendations

(viii) Our recommendations are:

(a) The revised dietary intakes estimated from the results of this survey support our conclusions of September 1997 that monitoring of iodine concentrations in cows' milk should continue;

(b) We have been informed that the UK Expert Group on Vitamins and Minerals will be reviewing the toxicity of iodine in due course and we may wish to reconsider these survey results in the light of the findings of that group;

(c) The revised dietary intakes increase the need for data on the different chemical forms of iodine in cows' milk and infant formulas and on the bioavailability of the iodine in iodophors. We therefore request that we see the results on the studies on the different chemical forms of iodine in cows' milk and infant formulas at the earliest opportunity and we reiterate our previous recommendation that the bioavailability of the iodine in iodophors is investigated.

Conclusions

(ix) We have made a number of recommendations, including investigation of the bioavailability of iodine from iodophors in milk. However, we conclude that the new data from this latest survey do not alter our previous advice that the concentrations of iodine in cows' milk are unlikely to pose a risk to health, even in those children who are high level consumers.

Table 1.1: Estimated dietary iodine intakes of adults and young children

	Dietary exposure (µg/person/day) *				Guideline intake (µg/day)[2]
	via milk only		via total diet[1]		
	Mean	97.5%ile	Mean	97.5%ile	
Summer					
Adult (16 – 64 years)	46	118	169	241	1000
Child (1½– 2½ years)	59	147	182	**270**	210
Child (2½ – 3½ years)	58	171	181	**294**	250
Child (3½ – 4½ years)	51	138	174	261	280
Winter					
Adult (16 – 64 years)	96	234	219	357	1000
Child (1½ – 2½ years)	142	**332**	**265**	**455**	210
Child (2½ – 3½ years)	129	**329**	**252**	**452**	250
Child (3½ – 4½ years)	114	266	237	389	280
Average					
Adult (16 – 64 years)	71	178	194	301	1000
Child (1½ – 2½ years)	98	**239**	**221**	**362**	210
Child (2½ – 3½ years)	92	**256**	215	**379**	250
Child (3½ – 4½ years)	81	207	204	**330**	280

* values in **bold** text exceed the relevant guideline intake

[1] To estimate total dietary intake the mean population dietary intake of iodine minus the contribution from milk (i.e. 209 mg/day [total diet] – 86 mg/day [milk] = 123 mg/day) was added to the intakes from milk alone estimated from this survey. For example, to estimate the average total dietary intake for adult consumers during the summer the intake from milk i.e. 46 mg/day, is added to 123 mg/day to give a final value of 169 mg/day

[2] This guideline intake, calculated for the purposes of this statement, is equivalent to the JECFA PMTDI of 0.017 mg/kg b.w. when expressed on a total body weight basis. For example, for children aged 1½ – 2½ years weighing 12.3 kg the PMTDI is equivalent to 210 mg/day (rounded to 2 significant figures)

Malachite Green and Leucomalachite Green in Farmed Fish

1.18 The Committee has previously reviewed the toxicity of malachite green and was asked to consider the results of a new survey of the concentrations of this compound and its metabolite, leucomalachite green in farmed fish. It prepared the following statement:

(i) The Committee has been requested by the Veterinary Medicines Directorate (VMD) of the Ministry of Agriculture, Fisheries and Food to consider the results of recent surveillance for the compounds malachite green and leucomalachite green in farmed trout. The Committee was invited to comment on the results and any possible consequences for the safety of consumers and the operators of fish farms.

Background

(ii) The triphenylmethane dye malachite green has been used in aquaculture throughout the world for the treatment of parasitic and fungal infections in fish and shellfish. Within the United Kingdom (UK) trout industry it has been used for many years as a general hatchery disinfectant, to treat fungal conditions on trout eggs and for the control of certain ectoparasites. Additionally,

it has been found to be effective in the treatment of 'proliferative kidney disease', a systemic protozoal disease in trout. Leucomalachite green is the major metabolite of malachite green.

(iii) Malachite green is used as a dye for fabrics and is freely available. It is not a licensed veterinary medicine and there are no regulations on dosage or mode of use in fish farming. The Committee understands that operators apply a solution of malachite green at the water inlet of the fish tanks or transfer the fish that require treatment to a separate container. Advice has been provided to the farmed and game fish industries about the precautions that should be taken to reduce the occurrence of malachite green and its metabolites in the fish that are sold on the retail market (*vide infra*).

The 1993 review

(iv) In 1993 the VMD sought the advice of the Committee on the possible risk to consumers from residues of malachite green that had been detected in some samples of farmed trout purchased at retail outlets. As only low concentrations, 1 to 7.4 µg/kg, of malachite green had been detected in about 7% of the samples the Committee concluded that these residues would not adversely affect the health of consumers. Nevertheless, the Committee had reservations about a) the chemical characterisation of the malachite green used in toxicological studies and in fish farming, b) similarities of the structure of malachite green to compounds that are carcinogenic and/or mutagenic, and c) the existence of evidence of foetotoxicity in rabbits, with there being some evidence to suggest that it may also be teratogenic. These reservations led the Committee to conclude that there were insufficient reliable toxicological data to permit a full safety assessment.[90]

(v) The Committee's advice was passed to the Veterinary Products Committee (VPC) in 1994. The VPC noted the Committee's views and agreed with its conclusions.

The 1995 review

(vi) In 1995 the Committee considered further data from 406 trout samples analysed in three surveys between July 1993 to March 1995. Sixty-seven of the samples, about 16%, contained malachite green at concentrations of 2 to 50 µg/kg. The Committee considered there were still insufficient data to reach a conclusion about the safety of this substance, and concern was expressed about the apparent increase in residue levels. Although the concentrations of residues found were considered to be unlikely to affect the health of the consumers if the substance continued to be used, the Committee recommended that toxicity data should be obtained and, in the first instance, appropriate genotoxicity studies should be carried out on malachite green and its major conversion products in fish, the carbinol derivative and leucomalachite green.[91]

Subsequent actions

(vii) The Committee has been informed that, in the light of the results in 1995, the British Trout Association (BTA) issued a briefing note to their members stressing the need to ensure that any trout placed on the market did not contain residues of malachite green. As part of a package of precautions, the BTA advised members that malachite green should not be administered to fry over 5 grams in weight. In support of this initiative, the VMD wrote to all registered trout farmers in the UK in July 1995 underlining its concerns about the results and repeating the BTA advice.

(viii) In 1996, the BTA further advised its members to limit malachite green to use only in the hatchery, on eggs and, if essential, to alevins and first feeding fry on grounds of welfare. So that due regard

for consumer safety should be taken into account, the fish should only be sold on to the next stage of the production chain once they were confirmed as being free of malachite green residues. The VMD endorsed this advice by writing in August 1996 to all registered trout farmers in the UK and to over 500 fisheries producing trout for game.

Additional survey data

(ix) We have been informed that malachite green was detected in 15 of 208 samples tested in a survey of retail trout in 1996. In 1997 there were only two trout, out of 137 tested, which contained residues of malachite green (Table 1.2). Subsequently a new method of analysis, capable of determining both malachite green and leucomalachite green, was tested on the samples from 31 of the 137 trout that had been tested in 1997. These samples were randomly selected although they included the two which had contained malachite green residues. Residues of leucomalachite green were detected and quantified in seven samples. The most recently received sample which had been positive for malachite green still contained residues of malachite green and also the highest concentration of leucomalachite green (Table 1.3). Surveillance for residues of malachite green and leucomalachite green in trout has been undertaken in 1998 using the new analytical method. Of the twenty seven samples collected in January to August, one was found to contain both malachite green and leucomalachite green and five others were found to contain leucomalachite green alone (Table 1.2).

(x) We have queried whether there is information about any additional sources of malachite green or leucomalachite green that might result in their accumulation in the fish but have been advised that such information is not available.

Additional toxicological data

(xi) We have had the opportunity of considering full reports of short-term (28 day) range-finding studies in rats and mice which have been carried out on malachite green and leucomalachite green by the National Center for Toxicological Research in the United States (US) in preparation for priority carcinogenicity testing of these compounds as part of the US National Toxicology Program.[92,93]

(xii) The results of these studies show that leucomalachite green caused a greater number of, and more severe, effects in rodents as compared to malachite green. Changes in body weight and liver to body weight ratio generally occurred at lower doses of leucomalachite green as compared to malachite green. In male rats, a dose-related increase in midzonal and centrilobular hepatocyte vacuolisation was observed in the groups fed leucomalachite green at 580 and 1160 milligrams/kilogram (mg/kg), whereas hepatocyte vacuolisation was observed in the malachite green-treated rats of either gender only in the group fed 1200 mg/kg diet. The dose levels of 1200 mg/kg of malachite green and 1160 mg/kg of leucomalachite green are approximately equimolar and were the highest doses tested.

(xiii) Morphological evidence of cell death in the transitional epithelium of the urinary bladder was observed in all female mice fed 1160 mg/kg diet of leucomalachite green, but not in female or male mice fed 1200 mg/kg diet of malachite green.

(xiv) There were histopathological changes in the thyroid glands of some female mice and male rats fed the highest concentrations of leucomalachite green. However, the number of animals affected was not statistically significant. Changes in the concentrations of total thyroxine (T4) and thyroid

stimulating hormone indicative of alterations of thyroid function were observed in male rats fed leucomalachite green at 1160 mg/kg diet for 4 or 21 days. Such changes were not observed in male rats fed malachite green at 1200 mg/kg diet although alterations to 3,5,3'-triiodothyronine and T4 concentrations were observed in the female rats after 21 days at this dietary concentration.

(xv) In a separate study, the formation of liver DNA adducts was demonstrated by ^{32}P-postlabelling in rats or mice fed either 100 or 600 mg/kg diet of malachite green for 28 days. Increased concentrations of adducts were observed in the livers of rats fed 96 or 580 mg/kg of leucomalachite green for the same period, but not in the livers of mice.

(xvi) Concerns about the possible genotoxicity of malachite green and its major conversion products, i.e. leucomalachite green and the carbinol, had led to our 1995 recommendation that appropriate genotoxicity studies should be carried out on these compounds. The additional data available now have led to our seeking the views of our sister committee, the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment, on the possible mutagenicity of malachite green and leucomalachite green. They have advised us that it would be prudent to consider both malachite green and leucomalachite green as potential *in vivo* mutagens.[94] The full statement of the Committee on Mutagenicity is given below.

Conclusions

(xvii)

(a) We *note* that recent surveillance data from the UK, together with a number of other studies, indicate that the predominant residue derived from malachite green in trout from fish farms is leucomalachite green rather than the parent substance. We *note* also that the possible presence of the carbinol derivative of malachite green has not been monitored and that there are no recent toxicological data on this compound.

(b) We *recommend* that the results of surveillance for malachite green and leucomalachite green should report separately the detected concentrations of these compounds.

(c) We *note* that leucomalachite green was not measured in surveys of farmed trout in the UK carried out between 1993 and 1996 inclusive.

(d) We are *concerned* that residues of malachite green continue to be detected in farmed trout. The toxicology data are insufficient to allow us to reach a full conclusion about the implications of these residues of malachite green and its metabolites for the health of the consumer of farmed trout. However, we *note* that, by comparison with the data provided at our review of 1995, there may have been a decline in the proportion of fish sampled that are found to contain residues of malachite green, see Table 1.2. We are aware that malachite green and leucomalachite green are currently undergoing carcinogenicity tests in the United States. We *consider* that, in the light of the opinion of the Committee on Mutagenicity (that it would be prudent to assume that malachite green and leucomalachite green are potential *in vivo* mutagens), there must be some concern about the health implications for those operators of fish farms who may be exposed to high concentrations of these compounds.

Table 1.2: Results of recent Surveillance in the United Kingdom by the Veterinary Medicines Directorate for Residues of Malachite Green and Leucomalachite Green in Trout Muscle

Sampling period	No. of samples analysed	No. containing residues#	Incidence%	Mean mg/kg	Range mg/kg	Concentration detected, mg/kg
Malachite Green						
January to December 1995*	210	35	16.7	9	2-35	2(3), 3(6), 4(2), 5(3), 6(6), 7, 8(3), 12, 13, 14, 16(4), 18, 23, 25, 35
January to December 1996	208	15	7.2	10	3-31	3(3), 4(2), 5(3), 6(2), 17(2), 21, 22, 31
January to December 1997	137	2	1.4	7	2-12	2, 12
Introduction of new analytical method						
Malachite Green						
January to August 1998	27	1	3.7	–	–	8**
Leucomalachite Green						
January to August 1998	27	6	22.2	76	4-150	4, 6, 70, 78**, 150(2)

Maximum Residue Limits (MRL) not set, Limits of Quantification (LOQ) = 2 mg/kg

* Contains data from samples collected in January to March 1995 previously considered by the COT

** Same sample contained both malachite green and leucomalachite green

Table 1.3: Results of reanalysis of samples of Trout Muscle collected in 1997 for residues of Malachite Green and Leucomalachite Green using the new analytical method

Sampling period	No. of samples analysed	No. containing residues#	Incidence%	Mean mg/kg	Range mg/kg	Concentration detected, mg/kg
Malachite Green						
September to December 1997	31	1	3.2	–	–	5*, **
Leucomalachite Green						
September to December 1997	31	7	22.0	66	4-330	4, 5, 7, 25, 41, 48, 330**

Maximum Residue Limits (MRL) not set, Limits of Quantification (LOQ) = 2 mg/kg

* When first analysed this sample had contained 12 µg/kg malachite green, see Table 1.2

** Same sample contained both malachite green and leucomalachite green

Mathematical Modelling

1.19 During 1999 the Committee held its first open meeting. The meeting took the form of a seminar at which the topic was 'Mathematical Modelling – Applications in Toxicology' and an audience with members from a variety of disciplines heard talks on *'Safety factors: what we think we are doing and what we are actually doing'* by Professor A.G. Renwick, University of Southampton (COT member); *'Population physiological pharmokinetic modelling – effect of ethanol on m-xylene exposure'* by Professor G.T. Tucker, Royal Hallamshire Hospital, Sheffield; *'Modelling consumer exposure of chemicals from food – future challenges and developments'*; Dr Naomi Rees, Joint Food Safety and Standards Group; and *'Intelligent learning about toxicity levels in the food chain after a catastrophe'* by Professor J.Q. Smith, University of Warwick.

Metals and Other Elements in Infant Foods

1.20 The results of a survey of the concentrations of various elements in infant foods was considered by the Committee, the members agreed the following statement:

(i) We have considered a draft Food Surveillance Information Sheet[95] describing the results of a survey carried out for the MAFF/DH Joint Food Safety and Standards Group on the concentrations of 13 elements (aluminium, antimony, arsenic, cadmium, chromium, cobalt, copper, lead, mercury, nickel, selenium, tin and zinc) in infant formulas, baby foods, rusks and infant drinks.

(ii) We have been informed that the concentrations of arsenic, copper, lead, tin and zinc in the infant foods did not exceed the statutory limits for those elements.

(iii) The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) has set Provisional Maximum Tolerable Daily Intakes or Provisional Tolerable Weekly Intakes for the metals aluminium, cadmium, copper, lead, mercury, tin and zinc.[96–98] We note that the estimated intakes by UK infants of these metals, other than zinc, in infant foods do not exceed the JECFA tolerable intakes.[96]

(iv) Although the upper range of the estimated intake of zinc by an infant aged 0-6 months exceeds the JECFA Provisional Maximum Tolerable Daily Intake we are aware that there is a nutritional requirement for zinc such that an infant consuming the Reference Nutrient Intake for zinc will exceed the JECFA tolerable intake. However, JECFA tolerable intakes are intended to apply throughout an individual's life with the exclusion of the 12 week period after birth. In addition, all the relevant samples contained a concentration which was within the statutory concentration range for zinc in infant formulas.

(v) The measured concentrations of antimony, chromium, cobalt, nickel and selenium in the infant foods are all consistent with earlier surveys of foodstuffs or infant formulas. For nickel the estimated high level (97.5th percentile) intake by infants aged 6-12 months would exceed by 2% a Tolerable Daily Intake (TDI) derived by the World Health Organization. However, we note that this TDI was derived using three 10-fold uncertainty factors to accommodate the lack of adequate data.[99]

Conclusions

(vi) We *note* that the estimates of intake by infants rely either on assumptions about feeding patterns (infants aged 0-6 months) or on survey data which may now be outdated (infants aged 6–12 months). We would welcome new studies to determine the patterns of consumption of foodstuffs in infants.

(vii) However, we *consider* that the consumption of the infant foods sampled in the survey will not result in the intake of such quantities of any of the analysed elements such as would give concern for the health of infants.

Methylcyclopentadienyl Manganese Tricarbonyl

1.21 The Committee was asked to advise on the toxicological implications of the possible use of the manganese-based additive methylcyclopentadienyl manganese tricarbonyl as an alternative to lead additives in petrol. The use of the additive would lead to a 15% increase in the ambient concentrations of manganese. This compound had already been considered as an additive in diesel fuel by the Committee in 1995.[100]

1.22 When this topic was first considered members had particular concerns about: a) the possible effects of manganese in susceptible sub-populations such as young children; b) the possible effects of manganese in anaemic subjects; c) whether particle size was important in determining the exposure of individuals to manganese; d) the lack of information on the relative proportions of manganese intake that were derived from air and the diet, and e) the need for clarification of the calculations that had been used to predict the increase of ambient concentrations of manganese.

1.23 These concerns were referred back to the company who sent a reply addressing these points. The Committee agreed that, of the five points raised, two (the possible effects of manganese in anaemic subjects and whether particle size was important in determining the exposure of individuals to manganese) had not been addressed adequately. It was agreed that the increase in the ambient concentrations of manganese was unlikely to constitute a risk to health. However, members recommended that a study be undertaken to assess the exposure to manganese of groups of workers heavily exposed to vehicle emissions, both before and after the introduction of the fuel additive.

