Committee on
TOXICITY

Committee on
MUTAGENICITY

Committee on
CARCINOGENICITY

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

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and the Environment

Annual Report

2000

Contents

ABOUT THE COMMITTEES

This is the tenth 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 a brief toxicological background to the Committees' decisions.

The year 2000 has seen changes in the administration of the Committees. With the formation of the Food Standards Agency on 1 April 2000, the Secretariats became the joint responsibilities of the Food Standards Agency and the Department of Health. The Food Standards Agency has prime responsibility for the COT and the Department of Health for the COM and COC. The terms of reference of the Committees has been changed to include the Food Standards Agency in the list of departments and bodies to which the Committees provide advice (see Annex 1).

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

A number of important changes have been made in recent years in the way Government departments are required to deal with appointments to the advisory committees for which they have responsibility. These principles are set out in the Nolan Report on Standards in Public Life. Arising from this, all future appointments to the COT, COM and COC will follow the best practice set out in Guidance issued by the Office of the Commissioner for Public Appointments (OCPA). Appointments are made on the basis of merit, to form committees with a balance of relevant skills and backgrounds. The appointment process will be open and Departments will be required to justify any departures from best practice. Members are appointed for fixed time periods, generally three years, and are eligible for reappointment at the end of their terms. It will, however, be unusual for a member to be appointed beyond the 10 year maximum allowed for in the OCPA Guidance.

In common with other independent advisory committees the members are required to follow a Code of Conduct which also gives guidance on how the commercial interests should be declared. 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. The Code of Conduct is at Annex 2 and Annex 3 describes the Committees' policy on openness.

Annex 4 contains a glossary of technical terms used in the text. Annex 5 is an alphabetical index to subjects and substances considered in previous reports. Previous publications of the Committees are listed in Annex 6.

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 independently of each other in their areas of expertise but will, if need be, work closely together. This is helped by the Secretariats' close working relationship, which is now maintained by the sharing of responsibilities between the Food Standards Agency and Department of Health. 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.

The Committees' procedures for openness include the publication of agendas, finalised minutes, agreed conclusions and statements. These are now published on the internet at the following addresses:

COT:	http://www.foodstandards.gov.uk/committees/cot/summary.htm
COM:	http://www.doh.gov.uk/com.htm
COC:	http://www.doh.gov.uk/coc.htm

This report contains summaries of the discussions and includes the Committees' published statements in full in order to fulfil the obligation to publish statements both electronically and in hard copy.

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

Preface

The COT has again discussed a wide range of toxicological problems over the past year. Many of these have related to chemicals that may be present in food, including additives (intense sweeteners), processing aids (enzymes), supplements (French Maritime Pine Bark Extracts), food contact materials (terephthalic and isophthalic acids), natural constituents (fluorine, bromine and iodine) and contaminants (dioxins and dioxin-like polychlorinated biphenyls). Dioxins and dioxin-like polychlorinated biphenyls have formed three separate discussion items, with consideration of results of general dietary exposure, of a specific survey of free-range eggs as an indicator of environmental exposure, and the start of a major review of the tolerable daily intake of these contaminants.

The COT has also been asked to advise on the safety of breast implants, on an environmental pollutant, on aspects of research commissioned by the Food Standards Agency and the Department of Health and on papers dealing with the workings of scientific advisory committees. We are pleased to note that the Committee already follows most of the procedures considered to constitute best practice for advisory committees.

2000 was also a busy year for COT Working Groups. An open meeting was held in February to consult on the draft report of the Working Group on Food Intolerance. The final report from this Working Group was published in July 2000, under the title “Adverse Reactions to Food and Food Ingredients”. Two new Working Groups commenced work during the year, on Phytoestrogens in April, and on Risk Assessment for Mixtures of Pesticides/Veterinary Medicines in December, and expect to report in 2001 and 2002, respectively.

The predominance of food-related issues in the COT agendas led to the decision that the lead responsibility for the Secretariat should be moved to the Food Standards Agency, on its formation in April 2000.

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

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

Adverse Reactions to Food and Food Ingredients

1.1 The COT Working Group on Food Intolerance completed its review of adverse reactions to food. A draft report was the subject of consultation at an open meeting of the COT in February 2000 and the final report was published in July 2000. The report, entitled "Adverse Reactions to Food and Food Ingredients" may be obtained from the COT secretariat at: The Food Standards Agency, Room 511C, Aviation House, Kingsway, London WC2B 6NH or by contacting Food Standards Agency Publications, PO Box 369, Hayes, Middlesex UB3 1UT (tel: 0845 606 0667; fax: 020 8867 3225).

Alitame

1.2 Alitame is an intense sweetener that was initially considered by the COT in 1989. Additional data were submitted during the period 1990-1994 and in 1998, both in response to requests made by the COT and as a result of requests made to the company by other regulatory bodies.

1.3 In 1998, the COT established an Acceptable Daily Intake of 0.3 mg/kg bw per day. This was subsequently confirmed in 1999, following submission of additional information by the company. The basis for determining the Acceptable Daily Intake was a significant elevation in liver weight in dogs treated with alitame for 18 months. There was a significant increase in liver weight at 500 mg/kg bw per day and a non-significant increase in male dogs receiving the next lowest dose (100 mg/kg bw per day). The COT considered that this finding was unusual and that it would be prudent to regard the dose of 100 mg/kg bw per day 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 inter-species and 10 for intra-individual variability were then applied, resulting in the Acceptable Daily Intake of 0.3 mg/kg bw per day.

1.4 The COT had also previously noted other observations of potential concern. Enzyme induction was reported in the dog study, with a NOAEL of 100 mg/kg bw per day. A study in diabetics given alitame at 10 mg/kg bw for 90 days, had reported a number of cardiovascular complications in some patients of both the alitame and placebo groups during the follow-up period.

1.5 In 2000, the company submitted the results of a new, more comprehensive study in which diabetics were administered alitame at 10 mg/kg bw per day. Particular emphasis was given to cardiovascular effects, the results indicating that alitame was well-tolerated and therefore the company asked for the Acceptable Daily Intake to be increased.

1.6 The COT noted that the diabetic studies had not included an assessment of enzyme induction, and the results of statistical analysis of the new study were questioned. The COT agreed that the new data were not adequate to justify decreasing the uncertainty factor and therefore did not warrant review of the Acceptable Daily Intake for alitame.

Breast implants

1.7 In 1999 the COT provided an emergency consideration for the Department of Health and the Medical Devices Agency (MDA) on the safety of breast implants containing soya bean oil. This contributed to the voluntary withdrawal of the Trilucent‘ breast implant due to a lack of adequate safety data. The MDA subsequently initiated a review on the safety and performance of all recently introduced breast implant fillers.

1.8 MDA reviewed the technical information provided by one manufacturer, Poly Implant Prosthesis, on their hydroxylpropyl cellulose hydrogel pre-filled breast implant. MDA asked the COT to consider the report of a study in which rats received subcutaneous injections of the filler material and were then observed for periods of up to 12 weeks.

1.9 The COT considered the rat study to be very limited in nature. There were serious deficiencies in the design, performance and reporting of the study. COT concluded that the findings of the rat study could not be discounted and suggested that a study employing a considerably longer period of observation should be conducted on the filler material. A statement outlining the conclusions reached by the COT is included at the end of this report.

1.10 On receipt of these conclusions MDA initiated regulatory activity which led to the voluntary withdrawal of these implants by the manufacturer. This was a precautionary measure until sufficient information to address MDA’s concerns over the manufacturer’s biological safety assessment of the device is available. The withdrawal was made public by an MDA Device Alert (MDA DA2000(07)) on 11 December 2000.

1.11 On 5 December MDA requested an emergency consideration of data on a second hydrogel filling material used in NovaGold‘ breast implants. This filling material was a polyvinylpyrrolidone and guar gum gel. As described in the 1999 Annual Report, emergency consideration can be undertaken with the agreement of the COT Chairman and provides the collated opinions of a limited number of individual members with particularly relevant expertise. The information was circulated to a number of members on 5 December. Their opinions were received by 8 December and were passed to MDA. MDA released an MDA Device Alert (MDA DA2000(08)) on 11 December,

which identified inadequacies in the manufacturer's biological safety assessment and concluded that as a precautionary measure these implants should not be implanted until the concerns have been addressed.

Code of Practice for Scientific Advisory Committees

1.12 In July 2000, the Office of Science and Technology published a consultation paper, inviting comments on a proposed code of practice for Scientific Advisory Committees. The paper outlined proposed guidelines for Scientific Advisory Committees and complemented a second document on "Review Of Risk Procedures Used By The Government's Advisory Committees Dealing With Food Safety", which was published in September 2000 and also discussed by the COT (see paragraphs 1.72 – 1.78). The consultation paper described the duties, rights and responsibilities of committee members and their independence from the committee's secretariat, stressing the need for inclusivity, transparency and proportionality and raising the issue of the manner in which confidential information is handled. It stressed the need for clear explanation of levels and types of uncertainty, and how this information is incorporated into advice, and called for training of committee members in communication skills.

1.13 The Secretariat noted that producing a set of uniform guidelines would enable interested parties, the public and the media to judge and comment on the standards required of such committees.

1.14 Members agreed that the COT already follows the procedures defined as far as is practicable. COT noted that considerable steps have been made to increase transparency. However there was concern that publication of some material or attribution of comments made during a meeting could compromise personal security.

1.15 Members also discussed the issue of confidential papers and noted that steps have been taken to reduce or eliminate use of material classified as confidential. Where commercial confidentiality precluded full publication, it was suggested that at least part of the paper and the Committee's deliberations should be published. The Secretariat informed Members that where material was "commercial in confidence", it is normal procedure to approach companies to determine whether they would be willing for part, if not all, of the papers concerning their product to be made publicly available. (Procedures for openness are outlined in Annex 3).

1.16 Members noted the suggestion that Chairs and Secretariats should review board members interests, taking into account: ”the proportion of the total equity value which is held” (where share holdings are under consideration). It was stressed that it is well-established COT procedure to tabulate Members’ interests in the annual report, but that it would be difficult to quantify interests.

1.17 With regard to Secretariat duties, Members noted that the COT would continue to encourage submission of Secretariat reviews to journals for publication in peer-reviewed journals.

Dioxins and dioxin-like PCBs - Dietary exposure

1.18 COT was asked to consider estimates of dietary exposure to dioxins and dioxin-like PCBs derived from the 1997 Total Diet Study. Dietary exposures to dioxins and dioxin-like PCBs had previously been estimated from the 1982 and 1992 Total Diet Studies. Since these earlier data were published, the WHO had recommended /revised Toxic Equivalency Factors (TEFs) for dioxins and dioxin-like PCBs, which were endorsed by the COT in 1998. Therefore, the 1982 and 1992 exposure estimates had been recalculated using these latest WHO-TEFs in order to be directly comparable with the new data.

1.19 In comparison with earlier similar surveys, dietary exposures to dioxin-like compounds, on a total toxic equivalent (TEQ) basis, for all three age groups, showed a continuing downward trend.

1.20 COT agreed the data indicated that changes in analytical sensitivity, or in the number of food groups analysed, had not contributed significantly to the decline in exposure. COT therefore agreed it should be stressed that the decline in exposure to these compounds was real and not an experimental artefact.

1.21 The COT agreed a statement (included at the end of this report) on dietary exposure to dioxins and dioxin-like PCBs, concluding that the current concentrations of dioxins and dioxin-like PCBs in food are unlikely to pose a risk to health.

Dioxins and dioxin-like PCBs in free range eggs

1.22 COT was informed of a Food Standards Agency survey of dioxins and dioxin-like PCBs in free-range hen and duck eggs. The aim of the survey was to examine the use of eggs as indicators of environmental contamination and the results were not considered as being representative of free-range eggs on sale throughout the UK.

1.23 Dietary exposure had been estimated using the concentrations of these compounds in free-range eggs together with age-specific food consumption data, and used different scenarios based upon average or maximum concentrations in the eggs.

1.24 Advice was sought from the COT on the public health significance of the estimated dietary exposures to dioxins and dioxin-like PCBs based on data from this survey of free-range eggs. Members were also asked to consider whether the survey was sufficiently robust to draw conclusions applicable to consumers of free-range eggs and whether the different exposure scenarios were realistic with respect to anticipated consumption patterns of free-range eggs.