Multielement Survey of Wild Fungi and Blackberries

1.24 A group of 12 elements were analysed in samples of fungi and blackberries which had been collected in the wild ('free foods'). The Committee produced the following statement:

(i) We have considered a draft Food Surveillance Information Sheet[101] describing the results of a survey carried out for the MAFF/DH Joint Food Safety and Standards Group on the concentrations of 12 elements (arsenic, cadmium, chromium, copper, lead, manganese, mercury, nickel, platinum, tin, titanium and zinc) in free food, namely wild edible fungi and blackberries.

(ii) Wild edible fungi were collected from 34 locations and wild blackberries from 48 locations within Great Britain. Samples were collected from urban and rural areas, and for blackberries also close to and away from roads. These free foods were then analysed for total concentrations of these elements, though no attempt was made to distinguish between different chemical species of each element. For example, the reported arsenic concentration is the sum of organic and inorganic arsenic.

Results of the survey

(iii) There were wide variations in the concentrations of the elements found in the different samples of free foods that were analysed. For a number of the elements there were differences when concentrations in samples from the different locations were compared. We *note* that the results for elements that may partially originate from vehicle exhausts, *i.e.* lead (from leaded petrol) and platinum (from catalytic converters), are indicative of higher concentrations in samples from localities with a greater density of motor traffic. We also *note* that chromium and tin concentrations appeared to be higher in fungi from urban areas whereas cadmium and zinc concentrations were higher in fungi from rural areas. For blackberries, the arsenic, chromium and tin concentrations were higher in urban samples, while manganese concentrations were higher in rural samples.

(iv) We *note* that there are no legal limits for metals and other elements in free foods that are collected for personal consumption. We also *note* that the concentrations of arsenic in the samples of fungi and blackberries were all less than 1 milligram/kilogram (mg/kg), the statutory limit for commercial foods. Similarly, the concentrations of tin in the samples were all much less than the legal limit of 200 mg/kg that applies to commercial products.

(v) Five of the thirty-four samples of fungi had lead concentrations higher than the legal limit of 1 mg/kg that would apply to commercial samples of fungi. However, we *note* that total lead intake of individuals who are high level (97.5th percentile) consumers of both wild edible fungi and blackberries suggest that such individuals would not exceed the Provisional Tolerable Weekly Intake (PTWI) for lead that has been determined by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA).[102]

(vi) JECFA have also determined PTWIs for cadmium, mercury, and tin.[96,97] Provisional Maximum Tolerable Daily Intakes (PMTDI) have been established by JECFA for copper and zinc.[96] In addition, a Tolerable Daily Intake (TDI) for nickel has been established by the World Health Organization (WHO).[99] Estimates of intake of these elements show that the 97.5th percentile consumer would not exceed the appropriate tolerable intake.

(vii) Estimates of total dietary intake of arsenic suggest that a high level consumer of wild edible fungi and blackberries might exceed the JECFA PTWI.[97] However, the JECFA PTWI would be exceeded by only 8%. Furthermore, it is estimated that the intake of arsenic via the consumption of wild edible fungi and blackberries only contributes 5% to the total estimated dietary intake. As was indicated in paragraph ii, estimated intakes of arsenic from the consumption of free foods as well as from the rest of the diet are for total (*i.e.* organic and inorganic) arsenic. Inorganic arsenic is more toxic than organic arsenic and this is reflected in the JECFA PTWI, which applies to inorganic arsenic only. However, most of the arsenic in the diet is present as the less toxic organic arsenic compounds, with fish and shellfish being the major sources.

(viii) Neither JECFA nor the WHO have determined tolerable intakes of chromium, manganese, platinum or titanium. We *note* that titanium was included as an indicator of surface contamination of the samples with soil and that there are no estimated total dietary intakes for titanium with which to compare intakes from the consumption of free foods. We have been informed that the concentrations of chromium, manganese and platinum in the wild edible mushrooms and blackberries would not contribute appreciably to the total intake of those elements from dietary sources as estimated from the Total Diet Studies of 1994 and 1997.[103–105]

Conclusion

(ix) Estimated total dietary intakes of the elements cadmium, copper, lead, mercury, nickel, tin, and zinc for high level consumers of wild edible fungi and blackberries do not exceed the appropriate tolerable intake and are therefore not a health concern. Although estimated total dietary intakes of arsenic may exceed the tolerable intake, this value applies to inorganic arsenic only whereas most of the arsenic in the diet is in the less toxic organic forms. Therefore, we do not consider the concentrations of arsenic in free foods to be a cause for concern. There are no relevant tolerable intakes for the metals chromium, manganese, platinum and titanium against which to compare estimated dietary intakes. However, where the results of previous surveys are available, the concentrations of each element in wild edible fungi and blackberries would not contribute appreciably to the total dietary intake of that element.

(x) Therefore, we *conclude* that the concentrations of the elements arsenic, cadmium, chromium, copper, lead, manganese, mercury, nickel, platinum, tin, titanium and zinc in fungi and blackberries collected in the wild do not provide any cause for concern for individuals eating these foods.

Multiple Chemical Sensitivity

1.25 The Health and Safety Executive sought the advice of the COT on a review of multiple chemical sensitivity (MCS) that had been commissioned from the Institute of Occupational Medicine at Edinburgh (IOM). The Committee were also provided with a draft report by the United States Interagency Workgroup on MCS and a book chapter in press on the topic.

1.26 Members noted that the IOM review was detailed and informative and that there was a degree of overlap between the report and areas under consideration by the Committee's Food Intolerance Working Group. It was noted that MCS was a condition largely defined by the patient and that there was no consistent pattern of symptoms or exposure data to define the condition.

1.27 The Committee agreed that, on the basis of current knowledge, there was insufficient evidence to make comments on potential mechanisms or to recommend further research in this area.

Openness

1.28 New procedures for greater openness of the work of the Committee were instituted at the start of this year. They include publication of the minutes and Committee papers, subject to commercial confidentiality. These procedures, which apply to all three committees, are described elsewhere in this report.

Organophosphates

1.29 During the year the Committee's Working Group on Organophosphates prepared a draft report which was submitted to and endorsed by the Committee. The final report was published in November.[106]

1.30 Organophosphates are a group of compounds widely used in veterinary medicines (particularly sheep dips) or in pesticide products for agricultural, horticultural or public hygiene use. In addition, one compound (malathion) is used in human medicine for head lice treatment.

1.31 The conclusions in the COT report are that the evidence did not support the view that low-dose exposure to organophosphates caused clinically significant neurophysiological effects or peripheral neuropathy.

1.32 The available data indicated that any risks involved in using sheep dips arose predominantly from exposure to the concentrated formulation. Subsequently, the Veterinary Products Committee recommended that current organophosphate sheep dip products be withdrawn pending the introduction of new containers that minimised exposure.

1.33 In the case of shampoos for head lice treatment there are no concentrated products available and the diluted products are for intermittent use only. The Committee on Safety of Medicines advised that there is no need for any regulatory action to restrict use of these products.

PCDDs, PCDFs and PCBs in Marine Fish and Fish Products

1.34 The Committee reviewed the results of a surveillance exercise for the presence of 'dioxin-like' compounds in fish and fish products, the following statement was prepared:

(i) We have been informed of the results of a survey conducted by the Joint Food Safety and Standards Group of the Ministry of Agriculture, Fisheries and Food and the Department of Health. Samples of marine fish, fish fingers and fish cakes were obtained from various sources between November 1995 and January 1996. All samples were analysed for the presence of the environmental contaminants known as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs).[107]

Tolerable Daily Intake

(ii) In 1992 we endorsed a Tolerable Daily Intake (TDI) for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) of 10 picograms/kilogram body weight (pg/kg b.w.) which had been recommended by the World Health Organization (WHO) Regional Office for Europe. We recommended also that when considering mixtures of PCDDs and PCDFs the TDI could be regarded as being expressed in Toxic Equivalents of 2378-TCDD (TEQs), calculated using internationally agreed Toxic Equivalency Factors (TEFs) for various PCDD and PCDF congeners. Because of the accumulation of such compounds within the body and their long elimination half-lives, we considered that it was appropriate to regard the 10 pg/kg b.w. figure as a time-weighted average tolerable intake.[108]

(iii) In 1997, as part of a consideration of the health hazards of PCBs, we considered that the use of TEFs for certain coplanar ("dioxin-like") PCBs offered a pragmatic approach to the evaluation of these compounds and that they should be considered in combination with the PCDDs and PCDFs.[109]

(iv) Recently, we have reviewed the data used by a 1997 consultation of the WHO European Centre for Environment and Health (ECEH) to derive new Toxic Equivalency Factors (WHO-TEFs) recommended for certain PCDD, PCDF and PCB congeners and *endorse* the use of these WHO-TEFs to calculate the TEQs in analysed samples.

(v) We are aware that a recent WHO International Program on Chemical Safety/ECEH consultation has recommended that a Tolerable Daily Intake (TDI) of the PCDDs, PCDFs and PCBs, expressed as Toxic Equivalents (TEQ), is 1-4 pg TEQ/kg b.w./day.[111,112] We have not yet had the opportunity to review the data used by the consultation to derive the recently recommended WHO TDI. We will undertake such a review when a full report of the consultation is available and will consider whether any specific subgroups of the United Kingdom (UK) population might be particularly at risk. In the interim we have considered the surveillance data using both the current UK TDI and the recently recommended WHO TDI.

Evaluation of exposure

Adults

(vi) We have been provided with estimates of the intake of PCDDs, PCDFs and PCBs by individuals eating marine fish as part of their diet.[107] We note that the measured concentrations of PCDDs, PCDFs and PCBs in fish and fish products do not result in the average consumer exceeding either the current TDI or the upper value of the recently recommended WHO TDI. However, a high

level (97.5th percentile) consumer of fish in combination with the rest of the diet, might ingest 5.6 pg TEQ/kg b.w. per day, because of the contribution from oily fish (*e.g.* herring, mackerel and salmon). This is slightly greater than the newly recommended upper value of the WHO TDI.

Schoolchildren

(vii) Estimates of intakes of PCDDs, PCDFs and PCBs by 10-15 year old schoolchildren based on a study of the diets of British schoolchildren[113] suggest that the average consumer in this age group will not exceed either the current UK TDI or the upper value of the recently recommended WHO TDI. We note that the high level (97.5th percentile) consumer in this age group has an estimated total daily intake of 4.7 pg TEQs/kg b.w. per day, just exceeding the upper value of the recently recommended WHO TDI.

(viii) However, we note that the data concerning the amounts and the various types of fish and fish products consumed by this age group are limited, as the survey population contained few children who consumed certain fish species. Therefore, these estimated intakes should be viewed with caution.

Toddlers

(ix) A survey of the diet of children aged 1½-4½ has been carried out.[114] However, because the detailed records of 'toddler-specific' foods consumed by the survey participants have not yet been coded into the appropriate species of fish, there is a lack of data to provide reliable estimates of dietary intake of the PCDDs, PCDFs and PCBs by children of this age range. We will review this matter when such data are available. Instead, we have been informed of the results of theoretical calculations made by scaling the consumption of fish and fish products by adults by the ratio of the caloric intakes of toddlers and adults so as to provide an estimate of the intake of PCDDs, PCDFs and PCBs from fish and the rest of the diet by the toddler age group.[107] We note that these estimates do not allow for possible differences in the proportion of fish in the diets of toddlers and adults nor for possible differences in the types of fish consumed by toddlers as compared to adults.

(x) Calculations using this method suggest that the average intake of a toddler would exceed the upper value of the recently recommended WHO TDI. The total intakes of PCDDs, PCDFs and PCBs are estimated as ranging from 5.5-6.5 pg TEQ/kg b.w. per day, depending on age. A toddler who was consuming at the 97.5th percentile level would have total intakes of PCDDs, PCDFs and PCBs estimated as being in the range of 8.3-10 pg TEQ/kg b.w. per day. As mentioned above, we consider that these estimates may be unreliable.

Environmental factors

(xi) We have previously noted[109] that dietary exposure to PCBs in the UK has declined, as evidenced by a decrease in the concentrations of PCBs between Total Diet Study samples collected in 1982 and those collected in 1992. This is compatible with reductions in the concentrations of PCDDs and PCDFs in samples of human milk collected in developed countries between 1988 and 1993.[115] We anticipate that the measures already taken in the UK to reduce emissions to the environment will result in a continued decline. We note that the WHO-IPCS/ECEH consultation recommended that continued efforts should be made to reduce exposure towards the lower end of the range of the TDI that it had determined. This recommendation was made because of the consultation's recognition "that subtle effects might already be occurring in the general population in developed countries".

Nutritional aspects

(xii) We have taken into account the advice of the Department of Health's Committee on Medical Aspects of Food Policy (COMA) that there should be an increase in the consumption of oily fish to an average of one portion per person per week. This recommendation was made on the basis of evidence that increasing the intake of certain long chain polyunsaturated fatty acids (for which oily fish are a rich source) reduces the risk of cardiovascular disease.[116] Adherence to this advice is likely to provide health benefits for most adults without exceeding the recently recommended WHO TDI and therefore we do not discourage the consumption of oily fish because of their content of the PCDDs, PCDFs and PCBs.

Conclusions

(xiii)

a) We *endorse* the use of WHO-TEFs for PCDDs, PCDFs and PCBs in the calculation of the TEQs for these compounds in analysed samples.

b) We will be undertaking a full review of the data considered by the WHO-IPCS/ECEH consultation in determining its recently recommended TDI.

c) We *welcome* the evidence that the average UK adult consumer will not exceed either the current UK TDI or the upper level of the recently recommended WHO TDI for PCDDs, PCDFs and PCBs.

d) We *note* that a high level adult consumer of oily fish will have an intake of PCDDs, PCDFs and PCBs which falls between the current UK TDI and the newly recommended WHO TDI. We note, however, that such a consumer will be eating more fish than is required to meet the recommendation made by COMA on health grounds.

e) We *note* that the estimated intakes of PCDDs, PCDFs and PCBs by schoolchildren and toddlers do not exceed the current UK TDI but that it is possible that some children, in particular toddlers, may exceed the newly recommended WHO TDI. We also *note* that there are assumptions made in deriving the estimated exposures which mean that they should be viewed with caution. However, we *reiterate* our view that further monitoring of concentrations of PCDDs, PCDFs and PCBs in components of the UK diet should be undertaken to confirm that the trend to lower values continues.

f) In order better to assess the implications of the intake of PCDDs, PCDFs and PCBs for the health of children, we would wish to review the data when additional information on the types and quantities of fish consumed by this age group is available.

g) We *recommend* that adults adhere to the COMA advice to eat one portion of oily fish each week as this will confer health benefits and yet will not result in the majority of individuals exceeding either the recently recommended WHO TDI or the current UK TDI for PCDDs, PCDFs and PCBs.

Phytoestrogens

1.35 The Committee reviewed the toxicity of phytoestrogens in 1996 as part of a consideration of a MAFF report on Inherent Natural Toxicants in Food.[117] At that time the Committee had noted that phytoestrogens could cause adverse effects in animals and had concluded that young children who consumed soya-based infant formulas could be exposed to concentrations of phytoestrogens that might elicit biological effects. The Committee had recommended that research should be undertaken to determine whether the ingestion of soya-based infant formulas carried any risk for infants.

1.36 A extensive programme of research had been started and members were informed that some of the results of the programme were being reported at an international meeting. Since a considerable quantity of data would be available within the next year it was agreed that this could best be reviewed in a Working Group established by the Committee.

Potatoes Genetically Modified to Produce *Galanthus nivalis* Lectin

1.37 The Advisory Committee on Novel Foods and Processes sought the advice of the COT on toxicological aspects of studies involving genetically modified potatoes as components of the diet of rats. The Committee prepared the following statement:

(i) The Committee was asked to provide advice to the Advisory Committee on Novel Foods and Processes (ACNFP) on the toxicological aspects of certain studies which had been carried out at the Rowett Research Institute. These unpublished studies involved the administration to rats of various potato-containing diets. The diets contained potatoes either with or without the addition of non-potato lectins (the snowdrop, *Galanthus nivalis*, lectin or the jack bean lectin, Concanavalin A) or potatoes which had been genetically modified to produce the snowdrop lectin.

(ii) These studies had received considerable publicity following statements about adverse effects on the rats made on a television programme. Accordingly, the Secretariat of the ACNFP had sought to obtain copies of detailed scientific reports relating to these studies. However, it had only been able to obtain certain documents that were already in the public domain and a manuscript submitted for publication by Dr Stanley Ewen and Dr Arpad Pusztai. The documents that were considered by the Committee on Toxicity are listed below.

(iii) The Committee was particularly asked to consider the significance of:

- the effects reported on the body and organ weights of the rats,
- the results of the lymphocyte proliferation assay, and
- the histological changes reported in the gastrointestinal tract.

(iv) Dr Pusztai and Dr Ewen were invited to attend the meeting of the Committee. Only Dr Ewen was able to be present. He made a presentation to the Committee on the histopathology of the gastrointestinal tract of rats from one of the studies on the genetically modified potatoes. He also answered questions from Committee members during the subsequent discussions.

The Committee's Discussions

(v) In the course of their consideration the Committee indicated that certain important information about the studies was not provided in the documentation available. Although Dr Ewen was able to provide additional details on the histopathology he was not able to provide answers to questions relating to the design of the studies or matters relating to the body and organ weight changes and the lymphocyte proliferation assay.

(vi) In response to a specific question, the Committee was informed by the ACNFP Secretariat that it was understood that the genetically modified lines of potatoes were not intended for release on the market and that an application for such a release had not been made to the ACNFP.