1.25 COT agreed that the survey should be considered as a hypothesis generating study, indicating that analysis of free-range eggs is a potentially useful technique for investigating environmental contamination. However, because of the sampling methodology and the limited number of samples taken, the data from this survey should not be used to estimate dietary exposures to these compounds for consumers of free-range eggs. There was also a paucity of data on concentrations of these compounds in other types of eggs, such as battery hen eggs, against which meaningful comparisons could be made.

1.26 The COT statement on the survey is included at the end of this report.

Di-isopropylnaphthalenes

1.27 Di-isopropylnaphthalenes (DIPN) are used as solvents for the colour former in carbonless copy-paper, which may be included in recycled paper used in making board for food-packaging. Treatment of the recycled fibres may fail to remove all of the DIPN and thus some may be present in the finished board and could migrate into food. The Committee gave consideration to a survey on DIPN during 1998, and agreed that the toxicological information was inadequate and that additional studies should be submitted within 3 years. (See paragraphs 1.5 – 1.6 of 1998 Annual Report).

1.28 COM reviewed new mutagenicity data on DIPN in February 2000. It concluded that DIPN could be regarded as non-mutagenic and that no further mutagenicity testing was required (see paragraphs 2.11 - 2.12 of this report). COT was asked whether, in light of the new mutagenicity data and the advice from the COM, it wished to revise its previous position on DIPN and its earlier requirement for a long-term study.

1.29 The COT considered that, even though DIPN could be regarded as non-mutagenic, there was still a need for further safety studies. In view of the fact that there were no longer concerns that DIPN may be a genotoxic carcinogen, and that intakes of DIPN were low, a carcinogenicity study was no longer required. Instead the COT agreed that a 28-day sighting study for dose selection, followed by a 90-day study would be acceptable. Because the data on human exposure levels are limited, the Committee stressed the importance of ensuring that appropriate dose levels were used in the proposed studies, achieving some toxicity at the highest dose.

1.30 COT re-iterated its previous advice that it would be prudent to ensure that the levels of DIPN in food packaging made from recycled paper and board should be kept as low as reasonably practicable.

Enzyme submission - Amano 90

1.31 COT considered the Amano 90 submission by postal consultation in 1999. At that time COT required further evidence to support the company's claim that no residual enzyme activity would be expected in bread after baking, further validation of the enzyme assays and an increase in the frequency of testing for mycotoxins and antibacterial activity. COT agreed to recommend a twelve month temporary clearance of Amano 90 for use in bread making, while awaiting the additional data from the company.

1.32 In response the company had submitted the results of a study aiming to demonstrate that Amano 90 is inactivated during the baking process, together with data on the repeatability of the assay used to determine enzyme activity. The company also provided written assurance that one in every four batches of Amano 90 would be tested for mycotoxins and antibacterial activity.

1.33 In the study submitted, Amano 90 was added to flour, at the recommended concentration and also at a 40-fold higher concentration, either prior to or subsequent to baking. Enzyme activity was only detected in the bread sample in which the higher concentration of Amano 90 was added after baking, being below the limit of detection in all the other samples. However, COT considered that the method routinely used to assay enzyme activity in production batches of Amano 90 was not sufficiently sensitive to demonstrate

enzyme inactivation during baking of bread containing the recommended concentration of enzyme. COT recommended that a more sensitive method should be developed, and a limit of detection in bread defined. The improved assay should then be used in a repeat study, with duplicate analyses.

1.34 COT considered that the data submitted were not adequate to demonstrate the validity of the enzyme assay. COT agreed that a better description of the data would be helpful and this should include data on the linearity of the enzyme assay.

1.35 COT welcomed the statement of intent to increase routine testing of mycotoxins and antibacterial activity in at least one in four batches but was unable to recommend full clearance of the enzyme preparation, Amano 90.

Enzyme submission - Chymosin

1.36 Chymosin had previously been evaluated by COT and had been granted clearance. The manufacturer had now developed a modified recovery and purification procedure for this enzyme preparation.

1.37 COT considered that additional information was required to confirm the similarity between the product of the modified purification process and the original product. In addition several technical issues relating to the modified process required further clarification, as did the current specification for the enzyme preparation. The Committee agreed to one year's temporary clearance whilst this further information was provided.

Enzyme submission - Lipase D

1.38 COT conducted a postal consultation of a submission seeking approval of an immobilised enzyme preparation, Lipase D, to be used in manufacture of yellow fat spreads. The responses were discussed and agreed by the full Committee. COT agreed that the level of detail submitted on the manufacturing processes was appropriate and recommended that:

- the production strain of *Rhizopus oryzae* should be deposited with a recognised culture collection;
- the specification for the immobilised enzyme should include limits for heavy metals;

- testing for moulds and yeasts should be conducted on every batch and mycotoxins and antibacterial activity should be analysed in at least one in four batches;

- in the absence of toxicological data on a polymer used in the process, evidence that this polymer is not found in the final inter-esterified product or products should be provided.

1.39 COT agreed to recommend a two-year temporary clearance for the use of immobilised Lipase D in production of yellow fat spreads, pending submission of the requested analytical data.

Enzyme submission - Newlase

1.40 Newlase was granted temporary clearance pending the submission of further data in 1994. Additional data were submitted in 1998 and COT recommended further temporary clearance, pending the submission of a satisfactory method for the detection of the mycotoxin rhizoxin. Data on the detection of rhizoxin, submitted to the COT in 1999, were considered inadequate. The company subsequently submitted a revised methodology.

1.41 COT considered the new methodology would be sufficiently robust to demonstrate the absence of rhizoxin, subject to some additional requirements. These modifications involved the analysis of an appropriate Newlase sample spiked with rhizoxin with each batch and specification of the percentage recoveries of spiked samples compared to the equivalent concentration analysed by direct injection as part of the same analytical run.