Body and organ weight changes

(vii) The Committee recognised that an exact knowledge of the composition of the diet and the use of appropriate statistical methods were crucial to the interpretation of the changes in body and organ weights that had been recorded in the studies. The known adverse effects on the health of laboratory rats of raw potato starch in the diet were pointed out. In addition, the importance of minor changes in composition and palatability of diets in determining the body weight in the rat was stressed. Although it was clear that attempts had been made to ensure that the animals had received an adequate diet, there was a lack of critical information that would allow the Committee to satisfy themselves that this had been achieved. In relation to this, concern was expressed about the marked differences in composition of the genetically modified potatoes used in some experiments in comparison with control potatoes, which could have had physiological and metabolic effects on the rats irrespective of the genetic modification.

Lymphocyte proliferation assay

(viii) The variability of the results from this assay has been described in the report of the Audit committee of the Rowett Research Institute.[118] The Committee noted that this variability meant that large numerical differences would not necessarily indicate a statistically significant effect and that it was necessary to relate any changes in the immune system to those occurring in other organs of the animals.

Histopathology

(ix) Dr Ewen made a presentation of the histopathology of the gastro-intestinal tract. In this he showed slides recording changes to the thickness of the mucosa in the stomach and sections of the small intestine occurring after feeding of diets containing genetically modified potato. A common feature was elongation of the crypt and villi of the jejunum and ileum. The Committee enquired as to whether measures that would discriminate between hypertrophy and hyperplasia had been made. They were informed that this had not yet been done. The histopathology related only to the gastrointestinal tract of the rats from one of the studies,[119] it was pointed out that it would be usual for a toxicology study to have incorporated a full examination of all the major organs. The Committee was of the view that this would have provided an opportunity for seeking an explanation of any changes to the immune system or of organ weights.

Conclusions

(x) On the basis of the information made available, the Committee expressed concern about the design of the studies, e.g. the limited numbers of animals used and the adequacy of the diets. It appeared that a limited set of studies had been used to address many questions relating to nutritional, toxicological and immunological matters. The design of the studies may not have been adequate for this purpose.

(xi) It was agreed that the studies could be used to provide an indication of the investigations and procedures that would be needed in any future work in this area. However, these studies could not be used, by themselves, for defining the effects on the rat of potatoes containing the transgene for the *Galanthus nivalis* lectin.

Documents considered by the Committee

Anon. (1999). The effects of raw and cooked transgenic (GNA-expressing) potatoes on metabolism of rats were evaluated in a 10 day feeding trial [D227], pp 6.[119]

Bourne FJ, Chesson A, Davies H and Flint H (1998). SOAEFD flexible fund project RO 818: Audit of data produced at the Rowett Research Institute (Date of audit: 21st August 1998).[118]

Bourne FJ, Chesson A, Davies H and Flint H (1999). The Audit Committee's Response to Dr Arpad Pusztai's Alternative Report of 22 October 1998. 16 February 1999.[120]

Ewen SWB and Pusztai A (1999). Diets containing genetically modified (GM) potatoes expressing Galanthus nivalis (GNA) lectin are associated with proliferation of the mucosal cells of the rat gut, unpublished manuscript.[121]

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Short and Long Chain Triacyl Glycerol Molecules (Salatrims)

1.38 In 1997 the Committee had commented, at the request of the Advisory Committee on Novel Foods and Processes (ACNFP), on the toxicological aspects of a voluntary submission of the Salatrims to the ACNFP.[125] Since that date the European Commission's Regulation on Novel Foods and Novel Food Ingredients has come into force and, in 1999, the company submitted the product to the United Kingdom Competent Authority for consideration under these regulations. The company had addressed matters of concern to the Committee that had been identified in the 1997 COT statement and the additional data was considered by the COT. The Committee prepared the following statement:

(i) In 1997 we issued a statement[125] on specific aspects of a submission made to the Advisory Committee for Novel Foods and Processes (ACNFP) on Salatrims, a family of low calorie fat materials. We had been asked by ACNFP to comment on the toxicological aspects of the data provided at that time under the voluntary system that existed for the evaluation of novel foods.

(ii) We concluded that there were three areas of concern that needed to be addressed further, namely:

(a) additional pharmacokinetic studies were required to evaluate blood levels of short chain fatty acids in volunteers following consumption of individual Salatrim products before any conclusions could be drawn regarding the teratogenic risk of Salatrims;

(b) a No Observable Adverse Effect Level (NOAEL) should be determined for effects of Salatrims on enzyme markers for liver dysfunction in humans; and

(c) a NOAEL should be determined for effects of Salatrims on gastrointestinal function in humans.

(iii) The EC Regulation on Novel Foods and Novel Food Ingredients was introduced in May 1997, after the initial consideration of Salatrims in 1995-1997. As Salatrims were not on the market in Europe at that time, they are regarded as novel foods and therefore they require clearance under the Regulation before they can be sold in Europe. An application has now been made to the UK Competent Authority for such clearance[126] and ACNFP has asked for our further advice on the additional information and analysis included in the submission to meet the concerns that we identified earlier.

(iv) Our first statement described the toxicological and clinical data available on Salatrims in 1997. This statement, which needs to be read in conjunction with our previous opinion, discusses the additional data and analyses provided in the submission made under the Novel Food Regulation in response to the concerns that we had identified.

Levels of short chain fatty acids and possible teratogenic risk

(v) The Committee had previously expressed a concern that the release of butyric acid from Salatrim which is rich in this short chain fatty acid might be sufficient to cause a rise in plasma butyrate levels. This possibility needed to be examined in the light of *in vitro* data on possible teratogenic effects of high levels of butyric acid. A new study has been conducted[127] in which the post-prandial response to a load of 30 grams (g) of butyrate-based Salatrim (known as 4SO) was investigated to determine, amongst other effects, whether free butyrate would reach the general circulation. Plasma was analysed for butyric acid at regular intervals up to 360 minutes after dosing and at no time could free butyric acid be detected.

(vi) The Committee was satisfied by these data.

Effects on enzyme markers for liver dysfunction in humans

(vii) The Committee had asked that further information be provided to enable a NOAEL to be set for the effects of Salatrims on enzyme markers of liver dysfunction seen previously in the free-living clinical study. The company has provided further statistical analyses of the serum enzyme data from this study and expert assessments of the implications of those statistical analyses.

(viii) Small, statistically significant increases were recorded in the mean activities of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) at the beginning of the exposure period in the free-living clinical study, although the mean values for the treatment groups did not fall outside the normal ranges for these enzymes at the laboratory conducting the study. The Committee noted that there was some concordance in the increases in liver enzymes AST and ALT within individuals and that some temporal trends were apparent. The Committee had also noted

that it was possible that the individuals who withdrew from the study may have been atypical and that this could have contributed to the variability of the data. However, the company submitted information that shows that those individuals who withdrew from the study, did so mainly because of adverse gastro-intestinal effects resulting from consumption of 60 g/day Salatrim. In addition, further analysis of the enzyme activities for those withdrawing from the study in comparison to those remaining shows that there were no significant differences in changes above baseline for serum hepatic enzyme activities whether or not those subjects withdrawing from the study were included.

(ix) In addition, data from a further study conducted by Nestel and colleagues[128] were provided. In this five-week crossover study, following a low-fat control phase, subjects consumed diets containing margarine rich either in Salatrim or in palm oil ('palmitate'). These subjects were selected on the basis of their having an elevated blood cholesterol level and the study was designed primarily to provide information on effects of Salatrim on blood lipid levels. However, additional analyses of serum enzymes from blood samples taken pre-dose and at the end of each dietary phase showed that mean activities of AST and ALT, and other indices of hepatic function (g-glutamyl transferase, alkaline phosphatase and lactic dehydrogenase) were no different at the end of the Salatrim and 'palmitate' dietary phases. Small increases in serum enzymes were noted after the 'palmitate' dietary phase in some subjects, which were of a similar magnitude to those seen in some subjects consuming Salatrim in the free-living clinical study described above.

(x) The Committee concluded that consumption of high doses of Salatrim did result in slight increases in AST and ALT activities in serum, but noted that these increases were within the normal reference range. The further analyses of the enzyme data satisfied the Committee that there were no differences in the trends seen whether those withdrawing from the study were included or not. These enzyme changes, in the absence of any other indications of liver damage, were not considered to represent a clear toxic effect. The data from the five-week crossover study[128] provided reassurance of a lack of any clear adverse hepatic effect. However, the Committee noted that the reported studies did not include investigations in children or in those with pre-existing liver disease.

Effects on gastrointestinal function in humans

(xi) The Committee had noted previously that gastrointestinal effects had been recorded in a number of subjects consuming Salatrim and had asked that further information be provided to enable a NOAEL to be determined. A further analysis of these observations was submitted by the company. The company suggested that the gastrointestinal disturbances reported were the consequence of sudden changes from an absorbable diet to one containing significant amounts of unabsorbable material. Metabolism of Salatrims rich in short chain fatty acids might result in rapid release of cholecystokinin, which would lead to slow gastric emptying, nausea and bloating. Analysis of the reasons for subjects withdrawing from the study shows that adverse gastrointestinal symptoms (such as nausea, cramps and gas) were the predominant reason for withdrawal from the study in the 60 g/day Salatrim group. However, there was no clear evidence of any increase in the incidence of such effects at intakes of 30 or 45 g/day Salatrim.

(xii) The Committee considered that the gastrointestinal disturbances seen after consumption of high doses of Salatrim could be the result of individual intolerances, noting the considerable variation between individuals and their tolerance to fibre and other poorly digested substrates. Nevertheless, these effects were seen in a significant proportion of individuals after consumption of 60 g/day Salatrim and needed to be considered in the context of estimates for adults of a mean intake of 11 g Salatrim/day and of a 97.5th percentile intake of 33g Salatrim/day. Salatrims are intended for use

in reduced calorie foods aimed at individuals choosing a diet for the control of weight.[126] The main target consumers would be adults over 16 years of age and the intake estimates therefore were derived only for adults using commercially available databases on UK food consumption in conjunction with information on the types of food in which Salatrim would be used and the likely levels of inclusion. The application under the Novel Food Regulation is restricted to use in confectionery and baked goods only.

(xiii) The Committee was of the view that the gastrointestinal effects seen were likely to be linked to intolerances to large amounts of this material rather than any specific toxic effect. However, gastrointestinal effects might be more common in children because of their relatively higher nutrient requirement and dietary intake. The Committee further concluded that it was not possible to set any no effect level on the basis of such subjective end points.

Conclusions

(xiv) On the basis of the new data and analyses now provided, the Committee concluded that:

(a) consumption of Salatrim did not result in any elevation in plasma butyrate levels and thus did not pose any teratogenic risk;

(b) consumption of high doses of Salatrim did result in slight increases in AST and ALT activities in serum, although these increases were within the normal reference range. There were no differences in the trends seen whether those dropping out of the study were included or not. These changes in enzyme activities, in the absence of any other indications of liver damage, were not considered to represent a clear toxic effect. The data from the five-week crossover study in humans provided an additional reassurance of the lack of any clear adverse effect;

(c) the gastrointestinal effects reported were likely to be linked to individual intolerances to large amounts of this material rather than to any adverse toxic effect. Furthermore, it is not possible to set any no effect level on the basis of such subjective end points.

(xv) The Committee noted that neither children nor those with pre-existing liver disease were included in the trial groups. However, the Committee understands that products containing Salatrim would be aimed at adults choosing a diet for the control of weight.

1999 Membership of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

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Professor H F Woods BSc BM BCh DPhil FFPM FIFST HonFFOM FRCP(Lon & Edin)
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Declaration of interests during the period of this report

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Professor H F Woods (Chairman)	Halifax Bank	Shares	University of Sheffield, Faculty of Medicine Wide range of national & international food & chemical companies.	University of Sheffield, Faculty of Medicine. Has extensive activity in teaching and research in nutrition and toxicology and in topics related to and supported by many companies in the food and chemical industry. Trustee of Hallamshire Therapeutic Research Trust Ltd, Harry Bottom Charitable Trust and Special Trustee for the former United Sheffield Hospitals.
Professor P J Aggett	Nestec Wyeth Borax	Ad hoc Consultancy Lecture and Chairing Meetings	Nestec FDF Abbot Unilever Meat and Livestock Commission	Departmental commissioned research and student placements
Professor N A Brown	Merck Glaxo Wellcome Searle Styrene Information Research Centre Du Pont	Consultancy Consultancy Consultancy Consultancy Consultancy	EC (DGXI and DGXII) Glaxo Wellcome US EPA	Research Support Research Support Research Support
Dr P Carthew	Provalis Unilever Cambridge Antibody Technology	Share Holder Share Holder Consultancy	NONE	NONE
Professor J K Chipman	Boots Healthcare International Quintiles	Consultancy Consultancy	Astra-Zeneca Glaxo-Wellcome Water Research Centre SmithKline & Beecham ICI	Research Support Research Support Research Support Research Support Research Support
Dr M Joffe	Ilzro	Research grant	NONE	NONE
Dr I Kimber	British Airways British Petroleum-Amoco ICI Halifax AstraZeneca AstraZeneca	Share Holder Share Holder Share Holder Share Holder Share Holder Employee	Unilever plc	Grant for Research
Professor A G Renwick	International Sweeteners Association	Consultant	Hoffmann-La Roche Unilever SmithKline Beecham Pfizer Flavor and Extract Manufacturers Association (FEMA)	Research Support Research Support Research Support Research Support Research Support

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Professor P A Routledge	Health Care Services Edinburgh	Fee	Paracetamol Information Centre	Member of Advisory Group
Professor I R Rowland	Colloids Naturels International (CNI) Rouen, France Danisco	Consultancy Consultancy	Various	Departmental teaching & research funded by various
Dr L Rushton	Institute of Petroleum Transport and General Workers Union	Consultancy, contracts and grants – completed Consultancy – completed	Concawe Eu	Contract to Institute for Environment and Health – Now completed Contract to Institute for Environment and Health
Ms J Salfield	Alliance & Leicester Halifax Woolwich Northern Rock	Shares Shares Shares Shares	NONE	NONE
Dr A Smith	Abbey National British Telecom Halifax Bank	Share Holder Share Holder Share Holder	Rhône Poulenc Glaxo-Wellcome	Research Support Research Support
Professor S Strobel	NONE	NONE	NONE	NONE
Dr A Thomas	NONE	NONE	NONE	NONE
Professor J A Timbrell	Shook, Hardy & Bacon (Law firm) Sorex Ltd	Occasional Fee Occasional Fee	Glaxo Wellcome Taisho Pharmaceutical Co	Research Support Research Support
Dr M Tucker	Zeneca	Pension	NONE	NONE

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

Preface

The Committee on Mutagenicity (COM) has two major functions:

a) To assess information on the mutagenic activities of chemicals present in food, consumer products and the environment and to advise on their hazards and risk to humans,

b) to advise on the general principles of test methods which are in use or newly developed, which are capable of assessing the mutagenic activity of chemicals.

This advice is provided to Government Departments and Regulatory Agencies.

The COM is made up of independent scientists and observers from Government Departments supported by a secretariat from the Department of Health. The secretariat provides the COM with comprehensive data packages upon which the Committee members use their expertise to evaluate both specific chemicals and the effectiveness of test methodologies used.

During the year, the COM provided advice on the environmental contaminant 2, 3, 7, 8 – tetrachloro-dibenzo-*p*-dioxin, (TCDD) and 3-monochloropropane 1,2-diol (3-MCPD) which may occur as a contaminant in flocculants used during water treatment. The COM also provided advice on the air pollutant ozone and malachite green and leucomalachite green which are used to control parasitic and fungal infections in fish and shell fish. The Committee's advice on 2-chlorobenzylidene malonotrile (CS) and CS Spray was published as part of a joint COT/COC/COM statement. The Committee has undertaken a major revision and updating of their 1989 advice on a strategy for evaluating chemicals for mutagenicity, this resulted in an additional meeting to specially progress this work.

Regarding test methodology the Committee considered the role of certain specific mutations in tumours as markers for chemical induced mutagenicity. Major changes in Committee procedures during the year to increase transparency of operations has lead to the publication of agendas prior to meetings and minutes and statements when completed. Thus, the conclusions reached by the Committee given in this Annual Report are already available on the COM web site (http://www.doh.gov.uk/com.htm). Members were also pleased to welcome a Lay Member to the Committee during this year.

Professor J M Parry (Chairman)
BSc PhD DSc

Malachite Green

2.1 Malachite green is a cationic triphenylmethane dyestuff used in a number of industries, including fish farming. It is used in freshwater fisheries for the treatment of external fungal and other infections, its main use being to stop fungal growth on the eggs. The COT have asked for advice on the mutagenicity of malachite green and the lipophilic metabolite leucomalachite green. In particular the COT has asked for advice on results from recent National Toxicology Programme (NTP) ^{32}P-post-labelling studies (sub-acute) carried out with range-finding studies prior to initiating carcinogenicity bioassays on malachite green and leucomalachite green in rats and mice.

2.2 The Committee commented on the paucity of the available mutagenicity data, particularly in respect of leucomalachite green. In many instances lack of information on the purity of the malachite green tested is a particular problem in assessing the adequacy of the available information. Members agreed that the structure of both malachite green and leucomalachite green contained groupings which provide structural alerts for potential mutagenicity and thus it was important to evaluate the potential mutagenicity of both malachite green and its lipophilic metabolite leucomalachite green. Members also noted the results from the 1997 surveillance investigations conducted for the Veterinary Medicines Directorate which, for the first time, present information on residues of leucomalachite green and suggest that, when found, residues of this chemical in fish were higher than those of malachite green. The COM conclusions were forwarded to the COT and have been incorporated into a statement published by that Committee. They are given below:

2.3 Malachite green

(i) There is one report of a *Salmonella* assay done to an acceptable protocol; malachite green oxalate (>90% pure) was shown to induce mutations in TA98 in the presence of an exogenous metabolic activation system.[129] There is also some evidence of clastogenicity in Chinese Hamster Lung cells.[130] Malachite green should thus be regarded as having mutagenic potential.

(ii) Negative results were obtained in a poorly reported bone marrow micronucleus test in mice using a single oral dose of malachite green oxalate (>90% pure) at the MTD (75% of the LD50).[129] Members agreed that the high dose level of 37.5 mg/kg was adequate, but it was difficult to assess the value of the negative results in the absence of appropriate information on bone marrow toxicity or data to show that malachite green and/or metabolites reached the bone marrow.

(iii) Recent ^{32}P-post-labelling studies using a 28 day dietary exposure and carried out with NTP range-finding studies (used in designing carcinogenicity bioassays) indicate that malachite green induces DNA adducts in the liver of rats and mice.[131,132]

(iv) The Committee concluded that although a limited negative *in-vivo* micronucleus test was available, the results of the recently conducted ^{32}P- post-labelling studies indicated that it would be prudent to assume that malachite green may be a potential *in-vivo* mutagen.

2.4 Leucomalachite green

(i) The Committee were concerned that only limited information was available on leucomalachite green. The lack of information on leucomalachite green prevents an adequate mutagenicity assessment for this compound.

(ii) The Committee recommended that *in-vitro* studies in bacteria for gene mutations, in mammalian cells for clastogenicity and a mammalian cell assay for gene mutation (preferably the mouse lymphoma assay) conducted according to current OECD guidelines should be undertaken as an important step to evaluating the mutagenic potential of leucomalachite green.

(iii) The Committee, however, concluded that the results of the recently conducted ^{32}P-post-labelling studies[131, 132] indicated that it would be prudent to assume that leucomalachite green may also be a potential *in-vivo* mutagen.

MCPD (3-Monochloropropane 1,2-Diol)

2.5 3-Monochloropropane 1,2,-diol (3-MCPD) can be present as a contaminant in epichlorhydrin/amine copolymers used as flocculants or coagulent aids in water treatment. These polyamine flocculants have been available for many years as approved products for use in water treatment and thus 3-MCPD may be present in drinking water due to the use of such flocculants. 3-MCPD is a member of a group of contaminants known as chloropropanols. This group includes some known genotoxic carcinogens in animals such as 1,3 dichloropropan-2-ol. The COM was asked to evaluate the available mutagenicity data on 3-MCPD and to provide conclusions for the Committee on Carcinogenicity (COC) who had been asked to consider the carcinogenicity data on 3-MCPD.

2.6 The Committee was aware that 3-MCPD had been detected as a contaminant of savoury food ingredients, including acid hydrolysed vegetable protein (acid-HVP) and that the EU Scientific Committee for Food had published an opinion in 1994 where it was agreed that 3-MCPD should be regarded as a genotoxic carcinogen.[133] The Committee also had access to published mutagenicity data on 3-MCPD, a safety evaluation prepared by CanTox.Inc (Ontario, Canada) for the International Hydrolysed Protein Council,[134] a review document published by the Institute of Toxicology, National Food Agency of Denmark,[135] and one *in-vivo* mutagenicity study submitted on an in-confidence basis.[136] In reviewing these documents, members commented that the available metabolism data on 3-MCPD were relatively old and focused on metabolic pathways following intraperitoneal administration. There was no data from oral studies using modern methods to measure absorption and elimination of 3-MCPD. The Committee considered the proposal by CanTox Inc regarding the formation of bacterial-specific mutagens and agreed that there was no evidence to support this speculation.

2.7 The Committee reached the following conclusions on the available mutagenicity data.

(i) 3-MCPD was mutagenic in *Salmonella typhimurium* in the absence of exogenous metabolic activation.[137,138,139,140] The addition of S-9 mix did not increase the mutagenic response observed.

(ii) Positive results have also been reported in the mouse lymphoma assay in the presence of metabolic activation but the full report of the study was not available to the Committee. Positive results were also reported in tests in *yeast (Schizosaccharomyces pombe)*[141] and in tests for Sister Chromatid Exchange in mammalian cells.[135]

(iii) The Committee concluded that 3-MCPD had mutagenic activity *in-vitro*.

(iv) Negative results have been reported from a bone marrow micronucleus assay in mice using a single oral dose of up to120 mg/kg bw and sampling of bone marrow at 24, 48 or 72 hours post administration. The authors stated that higher doses would result in significant weight loss and mortality. The Committee noted that there was no evidence for a reduction in the ratio of polychromatic to normochromatic erythrocytes (i.e. ratio of PCE/NCE) and thus there was no evidence to show that exposure of the bone marrow to the test material and its metabolites had occurred.[136]

(v) The Committee agreed that no conclusions could be drawn from the investigation of colonic micronuclei in mice[136] in view of the limited database available for this assay or from the inadequately reported dominant lethal assays.[142,143]

(vi) The Committee agreed that further negative results in an *in-vivo* mutagenicity test in a second tissue namely rat liver UDS were required in order to provide adequate reassurance that the activity seen *in-vitro* is not expressed *in-vivo*.

(vii) The Committee concluded that it would be prudent to assume that 3-MCPD is an *in-vivo* mutagen.

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin)

2.8 There are 75 possible chlorinated polychlorinated-para-dioxins (PCDDs) and 135 possible polychlorinated-para-dibenzofurans (PCDFs). A number of these substances (congeners), predominantly the tetra- through to octa-congeners may be found as trace contaminants in food. The toxicological properties and potencies of PCDDs and PCDFs mixtures have been estimated from the available information on TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) through the use of toxic equivalency factors (TEFs). TCDD thus serves as a model compound for the risk assessment of other PCDD/PCDF mixtures.[144]

2.9 PCDDs are produced as a trace contaminant during the manufacture of many organochlorine compounds such as 2,4,5-trichlorophenol (TCP), chlorophenoxy herbicides (2,4,5-T), hexachlorophene, chlorodiphenyl ether herbicides, and during certain industrial processes such as pulp bleaching using chlorine gas. They are also produced in a number of thermal reactions such as the incineration of municipal waste, sewage sludge, PVC as well as from automobile emissions. The highest exposures have predominantly occurred under occupational settings, often following accidental release, the exception being the large widespread release of TCDD to the environment with population exposure following the Seveso incident in 1976.

2.10 The International Agency for Research on Cancer (IARC) concluded in 1997 that TCDD should be regarded as a known human carcinogen and thus classified as Group 1.[145] Previously it had been classified as Group 2A (probable human carcinogen). This change prompted the Department of Health to request COC to review their earlier conclusion. The COC concluded in 1998 that there were insufficient epidemiological and toxicological data on TCDD to conclude a causal link with cancer in humans, but it would be prudent to consider TCDD as a 'probable weak human carcinogen'. The IARC working group concluded that the "experimental data indicated that 2,3,7,8-TCDD and probably other PCDDs and PCDFs are not direct-acting genotoxic agents and that TCDD is considered a non-genotoxic substance." The previous COM evaluation of TCDD was completed in 1987 and it was therefore timely for the Committee to reconsider its previous conclusions. The Committee considered a review of the relevant literature published since 1990 and noted that most of the newer studies had been negative.

2.11 The Committee agreed that negative results had been obtained in the *in-vitro* mutagenicity tests conducted in *Salmonella* and in L5178Y tk+/tk- cells mouse lymphoma cells.[146,147] However, TCDD induced micronuclei formation had been reported in one study in human lymphocytes and the cytochalasin B technique.[148] The same research group had also noted sister chromatid exchange (SCE) in a subsequent publication.[149] Members commented that it was not possible to draw any conclusions based on these results particularly in view of the unusually long incubation period of 71 hours prior to harvesting the cells. The Committee agreed that a repeat test would be desirable in order to validate the method used in these investigations.

2.12 The Committee agreed that negative results had been obtained in mouse hepatocytes following dosing of animals with up to 150 µg/kg (i.p).[150] No increase in SCEs in peripheral blood lymphocytes was noted in Rhesus monkeys, 2 years post administration of a diet containing 25 ppt TCDD for 4 years.[151] However, a small, but statistically significant increase in SCEs in peripheral blood lymphocytes had been documented in a limited study in rats given weekly gavage doses of 5 µg/kg for 2 weeks but not at 0.5 µg/kg.[152] Evidence of TCDD induced single strand DNA breaks in peritoneal lavage cells had been documented in rats given a single oral dose of 25 µg/kg bw up to 100 µg/kg.[153] In addition, a positive result had been reported in a deletion recombination spot test[154] but not in a separate study which used a similar dosing regime.[155] Members considered that no weight could be attached to this investigation in view of the limited study design, the concerns previously expressed by Members regarding these tests and the negative findings reported in a mouse spot test by a separate research group.

2.13 The Committee agreed the following overall conclusions.

(a) "In studies published since 1987, TCDD has continued to give largely negative results in tests for several different genetic endpoints, such as DNA damage, gene mutations, sister chromatid exchange and cell transformation. In a few studies TCDD has given a positive or equivocal result, often using assays with either highly non-standard or sub-optimal design. Currently the weight of the available experimental data continue to indicate that TCDD is not a genotoxic agent."

(b) The Committee wished to continue to monitor any further genotoxicity publications on TCDD.

Ozone

2.14 Ozone is an ubiquitous air pollutant due to its formation in the atmosphere by photochemical reaction between volatile hydrocarbons and nitrogen oxides. The Expert Panel on Air Quality Standards have recommended a very low standard (50 ppb as a running 8 hour average) based on its irritant effects on the respiratory tract at 100 ppb and above. Ozone is also one of a limited number of chemicals that have been shown to be carcinogenic in the mouse (producing lung adenomas/carcinomas in a strain sensitive to such tumour induction) but not the rat. The COC have reviewed the need for carcinogenicity bioassay data in the mouse, and as part of this work are considering the public health significance of such apparent mouse specific carcinogens. A key aspect of these is consideration of the genotoxic potential of such compounds. The COC felt it important to draw conclusions on the possibility of a genotoxic mechanism, particularly in the light of recent data on K-*ras* mutations in lung neoplasms from ozone exposed mice[156]; the advice of the COM was thus requested on this issue.

2.15 The following conclusions were drawn:

(i) Ozone is a powerful oxidising agent and reacts readily with most biological macromolecules including DNA.[157,158,159,160,161,162,163,164,165,166] It has been shown to produce gene mutation in *Salmonella typhimurium* TA102 in the presence or absence of rat S-9.[167,168,169] There are no data from *in-vitro* studies to adequately investigate its clastogenic potential[170] but it has been shown to induce SCEs in mammalian cells.[171,172,173] The available *in-vitro* data indicate that ozone has mutagenic potential.

(ii) The *in-vivo* clastogenicity of ozone in bone marrow or peripheral blood has been investigated in the Chinese hamsters,[174,175,176] mouse[174,175] and rat.[177] Although all these studies have limitations, the results do suggest that ozone is not clastogenic in bone marrow. Only one very limited study has been carried out in germ cells;[176] this was negative, which is consistent with the bone marrow data. However, clastogenic effects have been demonstrated in lung cells (macrophages) of rats exposed to ozone gas.[178]

(iii) In addition, examination of the K-*ras* mutational spectra of ozone induced lung neoplasms in mice indicated a high frequency of mutations, including A-T transversions in codon 61.[156] These appeared to be specific to ozone and did not occur in spontaneous lung neoplasms in mice. Thus, there is some evidence for *in-vivo* mutagenic activity at the target site for carcinogenicity in the mouse.

(iv) Members recommended that additional work to reproduce ozone induced A-T transversions that would provide additional reassurance regarding conclusion (iii). This would comprise an assessment of whether ozone induced mutational signatures *in-vitro* and analysis of lung tumours in mice exposed to an inert particle such as titanium dioxide.

(v) The COM concluded that it would be prudent to assume that ozone may have *in-vivo* genotoxic potential. Members agreed that it would be acceptable for the Committee to adopt the approach normally used for *in-vivo* mutagens, ie to assume a linear dose-response.

2-Chlorobenzylidene malononitrile (CJ) and CS spray

2.16 The COM provided advice on the mutagenicity of 2-chlorobenzylidene malononitrile during 1998 which was incorporated into a joint statement published by the Committees on Toxicity (COT), Mutagenicity (COM) and Carcinogenicity (COC) during 1999.

2.17 The conclusions reached by the COM (which should be considered together with those of the COC) are given below:

In vitro studies

2.18 The mutagenicity of CS has been extensively studied *in vitro*. Negative results were obtained in *Salmonella* assays, but there were reservations regarding the suitability of the standard protocols used in these tests with respect to CS in view of its very short half life.[179,180,181,182,183] Positive results were noted in assays in V79 cells for gene mutation and also in the mouse lymphoma assay.[180,184,185] Positive results were documented also in metaphase analysis for clastogenicity in V79 and CHO cells.[180,186] In addition, CS has been shown to induce SCEs (Sister Chromatid Exchanges) in CHO cells.[180] These data indicate that CS has clastogenic potential.

2.19 There is evidence from *in vitro* studies to indicate that CS has aneugenic effects. It has been shown to interfere with the spindle machinery and cell division in mammalian cells resulting in C-mitosis and metaphase block.[187,188,189,190,191,192] CS has also been shown to induce micronuclei in mammalian cells *in-vitro*.[25] These data suggest that CS has aneugenic potential.

2.20 The clastogenic effects seen appear to be due to CS itself, or an unknown short-lived intermediate.[186] The mechanism of aneugenicity appears to differ from the clastogenicity with 2-chlorobenzaldehyde being the important metabolite regarding aneugenicity but not in respect of clastogenicity.[189]

In vivo studies

2.21 Negative results were consistently obtained in bone marrow or peripheral blood assays for micronuclei induction using high dose levels and both the oral and intraperitoneal routes.[183,193] (These assays are capable of detecting clastogens and aneugens if the active metabolite reaches the bone marrow.) It was noted that no data were available to indicate if adequate amounts of CS or short lived reactive metabolites reached the target organ. Data from DNA binding studies in the liver and kidney did not help in this regard as no relevant analysis of tissues of initial contact (ie skin or nasal mucosa) were undertaken.[181] Studies using Drosophila (fruit flies) did not provide any meaningful data as the experimental design was unlikely to result in exposure of Drosophila to biologically active CS.[183] It was felt prudent for complete reassurance on the lack of mutagenic activity of CS *in-vivo* to have data from a study to investigate genotoxicity to measure potential mutagenicity at a site of contact, for example in the nasal mucosa. However, some members of COM recognised that the design of such an animal study would be difficult both from practical and ethical standpoints and were of the opinion that these studies were not necessary.

Test Strategies and Evaluation

Mutational spectra

2.22 DNA sequencing of mutations present in specific genes of tumours have indicated relationships between the sites within a gene, at which mutations have been detected (mutation spectra), and the individual chemicals implicated in the aetiology of a specific tumour. The Committee provided advice on the proposals regarding the minimum levels of data necessary to correlate a mutational spectra in a tumour type with a specific chemical. The

Committee agreed that it was theoretically possible that selection of spontaneous mutations with a particular effect or in particular genes could give rise to differences in tumour spectra between treated and control animals. The proposal outlined in the paper to establish whether a compound could induce specific mutations in neutral situations, i.e. where there is no selection through growth advantage as occurs in a tumour, was acceptable, although Members considered that it would be difficult to conduct such experiments with chemicals that were not stable under *in-vitro* conditions such as ozone. The Committee noted that there were a number of uncertainties to be resolved in developing a suitable methodology, for example, whether the use of specific non transcribed reporter genes (such as *lac* Z) would be non-selective resulting in important effects being missed.

Topics Under Consideration

2.23 The following topics, which were discussed by the Committee at meetings held in 1999, are still under review:

Revision of COM guidelines on a strategy for testing chemicals for mutagenicity
Hydroquinone & Phenol
Disopropylnaphthalenes

1999 Membership of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

Chairman

Professor J M Parry BSc PhD DSc
Professor of Genetics. School of Biological Sciences, University of Wales, Swansea

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J M Battershill BSc MSc
M Al-Derzi BSc MSc

Declaration of interests during the period of this report

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof J M Parry (Chairman)	Albright and Wilson Compass Catering JIB Insurance National Power Powergen Smith Kline Beecham	Share Holder Share Holder Share Holder Share Holder Share Holder Consultant	Boehringer Pfizer Welsh Water Glaxo Wellcome BAT Astra	Grant Grant Grant Grant Grant Grant
Prof J Ashby	Zeneca ML Labs Phytopharm ICI Astra Zeneca Astra Zeneca	Employee, Salary, Share option Share Holder Share Holder Consultant Consultant Share Holder	NONE	NONE
Dr P Bryant	British Gas Centrica Abbey National	Share Holder Share Holder Share Holder	NONE	NONE
Prof C Cooper	Halifax Norwich Union	Share Holder Share Holder	NONE	NONE
Prof D S Davies	Astra Zeneca plc ICI plc ML Laboratories plc Servier SmithKline Beecham	Share Holder Share Holder Non Executive Director and Share Holder Consultant Consultant	Astra Zeneca plc Bayer Plc Bristol Myers Squibb Glaxo Wellcome Hoechst Marion Roussel Knoll Pharmaceuticals Lilly Research Merck Sharpe & Dohme ML Laboratories Orion-Farmos Pfizer Rhone-Poulenc Rorer Roche Sanofi Winthrop Servier SmithKline Beecham Solvay	Fellowship Research & Meeting support Research & Meeting support Support for meeting Advisor Research Advisor Research Assistant Studentships Research Research & Meeting support Research Research & Meeting support Research & Fellowship Research Advisor Commissioned research Meeting support Advisor Research Support for meeting Research Meeting Support Research Studentship Advisor Meeting support Research

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof M Green	Dixons Foreign and Colonial Eurotrust HSBC Holdings Marks & Spencer National Westminister Second Alliance Investment Trust Shell Transport TR Property Investment Trust 3I	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	MRC Pension Scheme	Trustee Director
Ms M Langley	NONE	NONE	NONE	NONE
Prof R F Newbold	NONE	NONE	NONE	NONE
Dr D J Tweats	Glaxo Wellcome Halifax Co-operative Bank plc	Salary Employee Share Option Holder Share Holder Share Holder PEP Holder	NONE	NONE
Dr S Venitt	Abbey National plc Northern Rock	Share Holder Share Holder	NONE	NONE

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

Preface

The Committee on Carcinogenicity (COC) evaluates chemicals for their human carcinogenic potential at the request of the Department of Health and other Government Departments. The secretariat provides the COC with comprehensive data packages upon which the Committee members use their expertise to evaluate both chemical activity and the effectiveness of test methodologies. The Committee has begun to also review papers prepared by the DH Toxicology Unit at the Imperial College of Science, Technology and Medicine.