1.42 COT agreed to extend the temporary clearance for an additional two years, provided that the recommended amendments to the protocol were adhered to and that batches of Newlase in which rhizoxin was detected should not be marketed. COT requested that during this period, the company collate analytical data on routine rhizoxin analyses of at least one in every four Newlase batches. These data should be submitted so that the COT could be assured that the methodology was adequate for routine assay of production batches of Newlase.

Fluorine, bromine and iodine

1.43 COT was informed of the results of analyses of fluorine, bromine, and iodine on samples collected for the 1997 Total Diet Study. COT was also provided with estimates of mean population, and mean and high-level adult consumer dietary intakes of fluorine, bromine, and iodine. Dietary intakes for age groups other than adults had not been estimated.

1.44 It was noted that there are no guidelines for fluorine against which to assess estimated dietary intakes. COT will await the findings of a review of fluorine by the Expert Group on Vitamins and Minerals (EVM) before considering any potential effects associated with intake of this element.

1.45 COT noted that since the essentiality of bromide is unclear, the FAO/WHO ADI of 0-1 mg bromide/kg body weight should be considered as a Tolerable Daily Intake (TDI) of 1mg bromide/kg body weight. It was agreed that the estimated intakes of bromide were not a cause for concern.

1.46 With regards to dietary intakes of iodine, COT confirmed that its 1999 advice (See paragraph 1.17 of 1999 Annual Report) still applied ie these intakes of iodine are unlikely to pose a risk to health. However, it reiterated its previous recommendation on the need for investigation of the bioavailability of iodine in milk and indicated that it may wish to reconsider its advice in light of the forthcoming findings of the EVM review of iodine. A statement on this study is included at the end of this report.

Food Standards Agency funded research and surveys

1.47 COT was informed that most of the current research portfolio within the Food Standard Agency (FSA) is based on research programmes inherited from MAFF. At the request of the FSA Board, a Research Review Group had been established to review research within the FSA and ensure that the overall research strategy and priorities reflect the FSA future requirements. The Group is expected to hold three meetings and is due to report in the spring of 2001.

1.48 The Review Group had set up a Working Party, comprising senior Agency officials and outside independent experts including academics, other research funders, consumer groups and industry, to be responsible for the detailed review. COT was informed that the Working Party is conducting a consultation exercise and was invited to contribute. COT requested and received clarification relating to:

- funds for research on risk management/risk communication;
- funds for research on animal feedstuffs;
- the basis for collaboration with MAFF and DH.
- openness and responsiveness to researchers with suggestions for new areas to be included on research agendas

1.49 COT was not in favour of making long term commitments to specific "centres of excellence", which could deter other potential applicants and inhibit development of new areas of research. However, it was agreed that longer term funding is needed to attract and retain good research staff. The Committee were informed that most research contracts cover a three-year period.

1.50 The issue of quality assurance for research was raised: COT was informed that there is a need to develop quality assurance criteria for aspects such as ensuring management protocols within laboratories and independent appraisal of the quality of the work.

1.51 COT agreed that the design of surveys should take into account the need to interpret human health implications of the data.

French Maritime Pine Bark Extracts

1.52 COT had reviewed French Maritime Pine Bark Extract on previous occasions (see paragraph 1.7 of 1998 and paragraph 1.11 of 1999 Annual Reports) and noted the possibility that the product might contain allergenic proteins. The manufacturers had submitted new information addressing this issue.

1.53 COT considered that elemental analysis for nitrogen was not sufficiently sensitive and could not be used to exclude the possibility that the extract contained allergenic proteins. The SDS-PAGE analysis was considered to be more reliable but more information on the method used was needed, particularly in view of the fact that pine bark extract was a complex material. In addition, each analysis should include a concurrent control.

Health effects in populations living close to landfill sites

1.54 In 1998, COT commented on a SAHSU (Small Area Health Statistics Unit) proposal for a study on health effects in populations living close to landfill sites (see paragraphs 1.9 - 1.15 of 1998 Annual Report). The protocol had been revised following identification of all relevant sites, and the Committee was asked to consider the amended protocol and comment on whether it was appropriate to proceed with the study.

1.55 The Committee noted that the primary objective of the study was to test the hypothesis that living near a landfill site is associated with an excess risk of giving birth to a child with a congenital anomaly or of low birth weight, or with an excess risk of stillbirth. The secondary objective was to test the

hypothesis that living near a landfill site is associated with an excess risk of certain cancers.

1.56 After discussion of aspects of the study design and possible confounding factors, the Committee considered that it was appropriate to proceed with the study but urged caution in interpretation of the results.

Hexachlorobutadiene

1.57 Hexachlorobutadiene (HCBD) is formed as a by-product during the manufacture of chlorinated solvents. COT was informed of public health concerns related to possible prolonged exposure to HCBD in the vicinity of a disused waste dump in a quarry in Cheshire, and was asked to provide advice on the toxicity of HCDB.

1.58 COT noted that advice had been sought from some members of the COM, who had reviewed the mutagenicity data on HCBD. They had advised that it was prudent to assume that HCBD is an *in vivo* somatic cell mutagen. COT therefore agreed that it was not possible to establish a safe level in relation to cancer or to identify a TDI. Thus, it would be more appropriate to determine margins of exposure, by comparing the measured air levels with the doses producing effects in the toxicology studies.

1.59 On the basis of some conservative approximations, the NOAEL for non-cancer effects of 0.2mg/kg bw/day in animal studies was estimated to be equivalent to continuous inhalation of an air level of 60ppb HCBD. The effect level for non-cancer effects of 2mg/kg bw/day was estimated to be equivalent to 600ppb HCBD in air and the effect level for tumours of 20mg/kg bw/day was equivalent to 6,000ppb HCBD in air.

1.60 COT noted that there are qualitative similarities between humans and animals in the way that HCBD is distributed and metabolised in the body, and therefore continuous exposure to a concentration of less than 0.6 ppb HCBD in air (which allows for a 100-fold Margin of Exposure compared to the NOAEL equivalent of 60ppb) could be regarded as being without appreciable adverse health effects in respect of non-carcinogenic and reproductive effects.

1.61 This level was approximately 10,000 times lower than the dose that caused cancer in animals following lifetime dietary exposure and therefore COT considered that the carcinogenic risk at these low exposure levels was minimal and was not of appreciable health concern. However, given the uncertainties in the data, the Committee considered that exposure should be reduced to as low a level as reasonably practicable.