There have been several major pieces of work undertaken by the Committee this year. These include the statements on Drinking Water, Breast Cancer and exposure to organochlorine insecticides, 3-monochloropropane 1,2-diol (3-MCPD) which may occur as a contaminant in flocculants used during water treatment, 2-Chlorobenzylidene malononitrile (CS) and CS Spray and a review of the animal carcinogenicity data on Ozone. The Committee's advice on 2-Chlorobenzylidene malononitrile (CS) and CS Spray was published as part of a joint COT/COC/COM statement.

The Committee has started to publish the agenda, on the Internet prior to each meeting, and publishing minutes and completed statements and conclusions when they are agreed at the subsequent meeting.

The Committee received a presentation from Dr Denise Robinson of the Health & Environmental Sciences Institute (HESI) part of the International Life Sciences Institute (ILSI) on the collaborative research on alternative models for carcinogenicity assessment. This is an important area of work in which the Committee continues to take an interest in.

The major changes in Committee procedures during this year has lead to the publication of agendas prior to meetings and minutes and statements when completed. Thus the conclusions reached by the Committee given in this Annual Report are already available on the COC web site (http://www.doh.gov.uk/coc.htm). Members were also pleased to welcome a Lay Member to the Committee during this year.

Professor P G Blain (Chairman)
BMedSci MB PhD FRCP(Lond) FRCP(Edin) FFOM CBiol FIBiol

Drinking Water

3.1 In the United Kingdom, North America, and many other countries, chlorination has long been an important part of water treatment, intended to ensure that drinking-water contains no microbes hazardous to human health. In the mid-1970s, refinements in techniques of chemical analysis resulted in the detection in drinking-water of traces of chemicals formed when organic chemicals (such as those which may occur naturally in rivers, lakes, reservoirs and other water sources) are subjected to chlorination. In drinking-water, each of these chlorination byproducts (CBPs) is typically present at a concentration below 1 part per billion (1 µg/l). Some however, such as the trihalomethanes (THMs, ie chloroform, bromodichloromethane, chlorodibromomethane and bromoform), are often present at concentrations between 10 and 100 µg/l. Numerous CBPs have been identified, but many have yet to be detected or characterised.

3.2 Some CBPs, including some of the THMs, are known to be carcinogenic in laboratory mammals given doses far greater than human intakes from drinking-water. Some CBPs are genotoxic in test systems, including the bacterial "Mutagen X" (MX; 3-chloro-4-dichloromethyl-5-hydroxy-2(5H)-furanone). There have been many epidemiological investigations into the possible association between chlorination of drinking-water and cancer in humans and also many experimental studies regarding the mutagenicity and carcinogenicity of CBPs which have been considered in this statement.

3.3 1992 Evaluations

> The Committee concluded that the 1986 conclusions (i.e. up to 1992) were adequately founded and that information from subsequent investigations did not alter those conclusions. With regard to further epidemiological investigations within the UK the Committee pointed out that it would be very difficult to take account of consumption of chlorinated water in food, bottled water and other beverages. The Committee could not recommend that further epidemiological studies should be undertaken in the UK at the present time.

3.4 The COC also reviewed a meta-analysis[194] published in July 1992. The authors estimated an overall relative risk (RR) of 1.15 (1.09-1.20) {95% confidence interval, used throughout this statement} for all cancer sites together, with statistically-significant elevated RRs for bladder cancer (1.21 [1.09-1.34]) and rectal cancer (1.38 [1.01-1.87]) but not for the other ten categories of cancer which were evaluated. COC considered that the meta-analysis gave insufficient evidence for increased concern over the carcinogenic effects of chlorinated drinking-water. It was noted that no account had been taken of consumption of water other than tap water, but that in fact bottled water and water used in food was often chlorinated in the manufacturing plant. COC concluded that the meta-analysis did not change its conclusions, but added a proviso that *"the Committee could not recommend that further epidemiological studies should be undertaken in the UK at the present time, unless a population can be found with a distinctive exposure to chlorinated drinking-water"*.

3.5 In 1996, COC and COM considered the carcinogenicity and mutagenicity data on the THMs, and COC advised that *"The ratio between the lowest dose level giving rise to a carcinogenic effect in animals and the likely human exposure level from drinking-water for each of the four THMs considered by the Committee was in excess of 10,000. Thus the levels of these THMs in drinking-water in the UK are unlikely to provide a carcinogenic risk to humans"*.

New epidemiological studies

3.6 Futher epidemiological studies were considered by the COC in 1999. This comprised the twenty relevant epidemiological studies[195, 196, 198,–215] published since COC's 1992 evaluation.

3.7 A mixture of case-control, cohort and ecological studies has been employed to investigate the association between chlorinated drinking water and various cancers. Most of the recent epidemiological studies were carried

out in North America. None were from the United Kingdom. The focus for case control studies has been cancers at sites implicated in earlier epidemiological studies, and for which there may be, theoretically, a higher exposure to agents in drinking water.

3.8 Those carcinogenicity studies which have been performed on CBPs do not identify any CBP, or group of CBPs, which appears likely to cause cancer at these sites at the concentrations found in drinking-water. The Committee reaffirmed its view that since bottled water products may contain chlorinated water, it was not possible to identify an unexposed control group. In the absence of an identified aetiological agent, or a precise means of measurement, a number of different surrogates of exposure have been employed in these studies including the following comparisons:

- chlorinated vs non-chlorinated water sources
- duration of time exposed to chlorinated water
- surface vs groundwater sources
- trihalomethane levels (total and individual substances)
- high organic content vs low organic content
- high level of estimated water mutagenicity vs low level.

This consequently introduces uncertainty in exposure classification and makes comparison between studies, and interpretation of individual studies, more difficult.

3.9 In addition to these uncertainties, lifetime estimates of actual water consumption cannot be ascertained with any certainty, and exposure to substances occurring in drinking water via other routes (ie inhalation, dermal) or from other sources (eg food) may also not be properly considered. Consequently cancer epidemiological studies of chlorinated water suffer to a lesser or greater degree from deficiencies of study design.

3.10 It is also not uncommon, in those studies where statistically significant relative risks are observed, for these to be typically in the region of 2 or lower. Consequently, the strength of association between health outcomes and measures of exposure is considered to be weak, and the elevated risks may be within the range of uncertainty arising from possible confounding factors.

3.11 Of the 20 recent studies, only 4 were particularly well conducted. These comprised two case control studies dealing solely with bladder cancer,[195, 200] one case control study considering colon and rectal cancers[201] and a prospective cohort study of postmenopausal women[198] which looked at many different cancer sites including the bladder, colon and rectum. The remaining studies were either ecological in nature or had other serious limitations in design. Overall, however, all studies suffered to some extent from the difficulty of assessing long term exposure to potential aetiological agents in chlorinated drinking water. Additionally there was a lack of consistency of effect across studies dealing with different cancer end points. Many studies were also not directly comparable as they contained different measures of assessing exposure to chlorinated drinking water.

Bladder cancer

3.12 Previous epidemiological studies have suggested associations between bladder cancer and CBPs, and eleven of the twenty recent studies have investigated this hypothesis. Five were case-control studies,[195, 200, 204–206, 212] two were cohort studies,[198, 208] and four were ecological.[207, 213–215] Most report some statistically significant elevated relative risks for groups with the highest estimated duration or level of exposure, but the associations are generally weak, with relative risks below 2. Exceptions are found in subgroups in four of the case-control studies, but are not consistent between studies. Thus, in two studies[195, 200] the relative risk was confined to male smokers (respectively, odds ratios [ORs] of 2.3 for more than 60 years of use of chlorinated water, and 3.2 for more than 40 years use of municipal water). This contrasts with another case-control study[206] which found an elevated OR only in male non-smokers (OR 2.59 for 30 years exposure to drinking-water estimated as "substantially mutagenic"), and with an earlier large case-control study[216] which found associations primarily in non-smokers of both sexes. Members noted that a new ecological study[215] of chlorination of drinking water and cancer mortality in Taiwan had recently been published but agreed that such studies were only useful in the generation of hypotheses and not in respect of the evaluation of risk. A retrospective cohort study in Finland[208] found an elevated relative risk for women only (1.48 [1.01-2.18]) but, as noted above, the same group's case-control study[206] found an elevated OR in male smokers only. In another case-control study[205, 209] the highest ORs (2.28-2.58) were seen in groups with 35 or more years of unusually high consumption of water with estimated THM levels greater than 50 µg/l.

3.13 These recent studies of bladder cancer do not show any consistent dose-response relationship with estimated exposures to CBPs or THMs.

Colon and rectal cancers

3.14 Since the 1992 evaluation there have been 7 epidemiological studies which have examined an association with cancer of the colon and 8 studies investigating rectal cancer. Of these only two studies were considered to be particularly well conducted, a case-control study of colon and rectal cancers[201] and a prospective cohort study in postmenopausal women.[198] Findings from these two studies were inconsistent; for cancer of the colon, a moderately strong association with increasing duration of exposure was found in the case-control study but no significant association was found in the cohort study; conversely, for rectal cancer, a moderately strong association was found in the cohort study but not in the case-control study. Inconsistent findings were also evident in the other reviewed studies of these sites.

Other sites

3.15 Studies of the other sites were not considered to be of good quality and, although some elevated risks were identified, these studies overall also failed to demonstrate any consistent association.

Conclusion

3.16 Overall, the further epidemiological studies fail to provide persuasive evidence of a consistent relationship between chlorinated drinking-water and cancer. It remains possible that there may be an association between chlorinated drinking water and cancer which is obscured by problems such as the difficulty of obtaining an adequate estimate of exposure to chlorination by-products, misclassification of source of drinking water (including the use of bottled water), failure to take adequate account of confounding factors (such as smoking status), and errors arising from non-participation of subjects.

3.17 We therefore consider that efforts to minimise exposure to chlorination by-products remain appropriate, providing that they do not compromise the efficiency of disinfection of drinking-water.

Organochlorine Insecticides and Breast Cancer

Breast Cancer Risk and Exposure to Organochlorine Insecticides: Consideration of the Epidemiology Data on Dieldrin, DDT and Certain Hexachlorocyclohexane Isomers

Introduction

3.18 A number of articles in the scientific literature and general media have suggested that prolonged exposure to certain organochlorine insecticides (OCIs) may cause breast cancer. In 1995, the COC reviewed the available epidemiological studies on three chemicals (DDT and isomers/metabolites, and the hexachlorocyclohexane isomers γ-HCH (lindane) and β-HCH. The Committee agreed that the available evidence indicated no clear association. It was felt, however, that the matter should be kept under review. It is in this context that the Committee on Carcinogenicity was asked by the Department of Health to review the relevant information on four organochlorine insecticides (OCIs) in respect of the potential for an association with breast cancer. The additional chemical included in this statement is dieldrin, for which new epidemiological data have recently become available. However, it should be noted though, that only one of the OCIs considered by the Committee, namely lindane, is currently used in the UK, whilst pesticidal uses of the other chemicals considered were phased out over a decade ago.[217]

Overview of hypothesis that OCIs may cause breast cancer

3.19 The Committee agreed that a number of observations and assumptions had led some observers to suggest the hypothesis that OCIs and other organochlorine compounds may be associated with an increased risk of breast cancer. These could be summarised as follows:

(i) many of the known or proposed risk factors for breast cancer are related to endogenous or exogenous hormones (in particular oestrogen). These factors include age at first birth, at menarche, and at menopause, and obesity, parity and use of oral contraceptives and hormone replacement,[218]

(ii) there is some evidence available to suggest that the OCIs under consideration may have weak oestrogenic activity,[219, 220, 221, 222, 223, 224]

(iii) these OCIs have been shown to induce tumours (predominantly of the liver) in experimental animals,[225]

(iv) these OCIs persist in the environment and exposure of the population has occurred mainly via the diet.[221,226]

3.20 The Committee reviewed the evidence that dietary exposure to environmental levels of these OCIs might induce an oestrogenic response *in-vivo* through the consideration of three questions, namely;

(i) Do these OCIs have oestrogenic activity *in-vivo* and if so what is their potency relative to other sources of oestrogens?

(ii) Is there any evidence for synergistic effects?

(iii) Do these compounds persist in breast tissue?

3.21 The Committee used the information from this review to draw conclusions on the biological plausibility of the hypothesis that OCIs may cause breast cancer. The Committee then evaluated the available epidemiological investigations for evidence of an association between the four OCIs considered in this review and breast cancer. Final conclusions on each of these OCIs took account of the potential for an oestrogenic response *in-vivo*, the evidence for persistence in humans and the available epidemiological investigations.

Do these OCIs have oestrogenic activity in-vivo and if so what is their potency relative to other sources of oestrogens?

3.22 A tabulation of the Committee's assessment of the evidence for oestrogenic activity of the OC insecticides under consideration is given below:

Table 1: Assessment of oestrogenic activity of OC insecticide

OCI	**Evidence of oestrogenicity *in-vitro* through effects on**			***In-vivo* data**	**Conclusion**
	i) Oestrogen receptors	**ii) Oestrogen metabolism**	**iii) or by other mechanisms**		
β-HCH	Evidence of very weak oestrogenic activity[222, 227] (*ca* 40,000 x weaker than oestradiol)	No data available	Yes[222, 227]	Uterotrophic effects in rats and mice[228, 229]	Regard as a weak *in vivo* oestrogen. Mechanism unknown.
Lindane	No evidence of oestrogenic activity[224, 230]	Effects noted in MCF-7 cells[231, 232] but other investigators have been unable to identify xenoestrogens using this test system[233]	No data available	Difficult to interpret but equivocal evidence for oestrogenic and anti-oestrogenic effects in rats.[234, 235, 236, 237] No evidence of uterotrophic effect in reproduction studies in rats and mice.[238]	Considered not to have *in-vivo* oestrogenic activity.
DDT isomers/ Metabolites (in particular p,p′ DDE)	Evidence of weak oestrogenic activity.[224, 239] (*ca* 1000 x weaker than oestradiol)	Effects noted in MCF-7 cells[231, 232] but other investigators have been unable to identify xenoestrogens using this test system[233]	Yes [227, 239, 240, 252]	Oestrogenic effects in rodents reported with certain DDT isomers (eg o,p DDT p,p′ DDT, and o,p DDD) but not with p,p / DDE[221]	Regards some DDT metabolites and isomers as weak *in-vivo* oestrogens. Several mechanisms possibly involved.
Dieldrin	Evidence of very weak oestrogenic activity in the majority of studies *ca* 10,000-50,000 less potent that oestradiol [224, 241, 242, 243, 244, 245, 246, 247]	No data available	No data available	No evidence of uterotrophic effects in a number of studies in either rats or mice.[242, 245, 247]	Considered not to have *in-vivo* oestrogenic activity

3.23 The Committee agreed that the evidence supported the conclusion that β-HCH and DDT (in particular some metabolites and isomers) could have weak oestrogenic activity *in-vivo* which most probably occurs by several different mechanisms. Members agreed that dieldrin appeared to have weak oestrogenic activity *in-vitro*, but no evidence of an effect had been documented *in-vivo* in a number of studies in rats and mice. There was no convincing evidence that lindane had oestrogenic activity *in-vivo*.

3.24 The Committee agreed that it was important to compare the evidence for oestrogenic activity of the OCIs under consideration with other potential sources of exposure to oestrogenic substances. Members were aware that Dr Safe from the USA (Texas A&M University) had published estimates of total oestrogenic potency (i.e. oestrogenic equivalents EQs).[248] Thus although there were reservations regarding the use of these calculated data (as the method of determining EQs was based on *in-vitro* studies) it was agreed that the results presented a useful comparison of the relative importance of the sources of potential oestrogens to which women might be exposed.[248] The tabulated information from Dr Safe's paper (reproduced below as table 2) show that organochlorine xenoestrogens contribute a very small proportion of the potential total oestrogenic burden.

Table 2. Estimated mass balance of human exposures to environmental and dietary oestrogens and anti-oestrogens

Source of oestrogen	Oestrogen equivalents (µg per day)
Morning after pill	33,500
Birth control pill	16,675
Post-menopausal therapy	3,350
Flavonoids in foods (1,020 mg/day x 0.0001)	102
Environmental organochlorine oestrogens (2.5 x 0.000001)	0.0000025

(Abstracted from Safe H (1995). Environmental Health Perspectives, 103, 346–351.)

Is there any evidence for synergistic effects?