1.62 The Committee was informed that health studies are being undertaken of exposed individuals. There is also a proposal to develop a physiologically-based pharmacokinetic model for HCBD exposure. In addition, the Committee was informed that a technique is being developed to allow analysis for HCBD at parts per trillion concentrations in air. The Committee welcomed this information and considered that the results of these studies should inform a further review by the Committee, in due course, of its conclusions on the health significance of low-level exposures to HCBD.

1.63 The COT statement is included at the end of this report.

Multiple Chemical Sensitivity

1.64 COT last considered this item in 1999 when it agreed that there was a need to continue monitoring developments in the field so that the issues could be reconsidered when more information became available (see paragraphs 1.25 – 1.27, 1999 Annual Report). However, it noted that there were no consistent patterns of symptoms or exposure data to define the condition, and concluded that on the basis of knowledge current at the time, there was insufficient evidence to make comments on potential mechanisms or to recommend further research in this area.

1.65 COT was asked to consider a recent report published by the British Society for Allergy, Environmental and Nutritional Medicine (BSAENM) (Eaton *et al., J. Nutr. Environ. Med.* **10**, 39-84, 2000). This reviewed prevalence, possible mechanisms, clinical signs, diagnosis and patient management of Multiple Chemical Sensitivity (MCS). The review had generated considerable public interest and referred to links between MCS and allergy (including food allergy). However it contained little peer-reviewed data. One new paper (Kreutzer *et al*., *Am. J. Epidemiology* **150**, 1-12, 1999) which had not previously been considered by the COT, claimed a high prevalence (6%) of MCS, based on a telephone interview. COT was asked whether the BSAENM report warranted any change in its 1999 view.

1.66 COT remarked on the limited number of peer reviewed studies cited in the BSAENM report. There was discussion in the report of the need for tests to be developed to aid diagnosis but there was a major problem regarding the absence of any clear definition of MCS which was a condition based on patient-defined criteria with no consistent pattern of symptoms. The term is associated with a wide range of chemicals and symptoms so diverse that it is not possible to define mechanisms or formulate studies to consider possible mechanisms.

1.67 COT questioned the discussion in the BSAENM report of high dose effects of chemicals, such as depletion of nutrients, in considering low dose effects. The report also referred to TILT (Toxicant Induced Loss of Tolerance), and type B allergy, with reference to a collection of unexplained symptoms. COT noted that there is no scientific basis for these concepts, and that they are not accepted by the immunology community.

1.68 The new study of Kreutzer *et al* (1999) was based on telephone interviews with physician-assisted diagnoses. There was an indication that the participants may have been asked leading questions and the approach was considered to lack objectivity and accuracy. Another limitation was an almost complete lack of exposure data.

1.69 COT considered the case-files in the BSAENM paper. It was pointed out that when individual cases of MCS are investigated they often led to diagnosis of chemical allergy to a single specific chemical agent. People with allergy to one chemical may be more sensitive to effects of other chemicals. COT therefore agreed that problems with sensitivity to chemicals can occur but that these are not necessarily Multiple Chemical Sensitivity. The term "multiple" may be applied simply because a specific causal agent has not been identified.

1.70 COT also considered the suggestion that funds should be allocated on a ring-fenced basis for research on MCS. However, it considered that the lack of evidence for any mechanism of action prevented formulation of a sound research programme.

1.71 After careful consideration of the BSAEMN report COT concluded that there was no basis for modifying the view expressed in 1999.

Risk procedures used by the Government's Advisory Committees dealing with food safety

1.72 COT was informed that, at the Prime Minister's request, Sir Robert May (then Chief Scientific Advisor to the Government) together with the Chief Medical Officer, Professor Liam Donaldson, and the Chairman of the Food Standards Agency, Sir John Krebs, had carried out a review of risk procedures in scientific committees that deal with food safety. The review group also included representatives of the devolved administrations and Dr Jim McQuaid, former Health and Safety Executive (HSE) Chief Scientist and Chairman of the Interdepartmental Liaison Group on Risk Assessment (ILGRA). The completed review outlined how the committees approached risk analysis and provided recommendations for best practice.

1.73 Chairmen of the relevant committees (including the COT) were interviewed and asked to provide information on the committee approaches to risk assessment, information about risk communication, and its role in risk management.

1.74 COT considered that it is rigorous in its risk assessment and that the actual approach taken needs to be determined by the specific situation and not dictated by a formal systematic structure. However, a more structured framework for information gathering (for instance, details of literature searches) might help to increase transparency and confidence in the database.

1.75 There was considerable discussion over the issue of providing advice on risk management. It was agreed that, although the COT's primary aim is to provide advice on risk assessment, occasions can arise when it is necessary to review the toxicological implications of alternatives for risk management procedures. However, a clear distinction was made between technical assessments of policy options and making judgements on possible political trade-offs. It was noted that, in providing risk assessment advice to policy makers, committees need to clarify the assumptions made and the uncertainties involved in their assessments. Where the COT did provide views on possible risk management options these should be carefully delineated and not weighted by areas outside of Members' expertise.

1.76 COT considered that recent measures had greatly increased openness and that very significant moves had been made towards making the findings more accessible and transparent. It was agreed that minutes should remain anonymous because of personal security issues. It was stressed that COT reaches a collective decision and therefore unanimity is not an issue.

1.77 It was acknowledged that a degree of communication between expert committees arose mainly from cross membership of advisory committees. Members welcomed a suggestion that, at least on an occasional basis, they should meet with their counterparts on other committees.

1.78 Although Members were not usually called upon to discuss Committee conclusions with the media, it was agreed that training in risk communication would be helpful.

Sucralose

1.79 COT last discussed sucralose in 1999, when a new teratogenicity study in rabbits was presented. COT concluded that the "study was adequate and demonstrated that sucralose is not a specific developmental toxicant" and that the No-Observed Adverse Effect Level (NOAEL) for the study was

350mg/kg bw/day. COT was content to leave the determination of an Acceptable Daily Intake (ADI) to the Scientific Committee on Food (SCF).