3.25 The evidence for synergism between xenoestrogens, and in particular in respect of OCIs, arose from the results obtained in *in-vitro* experiments undertaken by one research group in the USA mainly involving mixtures of dieldrin with endosulfan or methoxychlor.[249, 250] The authors subsequently retracted their data after they were unable to replicate the original experiments.[251] Many other research groups were unable to repeat the finding of synergism using a number of *in-vitro*[237, 240, 248, 252] and *in-vivo* tests.[221, 240, 248] The results of these more recent experiments suggest, at most, an additive effect. There is thus no evidence to support the view that low levels of mixtures of xenoestrogens induce a biologically significant oestrogenic effect in mammals by acting synergistically. The Committee considered recent claims by one group of authors[253] regarding evidence of synergism between certain xenoestrogens and concluded that any significant synergistic interactions in mammals should have been identified by the available published experiments.

Do these compounds persist in breast tissue?

3.26 The observation that residues of certain OCIs persist in adipose tissue leading to bio-accumulation which might result in continuous exposure of breast tissue to weak oestrogenic substances has been cited as an essential part of the hypothesis that such compounds may cause breast cancer.[251] Many research groups have measured levels of OCIs in human adipose tissue or in human milk samples.[254, 255, 256, 257, 258, 259] The results published by the UK Working Party on Pesticide Residues (WPPR) show that p, p′ DDE, and β-HCH can be detected in samples of fat or milk from most individuals studied whilst dieldrin was detected in fewer individuals. Lindane was infrequently found in human fat (3%) and milk (1.8%) samples, mainly at low levels (i.e. only one human fat sample contained > 0.01 mg/kg). The available literature shows that lindane is more rapidly metabolised and eliminated in

mammals[260] than other OCIs such as dieldrin[261] and thus one possible explanation for the low frequency of detectable lindane residues in humans could be due to its metabolism.[254, 255]

3.27 The WPPR results support the conclusion that the mean concentration of p, p′ DDE and dieldrin in human fat samples has been decreasing for several decades. There is no evidence from the WPPR survey for a decrease in the mean concentration β-HCH in human fat up to 1982/3. However, the mean concentration β-HCH in human fat was substantially lower in samples taken in 1995-7 in comparison to the mean concentration of this chemical reported in human fat samples taken in 1982/3. In other surveys, a significant reduction in adipose tissue concentrations of β-HCH was reported in the USA[259] between 1970–1983 but not in a separate study undertaken in the Netherlands during 1968–1986.[257]

Table 3: Concentrations of OCI in human fat

OCI	Mean concentration (mg/kg) in human fat (Percentage of first reported residue level)				
	1963–64	1969–71	1976–77	1982–83	1995–97
Dieldrin	0.26 (100)	0.16 (61.5)	0.11 (42)	0.08 (30.7)	0.02 (7.6)
p.p'DDE	2.0 (100)	1.8 (90)	2.1 (100)	1.3 (65)	0.71 (35.5)
ß-HCH	No Data	0.28 (100)	0.27 (96.4)	0.31 (110)	0.12 (42.8)

Conclusions on biological plausibility

3.28 Regarding the specific chemicals under review, the Committee agreed that there was sufficient evidence to support the contention that some of the DDT metabolites and isomers and β-HCH had weak oestrogenic activity *in-vivo*, but dieldrin and lindane should not be regarded as having *in-vivo* oestrogenic activity. However, taking into account the low frequency of detectable levels of lindane in samples of human fat and milk, there appeared to be no convincing reason for including lindane as a xenoestrogen for examination in epidemiological studies of breast cancer.

3.29 The Committee concluded that the xenoestrogens considered, were, at most, very weak *in-vivo* oestrogens and agreed that there was no evidence of any synergistic effects between these chemicals. The impact of exposure to oestrogenic chemicals would be the product of oestrogenic potency and bioavailability. As OCIs are of low potency and occur at low concentrations, it is most unlikely that the effect of current exposures will represent a significant risk of breast cancer. Members were aware that other review groups[240] had reached a similar conclusion and noted that some recent methodological research had been published to identify appropriate biomarkers of total oestrogenic body burden from environmental sources.[262, 263, 264]

3.30 The Committee agreed that it would be appropriate if further investigations of the potential association between xenoestrogens and breast cancer concentrated on the evidence for risks associated with the effects of the total exposure to xenoestrogens.

Epidemiology

3.31 In 1995, the available epidemiological data on breast cancer and exposure to OCI were limited, comprising 6 case-control studies which investigated a total of 301 women with breast cancer using a variety of exposure analyses (in serum, plasma and breast adipose tissue).[265, 266, 267, 268, 269, 270] Only one of these studies used a prospective design.[270] All six studies investigated DDT (measured as the metabolite p, p′ DDE), with an equal number of positive and negative associations reported between (p, p′) DDE and the risk of breast cancer. The two investigations that considered β-HCH gave conflicting results, but no clear association was found. The two studies that considered lindane in breast tissue found no difference in lindane levels between cases and controls. There was no evidence of an asso-

ciation between the concentration of dieldrin in breast tissue and breast cancer in the one limited study before 1995 where dieldrin was measured.[266]

3.32 Since 1995, eight additional epidemiological studies have been published,[271, 272, 273, 274, 275, 276, 277, 278] which have considerably increased the number of women studied. Thus the total number of women with breast cancer studied with respect to (p, p′) DDE has now risen to over 1500. A brief description of these studies and their results is given in table 4 below. The conclusions reached by the Committee with regard to the epidemiological data are given below and in paragraph 17.

Table 4: Epidemiological studies of organochlorine insecticides and breast cancer published after 1995

STUDY (Reference No)	Description	Result/Comment
Lopez-Carrillo L et al (1997)[271] (Mexico)	Hospital based case-control investigation (n=141 cases and controls)	No evidence for an association between serum p,p′ DDE concentrations and risk of breast cancer. Age-adjusted odds ratio for serum DDE concentrations comparing lowest tertile to middle and upper tertiles were below 1.
Hunter DJ et al (1997)[272](USA)	Prospective investigation based on 121,700 nurses enrolled 1976. Blood samples provided by 32,836 (1989-90). Study based on 240 women with breast cancer (up to June 1992) and 240 matched controls.	No evidence for an association between plasma p,p′ DDE and risk of breast cancer. The median concentration of DDE in cases was below that of control.
Van't Veer P et al (1997)[273] (Germany, Netherlands, N. Ireland, Switzerland, Spain)	Multi-centre case-control investigation. (n = 374 cases and 374 populations hospital controls).	No evidence for an association between subcutaneous fat concentrations of p,p′DDE and risk of breast cancer. Mean concentrations of p,p′ DDE in needle aspirates of buttock fat were lower in cases compared with controls at all study centres.
Guttes S et al (1998)[274] (Germany)	Analysis of p,p′ DDT p,p′ DDE and α-,β-γ-HCH breast tissue samples from 45 women with breast cancer and 20 with benign breast disease at two centres.	After age adjustment a significant p,p′ DDE concentration was documented in breast cancer patients (p = 0.017 by analysis of covariance). A slightly lower mean β-HCH concentration was reported in cases. Lindane (γ-HCH) was only detected in 3/65 samples (≥ 1µg/kg).
Hoyers P A et al (1998)[275] (Denmark)	Prospective investigation based on 7712 women enrolled in Copenhagen Heart Study in 1976. 268 women developed breast cancer up to end of 1993. Analysis based on serum samples from 240 cases and 477 controls.	Dieldrin was associated with a significantly increased dose-related risk of breast cancer (adjusted odds ratio for highest tertile of exposure = 2.05 (95% CI 1.17 – 3.57)) β-HCH showed a slightly, but not statistically significant increase in odds ratio. No association was seen for a further 16 organochlorine pesticides studied (including lindane (γ-HCH) and p,p′ DDE). The Committee considered the result with deldrin may have been a chance finding in view of the large number of statistical comparisons (46) undertaken in this study.

Study (Reference No)	Description	Result/Comment
Olaya-Conteras P et al (1998)[276] (Colombia)	Hospital based case-control investigation (n = 155 cases, 153 controls)	After adjustment for confounding factors a significant increased odds ratio for serum p,p′ DDE concentrations and breast cancer was reported for the highest tertile of exposure (OR = 1.95 (95%) CI 1.11-1.32).
Dorgan JF et al (1999)[277](Colombia)	Prospective investigation based on 7224 women who donated a blood sample to the Missouri Breast Cancer Serum Bank between 1977/87. 105 cases identified at the end of 1989 but there was no follow-up of at least 70% of cohort beyond 1982/3. Analyses based on 105 cases and 208 controls but the criteria for matching for 17 cases were relaxed.	No evidence for an association between total DDT (α p,p′ DDT or p,p′ DDE). β-HCH dieldrin, lindane (γ-HCH) or for a further 13 other organochlorine insecticides was found.
Moyish KB et al (1998)[278] (USA)	Case-study of 154 post-menopausal breast cancer cases and 192 post-menopausal community controls.	No evidence for an association between serum concentrations of p,p′ DDE and risk of breast cancer.

3.33 The Committee was aware that a large number of epidemiological investigations were being conducted, mainly in the USA, under the sponsorship of the National Cancer Institute (NCI) and National Institute for Environmental Health Sciences (NIEHS); these studies would yield additional data over the next few years. It was agreed that completed published studies should be evaluated at a future meeting. Members considered that it would be valuable if such studies included an estimation of total body burden of environmental xenoestrogens as well as an analysis of individual chemicals. The Committee was also aware that it was likely that technological improvements will in the future enable the detection of lower levels of OCIs in tissue samples, although this would not necessarily imply that such concentrations would be associated with any harmful biological effect.

Conclusions: Epidemiology

3.34 The Committee agreed that the following conclusions regarding the epidemiological data for the OCIs reviewed in this paper could be drawn on the basis of the available evidence.

(i) *DDT* There is considerably more epidemiological data now than in 1995 on environmental exposure to DDT and its isomers and metabolites and a possible association with breast cancer. All of the eight studies[271-278] published since 1995 investigated p, p′ DDE, but only two[273,275] relatively small retrospective studies found evidence for an association between p, p′ DDE and increased risk of breast cancer. Overall, there is no convincing evidence from epidemiology studies for an elevated relative risk of breast cancer in association with DDT (as measured by p, p′ DDE).

(ii) *Dieldrin* There is very little epidemiological information available on dieldrin and its possible association with breast cancer. Of the two recent studies published after 1995, which considered this insecticide, one found no evidence for an association[277] and the other found a positive association,[274] which was considered likely to be a chance finding. Overall, there is no convincing evidence from epidemiological studies for an elevated relative risk of breast cancer associated with dieldrin.

(iii) *β-HCH* There is very little epidemiological information available on β-HCH and its possible association with breast cancer. Of the three recent studies published after 1995 which considered β-HCH,[274,275,276], none reported a statistically significant association with increased risk of breast cancer.

(iv) *Lindane* There is very little epidemiological information available on lindane (γ-HCH) and its possible association with breast cancer. Of the three recent studies published after 1995 which considered lindane,[274,275,277] none found evidence for an association with increased risk of breast cancer. The available evidence for environmental exposure to lindane suggests that body burdens of this chemical are very small, being undetectable in most individuals. It is therefore unlikely that further epidemiological investigations of breast cancer based on assessment of levels of lindane in adipose tissue, blood, or breast tissue would provide additional relevant information.

Overall conclusion

3.35 The Committee evaluated the hypothesis that OCIs might increase the risk of breast cancer by virtue of their claimed oestrogenic effects (*para 3.19*). The Committee concluded that the oestrogenic effects (if any) of these xenoestrogens were likely to be small in magnitude, especially compared with those of oral contraceptives or Hormone Replacement Therapy (HRT),[218,248] which entail much higher exposures to oestrogens (*para 3.24 and tables 1 and 2*). Moreover, the Committee concluded that there was no convincing evidence of oestrogenic synergy in mammals between different OCIs (*para 3.25*). There is also evidence that concentrations in human fat of the OCIs considered in this statement are decreasing in humans which provides some additional reassurance with regard to any potential risk of breast cancer (*para 3.27, table 3*). The Committee agreed, that further investigations of the potential association between xenoestrogens and breast cancer should concentrate on the evidence for risks associated with the effects of the total exposure to xenoestrogens (*para 3.30*).

3.36 The Committee was aware of further epidemiological research on the OCIs considered in this statement and agreed that the relevant reports should be reviewed when published. Regarding the specific chemicals under consideration (i.e. DDT (and isomers/metabolites), dieldrin, β-HCH, and lindane), the Committee came to the following overall conclusions, on the basis of the available information.

(i) *DDT* Some DDT isomers and metabolites should be regarded as having weak *in-vivo* oestrogenic activity. The stable metabolite p, p[1] DDE, a marker for exposure to DDT, can be found in samples of fat from most individuals. There is however, good evidence from investigations undertaken in the UK that concentrations of p, p′ DDE in human fat samples have been declining for several decades. There are now 14 epidemiological studies which have considered p, p′ DDE using both case-control and prospective study designs. There is no convincing evidence for an association with an increased risk of breast cancer. Overall the available data do not suggest that environmental exposure to DDT (and isomers/metabolites) is a cause for concern as a risk factor for human breast cancer.

(ii) *Dieldrin* Dieldrin is not considered to have *in-vivo* oestrogenic activity. There is thus no rationale to consider that exposure to this chemical should be associated with an increased risk of breast cancer. There is good evidence from investigations undertaken in the UK that conentrations of dieldrin in human fat samples have been declining for several decades. There is no convincing evidence from the five available epidemiological studies for an elevated risk of breast cancer in association with exposure to dieldrin. Overall the available data do not suggest that environmental exposure to dieldrin is a cause for concern as a risk factor for human breast cancer.

(iii) *ß-HCH* β-HCH should be regarded as having weak *in-vivo* oestrogenic activity. β-HCH can be found in samples of fat from most individuals. There is evidence from investigations undertaken in the UK for a decline in β-HCH concentrations in human fat samples after 1982/3. There is no convincing evidence from the five available epidemiological studies for an elevated risk of breast cancer in association with exposure to β-HCH. It is recommended that the published literature on this chemical should be kept under review.

(iv) *Lindane* Lindane (γ-HCH) is not considered to have any *in-vivo* oestrogenic activity. There is thus no rationale to consider that exposure to this chemical should be associated with an increased risk of breast cancer. The available evidence for environmental exposure to lindane suggests that body burdens of this chemical in the UK are very small, being undetectable in most individuals. None of the five available epidemiological investigations found evidence for an association with breast cancer. Overall the available data do not suggest that environmental exposure to lindane is a cause for concern as a risk factor for human breast cancer.

3-Monochloropropane-1,2-diol (3-MCPD)

3.37 3-Monochloropropane-1,2-diol (3-MCPD) can be present as a contaminant in epichlorhydrin/amine copolymers used as flocculants or coagulent aids in water treatment. These polyamine flocculants have been available for many years as approved products for use in water treatment and thus 3-MCPD may be present in drinking water from their use. 3-MCPD is a member of a group of contaminants known as chloropropanols. This group includes some known genotoxic carcinogens in animals such as 1,3-dichloropropan-2-ol. The COC was asked to evaluate and advise on the available carcinogenicity data on 3-MCPD by the Committee on Chemicals and Materials of Construction for use in Public Water Supply and Swimming Pools (CCM), a statutory committee which provides advice to the Secretary of State for the Environment on the approval of chemical substances in contact with public water supplies.

3.38 The Committee was aware that 3-MCPD had been detected as a contaminant of several foods and food ingredients, including acid hydrolysed vegetable protein (acid-HVP) and that the EU Scientific Committee for Food had published an opinion in 1994 where it was agreed that 3-MCPD should be regarded as a genotoxic carcinogen.[279] The Committee also had access to published mutagenicity data on 3-MCPD, a safety evaluation prepared by CanTox. Inc (Ontario, Canada) for the International Hydrolysed Protein Council,[280] and a review document published by the Institute of Toxicology, National Food Agency of Denmark.[281] The COC asked for advice from the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) in respect of the mutagenicity of 3-MCPD. In reviewing these documents, members commented that the available metabolism data on 3-MCPD were relatively old and focused on metabolic pathways following intraperitoneal administration. There was no data from oral studies using modern methods to measure absorption and elimination of 3-MCPD. The Committee considered the proposal by CanTox Inc regarding the formation of bacterial-specific mutagens and agreed that there was no evidence to support this speculation.

3.39 The Committee reached the following conclusions on the available mutagenicity data.

(i) 3-MCPD has a chemical structure, which suggests that it may be metabolised to genotoxic intermediates (particularly glycidol).

(ii) The COM has advised that 3-MCPD is an *in-vitro* mutagen. In the absence of a satisfactory evaluation of the potential *in-vivo* mutagenicity, it would be prudent to assume that 3-MCPD is an *in-vivo* mutagen. The COM had advised that further negative results in an *in-vivo* mutagenicity test in a second tissue namely rat liver UDS were required in order to provide adequate reassurance that the activity seen *in-vitro* is not expressed *in-vivo*.

(iii) 3-MCPD has been tested in four long-term animal carcinogenicity experiments, 2 in mice and 2 in rats.[282,283,284] However three of these studies[282, 283] are relatively old and were conducted to inadequate protocols. The conclusions reached by the COC therefore refer to the one study conducted to contemporary standards.[284] The Committee had access to the full study report[284] and to published critiques of this study.[281,282] The tumour data have been evaluated by a number of statistical methods. The analyses reported below refer to the Fishers pair-wise comparisons with controls.

(iv) In the study undertaken by Sunhara et al (1993)[284] 3-MCPD was administered via drinking water to groups of 50 male and 50 female (aged 6 weeks at start) F344 rats for a period of 104 weeks. Concentrations of 0, 20, 100, and 200 ppm were used. These equated to dose levels of 0, 1.1, 5.2, or 28 mg/kg bw/day in males and 0, 1.4, 7.0, or 35 mg/kg bw/day in females. 3-MCPD was also detected in the drinking water used in this study at 2.7 ppm and thus control animals were given doses of approximately 0.1 mg/kg bw/day. There was evidence that doses of 3-MCPD in the high dose group exceeded the Maximum Tolerated Dose level. The approximate decrease in body weights, relative to control animals for males and females at the high dose level was 33% and 35% respectively. There was no evidence of any treatment-related increase in mortality in this study. Survival to termination was acceptable (i.e.>50%) in all dose groups with the exception of the high male dose group where 21/50 animals survived to termination.