1.80 COT was informed that the SCF had completed its review and concluded that the effects on the gastrointestinal tract of the dams in the teratogenicity study were most likely to be attributable to high doses of poorly digestible substances, to which the rabbit is particularly sensitivity. The NOAEL identified by the study was therefore not considered to be relevant to setting the ADI. A NOAEL of 1500 mg/kg bw per day was identified from a number of dietary and gavage studies. Application of a 100-fold safety factor resulted in an ADI of 0-15 mg/kg bw per day.

1.81 COT noted and endorsed the SCF opinion and ADI of 0-15 mg/kg bw per day.

Terephthalic and isophthalic acids in food

1.82 Terephthalic acid (TA) and isophthalic acid (IA) are starting materials in the manufacture of polyester resins, which are used in coatings on the internal surface of some metal cans designed to come into contact with food.

1.83 The views of the COT were sought on the health implications of the results of a survey of TA and IA migration from can coatings into food. In particular the COT was asked to give its views on the possibility that these compounds might have endocrine disrupting activity.

1.84 COT was provided with estimates of intake of IA and TA by infants, toddlers and adults, based upon levels found in canned foods in this survey.

1.85 The toxicology of both TA and IA had been reviewed by the European Commission's (EC) Scientific Committee for Food (SCF). The SCF had set a restriction (for migration) of 5mg/kg food for IA and a TDI for TA of 0.125 mg/kg bw per day.

1.86 COT considered that the available toxicology data were old and not carried out to modern standards. In particular the Committee noted the presence of urinary bladder stones and associated tumours that developed in a long-term rat study carried out with a concentration of 5% TPA in the diet and requested that the views of the COM should be sought on the available *in vivo* genotoxicity data.

1.87 COT concluded that the concentrations of TA and IA that had been determined in foods analysed in the survey were not of concern for public health on the basis of available information. However, it was considered that

the available toxicity studies were not adequate to exclude the possibility of endocrine disruptor activity, and therefore appropriate studies should be conducted.

1.88 The COT statement on the survey is included at the end of this report.

Working Group on Risk Assessment of Mixtures of Pesticides

1.89 Risk assessment of pesticides has been carried out by measuring residue levels of individual pesticides in food and calculating whether intakes were likely to exceed the ADI for that pesticide. Usually this has not taken into account concurrent exposure to a number of pesticides via the same route (termed "cumulative exposure") or concurrent exposure to one or more pesticides via a different route (termed "aggregate exposure"). This has been a source of concern to a number of groups including consumers. Interest in an aggregate approach has been fuelled by the US Food Quality Protection Act, which mandates that intakes from all sources including food, drinking water and other sources should be considered. It also mandates that toxicological effects of exposure to more than one pesticide functioning by the same mechanism of action (eg cholinesterase inhibitors) should be considered. In addition, a considerable body of work has been carried out on the toxicology of mixtures in the US and the assumption is made that compounds with the same toxicological action will act in an additive fashion whereas those with different actions will act independently.

1.90 COT was informed that the Food Standards Agency considers that "combined" risk assessment of pesticides is a priority area. In order to consider cumulative and aggregate exposures, consideration needs to be given to the relative toxicity of the compounds, the magnitude of residues and the amounts of foods consumed. It may also be necessary to consider other sources of these chemicals such as drinking water and veterinary residues with similar action to the pesticide under consideration, and other means of exposure, such as occupational and domestic exposure. COT was asked to consider establishing a Working Group to review these issues.

1.91 COT agreed to the establishment of the Working Group and approved the terms of reference and membership. The Working Group expects to report within 18 months.

Ongoing work

Dioxins and dioxin-like PCBs - Consideration of the TDI

1.92 COT has commenced a review of the recent risk assessments of dioxins carried out by the World Health Organisation (WHO), the EU Scientific Committee on Food (SCF), and the United States Environmental Protection Agency (US-EPA). As part of this review COT will be reconsidering the tolerable daily intake (TDI). The Committee aims to complete its review of dioxins as soon as possible but accepted that it would not be complete before mid-2001 at the earliest. Without reviewing of the available data independently, COT was not content to accept that the studies selected by the WHO and SCF to inform their tolerable intakes were the most appropriate for this purpose.

1.93 COT proposed to review the evidence of effects other than cancer, taking into account the information provided by EPA, WHO and SCF. The Committee on Carcinogenicity (COC) was asked to review the evidence of carcinogenicity and the risk assessment procedure adopted by the US-EPA. COT agreed that it would be valuable to consult additional experts in other specialised areas.

Background on the three major assessments being considered by COT.

1.94 In 1998, a consultation of the WHO European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS) recommended a TDI for dioxins and dioxin-like PCBs in the range of 1-4 pg WHO-TEQ/kg. The WHO assessment was published in 2000 (van Leeuwen and Younes, *Food Additives and Contaminants* 17(4) 223-369). The WHO TDI was derived using the NOAEL/LOAELs of what were considered to be the most sensitive effects in experimental animals, and body burdens associated with these NOAEL/LOAELs (as opposed to daily intakes) were used to extrapolate between species. These body burdens were used in turn to calculate the estimated daily intake (EDI) considered to result in comparable steady state body burdens in humans. The use of body burdens was assumed to obviate the need for an uncertainty factor to account for species differences in toxicokinetics. TEFs were used to account for differences in toxicokinetics and potency between dioxin-like compounds. The consultation decided on an uncertainty factor of 10 to account both for interspecies and interindividual differences and the use of LOAELs instead of NOAELs. COT did not regard the information presented to be sufficient to make a judgement on whether the endpoints used by the WHO consultation to derive its TDI were the critical adverse effects.

1.95 A task force of the SCF reported its review of the TDI for dioxins in November 2000. It concluded that dioxins and dioxin-like PCBs should be allocated a temporary Tolerable Weekly Intake (t-TWI) of 7 pg WHO-TEQ/kg bw. The opinion is available on the SCF website at: http://europa.eu.int/comm/food/fs/sc/scf/outcome_en.html#opinions

1.96 The second draft of the US-EPA reassessment of dioxins was released during 2000. As in its previous (1995) assessment, the US-EPA considered that cancer is the critical endpoint and used low dose linear extrapolation to estimate the risk to humans. The validity of the US-EPA approach to the risks to health from exposure to of dioxins will be considered in addition to the TDI approach. The US-EPA draft reassessment is available on the EPA website at http://www.epa.gov/nceawww1/pdfs/dioxin/cd_index.html.