(v) In males, a statistically significant increase in the incidence of Leydig-cell adenoma was documented at the intermediate and high dose levels. Three animals at the high dose level had Leydig-cell carcinomas. A statistically significant increase in the incidence of mammary gland fibroadenoma was noted in the high dose male group. A statistically significant increase in mammary gland hyperplasia was recorded in the male mid and high dose groups. A small but not statistically significant increase in the incidence of preputial gland adenoma was recorded in the mid and high dose male groups. One animal in the intermediate dose group and two in the high dose group had preputial gland carcinomas. It is difficult to evaluate these findings since only a limited number of preputial glands were examined histologically (5-16/group) in this study. A small (not statistically significant) increase in renal tubular adenomas was documented in the intermediate and high dose male groups. A statistically significant increase in the incidence of nephropathy and renal tubular hyperplasia was also recorded at the intermediate and high dose levels in this study.

(vi) In females, a statistically significant increase in the incidence of renal tubular adenoma was recorded at the high dose level. A statistically significant increase in nephropathy and renal tubular hyperplasia was also recorded at the intermediate and high dose levels in this study. A slight but statistically non-significant increase in mammary gland hyperplasia was reported at the high dose level.

(vii) The Committee noted that the suggestion that all of the increases in tumours noted in this study in rats were mediated by non-genotoxic mechanisms involving either cytotoxicity (kidney) or hormonal disturbances.[280,281,284] Members agreed that the proposed non-genotoxic mechanisms advanced were plausible, but there was no compound specific data to support any of the contentions suggested. It was possible that evidence might be produced which would allow a firm conclusion to be reached in respect of the mechanisms of the tumours observed in rats.

(viii) The Committee concluded that it was not possible to draw a definite conclusion regarding the significance of the observed carcinogenic effects of 3-MCPD in the rat. However, in view of the COM conclusions and need to further investigate the potential for *in-vivo* mutagenic activity, it would be prudent to reduce exposures to as low as technologically practicable.

Ozone (Review of Animal carcinogenicity data)

3.40 Ozone is a ubiquitous air pollutant due to its formation in the atmosphere by photochemical reaction between volatile hydrocarbons and nitrogen oxides. The Expert Panel on Air Quality Standards have recommended a very low standard (50 ppb as a running 8 hour average) based on it's irritant effects on the respiratory tract at 100 ppb and above. Ozone is also one of a limited number of chemicals that have been shown to be carcinogenic in the mouse (producing benign lung tumours in a strain sensitive to such tumour induction) but not the rat.

3.41 The COC have reviewed the need for carcinogenicity bioassay data in the mouse, and as part of this work are considering the public health significance of such apparent mouse specific carcinogens.[285,286] A key aspect of these is consideration of the genotoxic potential of such compounds. The COC felt it important to draw conclusions on the possibility of a genotoxic mechanism, particularly in the light of recent data on K-*ras* mutations in lung neoplasms from ozone exposed mice;[287] the advice of the COM was thus requested on this issue.

3.42 The following conclusions were reached on the potential carcinogenic hazard of ozone to humans in the light of advice from COM and the available evidence from the animal carcinogenicity bioassays.

(i) Ozone has been tested for potential carcinogenicity in mice and rats in long term inhalation bioassays as part of the ongoing National Toxicology Programme (NTP) in the USA.[288]

(ii) There was no evidence of treatment related carcinogenicity in male or female F344 rats exposed to 0.12, 0.5.1.0 ppm 6 hours/day, for 5 days/week, for up to 105 weeks or in a similar study where rats were exposed to 0.5 or 1.0 ppm for 6 hours/day, 5 days/week, for up to 125 wks. Ozone induced inflammation and non-neoplastic pathology (hyperplasia and metaplasia) throughout the respiratory tract in both sexes, particularly at the highest concentration.

(iii) Long term inhalation bioassays were also undertaken in B6C3F1 mice using exposures of 0.12, 0.5 or 1.0 ppm 6 hours/day, 5 days/week for 105 weeks. A separate life-time bioassay was performed using exposures of 0.5 or 1.0, for 6 hours/day, 5 days/week, for 125 wks. The NTP reported the statistical analyses of the carcinogenicity data based on evaluation of the combined incidence of lung adenomas and carcinomas. The NTP consider that pulmonary tumours in mice form a spectrum of lesions and adenomas appear to progress into carcinomas with time. The results reported in this statement refer to the outcome of the NTP evaluation based on combinining the incidence of adenoma and carcinomas consistent with this approach to the assessment of data.

(iv) A statistically significant increase in the incidence of alveolar/bronchiolar adenoma or carcinoma (combined) was found in female B6C3F$_1$ mice in the long-term bioassay at 1.0 ppm. This occurred in the presence of non-neoplastic pathology (inflammation, hyperplasia and/or metaplasia) throughout the respiratory tract. Evidence of chronic inflammation was documented in some female animals at 0.5 ppm but no increased incidence of lung tumours (combined adenoma and carcinomas) was reported at this dose level. No evidence of a carcinogenic or inflammatory response was documented in female mice at 0.12 ppm. A slight but statistically non-significant increase in the combined incidence of lung tumours (combined adenoma and carcinoma) occurred in male mice at 0.5 ppm and 1.0 ppm.

(v) A slight but statistically non-significant increase in lung tumours (combined adenoma and carcinoma) was documented in the life-time bioassay in male mice at 0.5 ppm and 1.0 ppm and in female mice at 1.0 ppm. In separate analyses the increase in incidence of lung adenoma in female mice reached statistical significance at 1.0 ppm.

(vi) Ozone was carcinogenic by inhalation in female B6C3F[1] mice producing lung tumours; this effect was associated with chronic irritation of the lung. There was equivocal evidence for a carcinogenic effect in male mice. Ozone was not carcinogenic in male or female F344 rats.

(vii) The COM has advised that there is some evidence for *in-vivo* mutagenic activity at the target site for carcinogenicity in the mouse. Additional work to reproduce ozone induced A-T transversions would provide additional information regarding this conclusion. The COM concluded that it would be prudent to assume that ozone may have *in-vivo* genotoxic potential.

(viii) The COC concluded that the mechanism of ozone carcinogenicity in the mouse is currently uncertain, leaving a question about whether the carcinogenic action is due to a genotoxic event(s) or chronic irritation. The carcinogenic activity of ozone appears to be specific to the mouse in the presence of chronic irritation and has not been observed in the rat. The animal data are thus inadequate to draw any conclusions regarding the potential carcinogenicity of ozone in humans.

(ix) The Committee agreed to review the situation in the future should more evidence of an *in-vivo* mutational effect by ozone become available.

Test strategies and Evaluation

3.43 The Committee was pleased to receive a presentation from Dr Denise Robinson (Health And Environmental Sciences Institute (HESI), International Life Sciences Institute (ILSI) on the ongoing collaborative program for the validation of short-term carcinogenicity tests using transgenic animals. This is an important area of test method development previously considered by the Committee in 1997. A full report of the presentation and members discussion is given below. The Committee also considered the proposal from the International Programme on Chemical Safety (IPCS) for a framework for the for considering mode of action of chemical carcinogens. It was agreed that the IPCS should be encouraged to carry out further work in exploring this framework. Members noted that the framework for considering mode of action was most suited to data rich chemicals such as those undergoing regulatory approval.

Nongenotoxic Carcinogens: Identification And Significance

3.44 The Committee considered a review paper on nongenotoxic carcinogens: Identification And Significance. It has been estimated that approximately 50% of all chemicals tested in animal carcinogenicity bioassays induce an increase in tumours in at least one tissue. A proportion of these animal carcinogens induce tumours via a direct interaction of the chemical or its metabolites with DNA; these are referred to as genotoxic carcinogens. It is prudent to assume that for these carcinogens there is no threshold dose below which no carcinogenic effect would be expected to occur and that they are therefore of particular concern to public health. The remainder of the animal carcinogens act through non-genotoxic mechanisms, for which a threshold may operate. Non-genotoxic carcinogens tend to be tissue and/or species specific. In some cases these are not relevant to human health. At present there are no short term tests that can be used to identify such compounds and long term bioassays in animals are necessary. The development of rapid methods for the identification of chemicals that induce cancer by non-genotoxic mechanisms that are relevant to humans had been idenitifed by the COC as a priority area for research in 1996.

3.45 They considered information available on the known human carcinogens as evaluated by the International Agency for Research on Cancer (IARC) to identify non-genotoxic carcinogens and information on mechanisms of carcinogencity which were relevant to humans. The aim of the work had been to review experimental approaches which could be used to identify non-genotoxic mechanisms of carcinogenicity and to select the most appropriate short-term testing approaches which could be considered for further research. One important outcome of this work was to exclude rodent-specific carcinogenic mechanisms, such as chemically induced deposition of α2u globulin in the kidneys of male rats, which are thought to be unimportant with respect to hazard identification for humans.

3.46 Members noted that mutations are found in all chemically induced cancers. Members agreed that the most important non-genotoxic mechanisms could be placed into one of four groups, (i) persistent cytotoxicity accompanied by proliferative regeneration, (ii) chronic inflammation accompanied by the production of reactive oxygen species, (iii) hormomimmetic activity and (iv) ligand binding with xenobiotic induction receptors. Members considered that increased cellular proliferation was of particular significance.

3.47 The Committee agreed that it was not practical to design a test to detect all non-geonotoxic carcinogens. However, by focusing on a number of relevant mechanisms it should be possible to identify key toxicological responses in sub-acute studies which might provide an indication of carcinogenic activity. It was very important in designing a research programme to select appropriate reference compounds and to include as wider range of mechanisms and target organs as was practicable to study. Members considered the IARC list of group 1 carcinogens as useful start but felt that consideration ought to be given to chemicals listed as IIA by IARC and under the EU Dangerous Substances Directive (EEC/67/548) as category 1 and 2 carcinogens.

3.48 Members considered that future consideration of the application of gene arrays and other approaches to transcription profiling would become important in studies of non-genotoxic carcinogenesis but at present there was little experience in the use of such methods. Members agreed there was little evidence to support the development of *in-vitro* methods such as cell transformation assays, or the use of the currently available transgenic animal models (such as the Tg.AC mouse). It was possible that appropriate transgenic animal models for certain classes of non-genotoxic carcinogens could be developed in the future.

Presentation on validation of short-term carcinogenicity tests using transgenic animals

3.49 The Committee was pleased to hear a presentation from Dr Denise Robinson (Health And Environmental Sciences Institute (HESI), International Life Sciences Institute (ILSI) on the ongoing collaborative program for the validation of short-term carcinogenicity tests using transgenic animals. This is an important area of test method development previously considered by the Committee in 1997. A full report of the presentation and members discussion is given below. In 1997, the Committee had reached a number of conclusions on the utility of short-term carcinogenicity testing using transgenic animal models following an assessment of the available published literature (Mutation Research 403, 259-263, 1998). Dr Robinson had agreed to provide the Committee with an overview of the HESI program of work and the likely times for completion of the work.

Presentation

3.50 Dr Robinson thanked the Committee for the invitation to make a presentation. Members were told that ILSI was a non-profit scientific research and education foundation with a number of work programs related to public health. Each program involved participation from industry, government and academia and the topics identified for research programs were those most likely to have an impact on public health. HESI was established in 1989 with the aim of investigating pathology, toxicology, human health and environmental risk issues. HESI identified the utility of short-term animal carcinogenicity tests as a key topic for discussion following the adoption by the International Conference on Harmonisation (ICH) of guidelines for the carcinogenicity testing of pharmaceuticals. These included the option for a short or medium term rodent test in addition to a long-term carcinogenicity test in a rodent species instead of requesting long-term carcinogenicity tests in two species. HESI established the Alternatives to Carcinogenicity Testing (ACT) committee in June 1996 as a steering group for this work program. It had been established that there was an inadequate database on performance of alternative assays, there was no accepted rationale for selection of a model for a second test and there were major differences between regulatory authorities in North America and those in Europe and other areas on the acceptability of the new models.

3.51 ACT set a number of objectives for the work programme, which included expanding the database on the alternative methods, establishment of common experimental approaches, information on the scientific basis of the new models and the need for public debate on the evolving state of the science. Advisors were appointed to the work programme who had been involved in the early stages of the test model development. Fifty-one laboratories from around the world, with approximately equal numbers from U.S.A, Europe (including U.K.) and Japan agreed to take part. The outline of the program was considered which included the following issues; i) identification of assay systems to be evaluated, ii) establishment of standardised protocols, iii) selection of test agents, iv) data collection systems, v) data review process, vi) joint data analyses. Some comments made by Dr Robinson on each of these six issues are given below :

3.52 The test systems selected for evaluation were those identified in the ICH guidelines.

Test System	Tumour Response
P53 Knock-out mouse	Genotoxic chemicals. 6 month treatment schedule appears adequate for positive controls
Tg.AC transgenic mouse	Genotoxic and some non-genotoxic carcinogens. Originally a dermal test, but need to also screen for response to oral administration in respect of pharmaceuticals. Noted that recent results suggested the dermal model did not respond to glycidol which was an unexpected finding.
Ras H2 Transgenic mouse	Genotoxic and some non-genotoxic chemicals. Broadest available published database. Evidence for target organ selectivity (lung, forestomach). Mechanism of response unclear.
XPA Repair Deficient mouse	UV light and genotoxic carcinogens. Very limited database available.
Neonatal mouse	Limited database available. Responds to genotoxic carcinogens.
SHE cell transformation assay	Some data already available in published literature on this assay.

3.53 Dr Robinson noted that transgenic mice cost on average approximately five times more than wild type mice. Given the scale of the HESI program, a number of pragmatic decisions were made regarding protocols. Thus three dose levels were studied per chemical and the number of animals used per dose level was 15 males and 15 females. A vehicle and high dose control in wild type animals were also included. Information from NTP bioassays and a 4-week range finding assay were used to assist in dose selection. In most instances this has resulted in adequate survival. One exception was chloroform where a number of animals had died at the high dose level within the 26 week-dosing period. Possible differences between wild type and transgenic mice with respect to the metabolism of chloroform were to be investigated. Expanded histopathology is being undertaken on control and high dose animals, with tissues from animals in the intermediate dose group retained. More recently it had been agreed that histological analysis of the jaw would be undertaken in Tg.AC mice due to several instances of odontoma (a rare tumour in mice) in previous studies. Genotyping of tumours will be undertaken.

3.54 HESI had selected well-studied compounds with extensive human data, predominantly pharmaceuticals.

3.55 An Assay Working Group (AWG) had been established for each of the models under consideration in order to establish criteria for evaluating tests, to ensure a common format for data collection and consistency in histopathology. The AWGs would also act as a focal point for data review and analyses.

3.56 The AWGs suggested additional areas for research. Proposals were reviewed from approximately 30 laboratories in respect of drug metabolism in transgenic mice, mutational analyses of tumours, tissue specificity of mechanisms, and compound specific mechanisms. Thus proposals funded in 1999 included, i) mechanisms of hormonal carcinogenesis in transgenic mouse models, ii) an evaluation of drug metabolising enzymes in transgenic and parental strains of mice, and iii) mechanism of carcinogenicity in p53 mice (tumour analysis).

3.57 Data review(s) were expected in late 1999/early 2000 and, histopathology database development by mid 2000. Meetings of the Joint Steering Committee/AWGs in early 2000. Data analyses by around June 2000. An open public workshop was planned in Washington DC around November 2000 to tie in with the simultaneous ICH meeting.

3.58 The ICH guidelines had made an impact on the strategy for carcinogenicity tests being adopted by pharmaceutical companies. Information from the Centre for Medicines Research showed that 18 out of 38 companies asked were using alternative short-term tests. Out of the 49 companies using short-term tests, the predominant models being used were the p53 knock-out (26.6%) and *ras* H2 (20.4%), although tests using rat initiator/promoter (18.4%), neonatal rodent assay (16.3%), Tg.AC (12.2%), and the XPA deficient (6.1%), mouse models were also being undertaken. Members heard that some data were available to show a drift in the response of Tg.AC hemizygous mice to the positive control (12-O-tetradecanoylphorbol 13 acetate) TPA. Southern Blot analysis of responder and non-responding Tg.AC mice showed evidence for a 2kb deletion in the transgene. It was not clear how prevalent the transgene instability was in earlier tests, although action was taken to re-derive the breeding colonies of mice. Dr Robinson noted that the transgene deletion in Tg.AC mice had been detected before any significant impact in the HESI program could have occurred.

3.59 A full summary of this presentation has been published on the COC internet site.

Topics Under Consideration

3.60 The following topics, which were discussed by the Committee at meetings held in 1999, are still under review:

Alcohol & Breast Cancer
Cancer incidence near Municipal Incinerators
Trends in mortality from Intrahepatic cholangiocarcinoma
Use of Accelerator mass Spectrometry in cancer risk assessment.