Hyperactivity and Food Additives

1.97 COT was asked to consider the results of a research project entitled "Do food additives cause hyperactivity and behaviour problems in a geographically defined population of three-year-olds?" A short statement was drafted, its release to coincide with release of the study results.

Polycyclic aromatic hydrocarbons (PAHs) - Pragmatic guideline limits in food for use in emergencies.

1.98 COT was asked to consider the appropriateness of setting pragmatic guideline limits for PAHs in food. Guideline limits would be helpful in formulating advice on dealing with incidents, such as fires or oil spills, which resulted in PAH contamination of food. In such situations, it may be necessary to make decisions on possible restriction of harvesting or marketing the affected foodstuffs.

1.99 COT noted that some of the PAHs are generally accepted to be experimental carcinogens and occupational exposure to mixtures of PAHs have been shown to be associated with human cancer. The COC had identified three compounds as being of greatest concern in respect of carcinogenic hazard on the basis of *in vivo* mutagenicity and/or multi-site carcinogenicity. These are benzo(a)pyrene, benz(a)anthracene and dibenz(a,h)anthracene.

1.100 The COT was informed by published data on reported concentrations of individual PAHs that have been detected in various foods, and noted that smoking of food and some cooking processes, such as grilling and barbequing, may result in higher concentrations being detected.

1.101 COT is considering a statement on pragmatic guidelines for PAHs in food for approval and release in 2001.

Statements of the COT

Statement on a Toxicity Study in the Rat of a Hydrogel Filler for Breast Implants

Statement on Dietary Exposure to Dioxins and Dioxin-Like PCBs

Statement on Dioxins and Dioxin-Like PCBs in Free-Range Eggs

Statement on the 1997 Total Diet Study – Fluorine, Bromine and Iodine

Statement on Hexachlorobutadiene

Statement on Terephthalic Acid and Isophthalic Acids from Can Coatings

Statement on a Toxicity Study in the Rat of a Hydrogel Filler for Breast Implants

Introduction

1. The Committee was informed that, because of concerns raised by clinicians about the safety of the fillers used in breast implants, the Medical Devices Agency (MDA) had decided to review the safety data on a hydrogel pre-filled breast implant manufactured for Poly Implant Prostheses. The MDA had asked the Committee to consider the report of a study in the rat in which the animals had received subcutaneous injections of the filler material and had then been observed for periods of up to 12 weeks.[1]

The implant

2. The filler comprises 92% of physiological saline gelled with 8% of a polysaccharide. It is understood that the polysaccharide is based on a cellulose derivative and forms long, linear chains linked by bridges. This gel is contained within a silicone elastomer shell.

The rat toxicity study

3. The Committee was advised that the only toxicity study of any duration was one in which groups of five rats were injected once subcutaneously on either flank with the gel filler material or with saline as a control. Groups of dosed and control rats were killed after 3 days, 4 weeks and 12 weeks. Limited observations were made during life and at necropsy. In the groups of rats that were killed at 4 and 12 weeks no abnormal clinical signs or differences in body weight were reported for either treated or control animals. However, in the treated animals residues of the gel and poorly characterised tissue damage were observed at the injection site. At these times there were histopathological changes in lymph nodes, livers and, to a lesser extent, the kidneys of the treated animals.

4. The Committee considered that, despite having been carried out in 1996, the study was unsatisfactory in its design, execution and reporting. It was the view of the Committee that the changes in the lymph nodes represented a real effect and were consistent with a chronic inflammatory response. These changes require further study, including investigation of lymph nodes close to and distant from the site of injection. In addition, there should be investigation of the lesions reported in the liver and kidney and of the reversibility of any changes observed.

Conclusions

i) The Committee considered that the conclusion of the study, namely that there were no pathological findings in the organs examined, was not supported by the limited experimental results provided, which were considered to be imprecise and inadequate.

ii) The Committee agreed that the findings from the study could not be discounted. The Committee was not able to exclude the possibility that the reported lesions were indicative of a toxic or immunologically-mediated response.

iii) The Committee considered that further testing should be undertaken involving the administration of single doses of the filler gel with longer-term follow-up and with more detailed reporting compatible with current guidelines for chronic toxicity tests.

September 2000
COT Statement 2000/09

Reference

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Statement on Dietary Exposure to Dioxins and Dioxin-Like PCBs

Introduction

1. We have been informed of the results of a study conducted by the former Joint Food Safety and Standards Group of the Ministry of Agriculture, Fisheries and Food and the Department of Health in which Total Diet Study (TDS) samples collected in 1997 were analysed for the presence of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), collectively referred to as dioxins, and polychlorinated biphenyls (PCBs).[1]

Tolerable Daily Intake

2. In 1992 we endorsed a Tolerable Daily Intake (TDI) for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) of 10 picograms/kilogram body weight (10 pg/kg bw) that had been recommended by the World Health Organization (WHO) Regional Office for Europe. We also recommended that when considering mixtures of dioxins the TDI could be regarded as being expressed in Toxic Equivalents of TCDD (TEQs), calculated using internationally agreed Toxic Equivalency Factors (TEFs) for dioxin congeners, ie 10 pg TEQ/kg bw.[2]

3. In our 1997 review of the health hazards of PCBs, we were unable to set a TDI for total PCBs. However, we considered that the use of TEFs for certain dioxin-like PCB congeners offered a pragmatic approach to assess the potential toxicity of these dioxin-like PCBs and that they should be considered in combination with dioxins.[3]

4. Recently, we have endorsed the TEFs recommended by a WHO European Centre for Environment and Health (ECEH) consultation for the seventeen 2,3,7,8-substituted dioxin congeners and twelve dioxin-like PCB congeners.[4, 5]

5. We are aware that a recent WHO International Program for Chemical Safety (IPCS)/ECEH consultation has recommended a TDI range for dioxins and dioxin-like PCBs of 1-4 pg TEQ/kg bw.[6] 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. In the interim we have considered the results of the 1997 TDS survey using both the current UK-TDI and the recently recommended WHO-TDI.