1999 Membership of the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

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Declaration of interests during the period of this report

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof P G Blain (Chairman)	NONE	NONE	Unilever plc	Research Studentship
Prof C Chilvers	ICI plc Tomkins Morgan Crucible Lloyds TSB British Telecom UTD Assurance Group Hardy's & Hanson Glaxo-Wellcome Shell Cadbury Schweppes SmithKline Beecham Ivory & Sime	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	NONE	NONE
Prof C Cooper	Halifax Norwich Union	Share Holder Share Holder	NONE	NONE
Prof A D Dayan	British Petroleum Glaxo-Wellcome Schering Plough SmithKline Beecham TI British Gas Research & Technology Daw Agrosciences	Share Holder Pension Consultant Consultant Share Holder Consultant Consultant	NONE	NONE
Prof P B Farmer	Chiroscience Abbey National plc Woolwich Alliance & Leicester	Share Holder Share Holder Share Holder Share Holder	Glaxo Scotia Zeneca	Research Studentship & Research Support Research Support Research Support
Prof D Forman	Halifax Woolwich	Share Holder Share Holder	NONE	NONE
Dr S J Kennedy	Unilever Quintiles	Share Holder Share Holder	NONE	NONE
Ms M Langley	NONE	NONE	NONE	NONE
Prof R F Newbold	NONE	NONE	NONE	NONE
Prof J M Parry	Albright and Wilson Compass Catering JIB Insurance National Power Powergen SmithKline Beecham	Share Holder Share Holder Share Holder Share Holder Share Holder Consultant	Boehringer Pfizer Welsh Water Glaxo/Wellcome BAT Astra	Grant Grant Grant Grant Grant Grant
Prof I F H Purchase	Astra Zeneca Bayer Celltec Rhone Poulenc	Consultant Share Holder Consultant Share Holder Consultant	NONE	NONE
Prof A G Renwick	International Sweeteners Association	Consultant	Hoffmann-La Roche Unilever SmithKline Beecham Pfizer FEMA	Research Support Research Support Research Support Grant Grant
Dr S Venitt	Abbey National plc Northern Rock	Share Holder Share Holder	NONE	NONE

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof G T Williams	Abbey National plc Norwich Union plc AMP Ltd Aphton Corporation	Share Holder Share Holder Share Holder Consultant	NONE	NONE

Annexes

Annex 1

Terms of Reference

To advise at the request of:

Department of Health
Ministry of Agriculture, Fisheries and Food
Department of the Environment, Transport and the Regions
Department of Trade and Industry
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 Executive
National Assembly for Wales
Northern Ireland Assembly
Other Government Departments

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

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

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

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

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

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

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

Annex 2

Declaration of Interests: a Code of Practice for Members

Introduction

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

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

Definitions

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

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

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

   b. trade associations representing companies involved with such products,

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

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

Different types of interest

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

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

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

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

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

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

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

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

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

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

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

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

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

7. Members should inform the Department in writing when they are appointed of their *current personal and non-personal* interests. Only the name of the company and the nature of the interest is required; the amount of any salary, fee, shareholding, grant etc need not be disclosed to the Department. An interest is current if the member has an on-going financial involvement with the chemical industry, eg if he or she holds shares in a chemical industry, has a consultancy contract, or if the member or the department for which he or she is responsible is in the process of carrying out work for the chemical industry. Members are asked to inform the Department at any time of any change in their *personal* interests, and will be invited to complete a declaration form once a year. It would be sufficient if changes in *non-personal* interests are reported in the annual declaration form following the change. (Non-personal interests involving less than £1000 from a particular company in the previous year need not be declared to the Department.)

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

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

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

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

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

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

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

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

Record of interests

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

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

Annex 3

Glossary of Terms

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

ACUTE TOXICITY Effects that occur over a short period of time (hours or few days) immediately following exposure.

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).

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

ALANINE AMINOTRANSFERASE An enzyme that, when elevated activity is detected in serum, may indicate damage to certain organs.

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

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

ANEUGENIC Inducing aneuploidy (qv).

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

ASPARTATE AMINOTRANSFERASE An enzyme that, when elevated activity is detected in serum, may indicate damage to certain organs.

ASSAY A procedure for measurement or identification.

B6C3F1 MICE a particular strain of mice.

BIAS An inference which at any stage of an epidemiological investigation tends to produce results that depart systemically from the true values (to be distinguished from random error). The term does not necessarily carry an imputation of prejudice or any other subjective factor such as the experimenter's desire for a particular outcome.

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

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 biological concentration or similar quantity which acts as a surrogate for a biological effect.

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').

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

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

CELL TRANSFORMATION ASSAY See Transformation.

CENTRILOBULAR HEPATOCYTE VACUOLISATION Vacuolation of cells surrounding the central vein in a liver lobule.

CHROMOSOME ABERRATION Collective term of particular types of chromosome damage induced after exposure to exogenous chemical or physical agents which damage the DNA. (see clastogen).

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

CLASTOGEN An agent that produces chromosome breaks and other structural aberrations such as translocations (qv). Clastogens may be viruses or physical agents as well as chemicals. Clastogenic events play an important part in the development of some tumours.

COHORT A defined population.

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

CONGENER Compounds varying in chemical structure but with similar biological properties.

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.

CYTOCHROME Haem proteins that catalyse electron transfer reactions. Cytochrome P450 is a collective term for an extensive family of haem proteins involved in enzymic oxidation of a wide range of substances and their conversion to forms that are more easily excreted. In some cases the metabolites produced may be reactive and may have carcinogenic potential.

CYTOGENETIC Concerning chromosomes, their origin, structure and function.

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

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

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

DOMINANT LETHAL ASSAY See Dominant Lethal mutation.

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

ENDOMETRIAL Relating to the lining of the uterus.

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

EPIDEMIOLOGY Study of the distribution and, in some instances, the causal factors of disease in communities and populations.

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

ERYTHEMA Reddening of the skin due to congestion of blood.

ERYTHROCYTE Red blood cell.

EXOGENOUS Arising outside the body.

FLUORESCENCE IN-SITU HYBRIDISATION A technique which allows individual chromosomes and their centromeres (qv) to be visualised in cells.

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

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

FORESTOMACH (See glandular stomach).

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

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

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

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

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

HEPATIC Pertaining to the liver (see hepatocellular).

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

HEPATOTOXIC Causing damage to the liver.

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

HYPERTROPHY An increase in the size of cells or tissues.

INTRAPERITONEAL Within the abdominal cavity.

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

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

IPCS The World Health Organization's International Programme on Chemical Safety.

ISOMERS See ß-isomer.

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

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

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

LIGAND A molecule which binds to a receptor.

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

LIPOPHILIC 'Lipid liking' – a substance which has a tendency to partition into fatty materials.

LYMPHOCYTE Type of white blood cell.

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

MALIGNANCY See 'tumour'.

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

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

METABOLISM Changes made to a compound by biological systems to modify its properties.

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

METAPHASE Stage of cell division (mitosis and meiosis) during which the chromosomes are arranged on the equator of the nuclear spindle (the collection of microtubule filaments which are responsible for the movement of chromosomes during cell division). As the chromosomes are most easily examined in metaphase, cells are arrested at this stage for microscopical examination for chromosome aberrations (qv) – known as metaphase analysis.

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

MICRONUCLEI Isolated or broken chromosome fragments which are not expelled when the nucleus is lost during cell division, but remain in the body of the cell forming micronuclei. Centromere positive micronuclei contain DNA and/or protein material derived from the centromere (qv). The presence of centromere positive micronuclei following exposure to chemicals can be used to evaluate the aneugenic (qv) potential of chemicals.

MICRONUCLEUS TEST See Micronuclei.

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

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

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

MRC Medical Research Council.

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'.

NEOPLASTIC Abnormal cells, the growth of which is more rapid that that of other cells.

NEUROBEHAVIOURAL Of behaviour determined by the nervous system.

NEUROTOXICITY Toxicity to the nervous system.

NOAEL No Observed Adverse Effect Level, the highest administered dose at which no toxic effect has been observed.

NO OBSERVED ADVERSE EFFECT LEVEL (NOAEL) The highest administered dose at which no toxic effect has been observed.

NON-GENOTOXIC See 'carcinogens'.

ODDS RATIO (OR) A measure of association which is interpreted similarly to the Relative Risk (see Relative Risk); it is similar in magnitude to the Relative Risk in the case of rare diseases.

OECD Organization for Economic Cooperation and Development.

OEDEMA Excessive accumulation of fluid in body tissues.

OESTROGEN Is the hormone which develops and maintains female bodily characteristics.

OESTROGEN ACTIVITY Hormonal activity of the female steroid hormone oestrogen or its analogues.

ORGANOCHLORINE A group of chemical compounds used as pesticides.

^{32}P POSTLABELLING A sensitive experimental quantitatively method designed to measure low levels of DNA adducts induced by chemical treatment.

PHYTOESTROGEN Phytoestrogens are plant chemicals that are similar to the human female hormone oestrogen but are much less potent (10,000–140,000 times less potent in animal models).

PLASTICISER A substance which increases the flexibility of certain plastics.

POLYMER A very large molecule comprising a chain of many similar or identical molecular sub units (monomers) joined together (polymerized). An example is the polymer glycogen, formed from linked molecules of the monomer glucose.

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

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

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

RELATIVE RISK A measure of the association between exposure and outcome. The rate of disease in the exposed population divided by the rate of disease among the unexposed population in a cohort study. A RR of 2 means that the exposed group has twice the disease risk compared to the unexposed group

RENAL Relating to the kidney.

SCF The European Commission's Scientific Committee on Food.

SERUM The fluid remaining after blood has clotted.

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

TDI See 'Tolerable Daily Intake'.

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

TERATOGENIC RISK Risk that a compound will cause developmental abnormalities in the foetus.

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

TOLERABLE DAILY INTAKE (TDI) An estimate of the amount of contaminant, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risks.

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

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

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.

TRANSGENIC Genetically modified to contain genetic material from another species (see also genetically modified organism).

TRANSGENIC ANIMAL MODELS Animals which have extra (exogenous) fragments of DNA incorporated into their genomes. This may include reporter genes to assess *in-vivo* effects such as mutagenicity in transgenic mice containing a recoverable bacterial gene (*lac Z or lac I*). Other transgenic animals may have alterations of specific genes believed to be involved in disease processes (eg cancer). For example strains of mice have been bred which carry an inactivated copy of the p53 tumour suppressor gene or an activated form of the *ras* oncogene which may enhance their susceptibility of the mice to certain types of carcinogenic chemicals.

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

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

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.

WHO-IPCS/ECEH The World Health Organization's European Centre for Environment and Health and the WHO's International Programme on Chemical Safety.

XENOESTROGENS A 'foreign' compound, i.e. not natural to the body, with oestrogenic activity.

XENOBIOTIC A chemical foreign to the biologic system.

XENOESTROGENS Oestrogens that are foreign to the body.

Annex 4

Index to Subjects and Substances considered in previous Annual Reports of the Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

Annex 5

Index of Considerations by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment of MAFF Food Surveillance Papers

Annex 6

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

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

1992 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321604-1 Price £11.70.

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Annex 7

Improved Arrangements for Openness

Introduction

1. The Committee on Toxicity (COT) and its sister committees the Committee on Mutagenicity (COM) and Committee on Carcinogenicity (COC) are non-statutory independent advisory committees who advise the CMO and, through the CMO, the Government on a wide range of matters concerning chemicals in food, consumer products and the environment.

2. The Government is committed to make the operation of advisory committees such as the COT/COM/COC more open and to increase accountability. Proposals have been published in "Quangos-Opening the Doors" (Cabinet Office, July 1998). The COT/COM/COC have recently considered a number of options for greater openness of Committee business. There was a high level of agreement between the COT/COM/COC regarding the adoption of proposals for greater openness.

3. In discussing these proposals the Committees were aware that the disclosure of information which is of a confidential nature and was communicated in circumstances importing an obligation of confidence is subject to the common law of confidentiality. Guidance is set out in the Code of Practice on Access to Government Information (second edition, 1997). Thus an important aspect of implementing initiatives for greater openness of Committee business concerns setting out clear guidelines for the handling of information submitted on a confidential basis.

General procedures for greater openness

4. The Committees agreed that the publication of agendas, finalised minutes, agreed conclusions and statements (subject to the adoption of appropriate procedures for handling commercially sensitive information) and appointment of a lay/public interest member to each Committee would help to increase public scrutiny of Committee business. The Committees also agreed that additional open meetings on specific topics where interest groups, consumer organisations etc could attend and participate should be held.

5. A summary of the proposals is tabulated below. A more detailed outline of procedures regarding products where confidential data has been reviewed is given in paragraphs 11-13.

6. The Committees stressed that, in view of the highly technical nature of the discussions, there was a need for all documents released to be finalised and agreed by the Committee, ie any necessary consultation with Members and Chairman should be completed before disclosure.

7. Statements and conclusions should summarise all the relevant data, such as information regarding potential hazards/risks for human health in respect of the use of products and chemicals, and any recommendations for further research.

8. The Committees will be asked for an opinion based on the data available at the time of consideration. It is recognised that, for many chemicals, the toxicological information is incomplete and that recommendations for further research to address these gaps will form part of the Committee's advice.

9. The release of documents (papers, minutes, conclusions and statements) where the COT/COM/COC has agreed an opinion on the available data but where further additional information is required in order to finalise the Committee's conclusions, needs to be considered on a case-by case basis. The relevant considerations include the likelihood that such additional data would alter the Committee's conclusion, any representations made by a

company about, for example, commercial harm that early disclosure could cause and also the public interest in disclosure.

10. In the event that the Committees need to consider an item over several meetings, it might be necessary to keep relevant documents (eg papers and minutes) confidential until an agreed opinion (eg statement) is available.

Summary of proposals for committee openness.

Issue	Proposals	Comment
Open meetings on specified topics (eg invited audience, interest groups, consumer organisations, professional societies).	Agreed. Suggestions include meeting at time of release of Annual Report. External consultation on identifying topics for such meetings.	Meetings would be on generic issues in chemical toxicology, carcinogencity, mutagenicity and risk assessment. There would be no discussions of individual commercial products.
Agenda	Agreed	Made publicly available via Internet site prior to meeting.
Papers	Agreed	Finalised papers in to be made available upon request. Confidential information/annexes to be removed.
Minutes*	Agreed	Anonymised minutes made available upon request and on Internet site after appropriate consultation with members and agreement by the full committee.
Conclusions/statements*	Agreed	Agreed conclusions/statements published as appropriate including via the Internet and also made available on request.
Annual Report*	Agreed	Publish in accordance with procedures for previous years.

* Procedures for handling confidential information outlined in para 11-13 below.

Procedures for handling confidential information

Background

11. COT/COM/COC quite often consider information which has been supplied in confidence. For the most part this comprises information which is commercially sensitive. For example, this could include product formulations/specifications, methods of manufacture, and reports of toxicological investigations and company evaluations and safety assessments.

12. The normal procedure in the past has been to publish a summary of the Committee's advice in the Annual Report and to ask companies to release full copies of submitted reports for retention by the British Library at the completion of a review. Given the clear Ministerial commitment to the publication of detailed information regarding the activities of advisory committees, and in particular following the assessment of products which are already available to the general public, the COT/COM/COC have begun to adopt where possible a more open style of business where detailed statements have been published via the Internet soon after they have been finalised.

13. Except in cases where there is legislation under which information has been submitted and which deals with disclosure and non-disclosure, the general principle of the common law duty of confidentiality will apply. This means that any information which is of a confidential character and has been obtained in circumstances importing a duty of confidence may not be disclosed unless consent has been given or there is an overriding public interest in disclosure (such as the prevention of harm to others). The following procedure will be adopted which allows confidential information to be identified, assessed and appropriate conclusions/statements to be drafted and published on the basis of a prior mutual understanding with the companies. There is scope for companies to make representations also after submission of the information and prior to publication regarding the commercial sensitivity of data supplied and to comment on the text of statements which are to be published. However, companies would not have a right of veto in respect of such statements.

Procedures prior to committee consideration

Initial discussions

Upon referral to COT/COM/COC the Secretariat will liaise with the relevant company supplying the product in the UK to;

i) Clearly state the policy of Committee openness (as summarised above).

ii) To identify and request the information needed by the COT/COM/COC (eg test reports, publications etc).

Confidential data

iii) The company will be asked to clearly identify any confidential data and the reason for confidentiality.

Handling confidential data

iv) The procedures by which the COT/COM/COC will handle confidential data and the public availability of papers, minutes, conclusions and statements where reference is made to such data will be discussed with the company prior to submission of papers to the Committee(s). The general procedures for handling documents are outlined in paragraphs 4-10 above. Companies will be informed that confidential annexes to Committee papers (eg where detailed information supplied in confidence such as individual patient information and full study reports of toxicological studies) will not be disclosed but that other information will be disclosed unless agreed otherwise with an individual company.

v) The following is a suggested list of information which might be disclosed in COT/COM/COC documents (papers, minutes, conclusions and statements). The list is not exhaustive and is presented as a guide.

a) name of product (or substance/chemical under consideration),

b) information on physico-chemical properties,

c) methods of rendering harmless,

d) a summary of the results and evaluation of the results of tests to establish harmlessness to humans,

e) methods of analysis,

f) first aid and medical treatment to be given in the case of injury to persons.

g) surveillance data (eg monitoring for levels in food, air, or water).

Procedures during and after Committee consideration.

vi) The timing of release of Committee documents (papers, minutes, conclusions and statements) where the item of business involved the consideration of confidential data would be subject to the general provisions outlined in paragraphs 4-10 above. Documents would not be released until a Committee – agreed conclusion or statement was available.

vii) The most important outcome of the Committee consideration is likely to be the agreed statement. Companies will be given an opportunity to comment on the statement prior to publication and to make representations (for example, as to commercial sensitivities in the statement). The Chairman would be asked to consider any comments provided, but companies would not be able to veto the publication of a statement or any part of it. Companies will continue to be asked to release full copies of submitted reports for retention by the British Library at the completion of a review.

Introduction of new arrangements

14. These new arrangements will be announced by press release and publication on the Committees' Internet sites and will be adopted by the COT/COM/COC during 1999.

Annex 8

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