Estimated dietary exposure to dioxins and dioxin-like PCBs

6. We have been provided with estimates of dietary exposure to dioxins and dioxin-like PCBs of adults, schoolchildren and toddlers ie children aged $1\,^1/_2$ to $4\,^1/_2$. The same methodology has been used to estimate dietary exposures from the 1997 TDS as was used to estimate exposures for these age groups from the 1982 and 1992 TDS.[1] We note that where concentrations of these compounds in food were below the limit of detection, the concentration has been assumed to be at the limit of detection. It is considered that this approach overestimates dietary exposures to dioxins and dioxin-like PCBs. We have been informed that dietary exposure of adults and schoolchildren has been estimated using food consumption data for these specific groups.[7, 8]

7. While food consumption data for toddlers do exist,[9] due to a current limitation in the methodology used to estimate exposures, the consumption of 'toddler-specific' foods cannot yet be determined. As a result, toddler food consumption data were not used directly to estimate toddler dietary exposures from previous Total Diet Studies.[10] Toddler dietary exposure has been estimated previously by scaling the estimated dietary exposure of adults by the relative energy contents of adult and toddler diet. The energy content of the latter was calculated from the toddler food consumption data.[9] For comparative purposes, this approach has also been used to estimate dietary exposure of toddlers from the 1997 TDS.[1] However, toddler exposures have now also been estimated from the 1997 TDS (and retrospectively from the 1982 and 1992 TDS for comparative purposes) directly using toddler food consumption data. We note that this approach does not take into account exposures resulting from the consumption of 'toddler-specific' foods but we consider that it provides a more robust estimate of toddlers' dietary exposure than the earlier approach. However, we recommend that the methodology is revised as soon as possible so as to take account of consumption of 'toddler-specific' foods and we ask to see these revised exposure estimates at the earliest opportunity.

8. Dietary exposure to dioxins and dioxin-like PCBs, estimated from the 1982, 1992, and 1997 Total Diet Studies, for average and high-level (97.5th percentile) adult, schoolchild, and toddler consumers (using both approaches) are presented in the Table. The Table presents toddler dietary exposures estimated from toddler food consumption data and also presents exposures estimated by scaling adult consumption patterns by the energy content of the toddler diet. Dietary exposures estimated using toddler food consumption data are higher than when estimated by scaling adult consumption patterns by the energy content of the toddler diet. Exposures estimated from the 1982 and 1992 TDS have been recalculated using the new WHO-TEFs so that the data are comparable to dietary exposures estimated from the 1997 TDS.

Table: Estimated dietary exposures to dioxins and dioxin-like PCB from TDS samples
(pg TEQ/kg bw per day)

Year	1982		1992		1997	
Consumer type	**Average**	**High-level**	**Average**	**High-level**	**Average**	**High-level**
Age-group						
Adults	7.2	13	2.5	4.3	1.8	3.1
Schoolchildren	8.6	15	3.0	4.7	2.2	3.5
Toddlers (estimated using toddler food consumption data)						
$1^1/_2$ to $2^1/_2$	23	49	7.5	14 5.	1 1	0
$2^1/_2$ to $3^1/_2$	19	41	6.3	11	4.4	8.4
$3^1/_2$ to $4^1/_2$ (boys)	17	33	5.6	9.2	4.0	6.9
$3^1/_2$ to $4^1/_2$ (girls)	17	34	5.6	9.6	4.0	7.2
Toddlers (estimated by scaling adult consumption patterns by the energy content of the toddler diet)						
$1^1/_2$ to $2^1/_2$	18	28	6.3	9.8	4.6	7.2
$2^1/_2$ to $3^1/_2$	17	25	5.8	8.6	4.2	6.3
$3^1/_2$ to $4^1/_2$ (boys)	16	23	5.7	8.0	4.1	5.8
$3^1/_2$ to $4^1/_2$ (girls)	15	23	5.3	8.0	3.9	5.8

9. The estimated dietary exposures to dioxins and dioxin-like PCBs for both average and high-level consumers from the three age groups are at or within the current UK-TDI of 10 pg TEQ/kg bw. Furthermore, the estimated average and high level dietary exposures for adult and schoolchild consumers are also below the upper value of the recently recommended WHO-TDI of 1-4 pg TEQ/kg bw. However, the estimated dietary exposures for toddlers who are average consumers are at or slightly above the upper value of this TDI. The upper value of this TDI is exceeded approximately two-fold by all toddlers who are high-level consumers.

10. The estimated dietary exposures to dioxin-like compounds, on a total TEQ basis, for all three age groups show a continuing downward trend, albeit less steeply compared with the decline between 1982 and 1992. However, the dietary exposures to dioxin-like PCBs estimated from the 1997 TDS are very similar to those estimated from the 1992 TDS.

11. We have seen data which indicate that the decline in dietary exposure is real and not attributable to changes in analytical sensitivity or number of food groups analysed in different Total Diet Studies. This decline in dietary exposure is primarily due to either a reduction in emissions to the environment or a change in food consumption patterns, or both.

Environmental controls

12. Abatement measures have been taken to control the emission of dioxins to the environment and hence foods. In particular the imposition of strict emission limits on municipal waste incinerators have reduced emissions from this sector by an estimated 90%. The UK is introducing Regulations to give effect to EC Directive 96/59, which requires the phasing out and disposal of remaining identifiable PCBs. The Regulations follow on from consultation last year, and the publication of the UK action plan in 1997.[11] It is anticipated that as a result of these measures dietary exposure to dioxins and dioxin-like PCBs will continue to decline gradually. We understand that the Government is in the process of producing an UK position paper on dioxins and dioxin-like PCBs, which will assess the effectiveness of current and future abatement measures.

Recommendations

13. We are *reassured* by the evidence of a continuing decline in dietary exposure to dioxin-like compounds. We welcome the evidence that average and high-level adult and schoolchild consumers do not exceed the current UK-TDI or the upper value of the recently recommended WHO-TDI.

14. We *note* that estimated dietary exposures of toddlers do not exceed the current UK-TDI but that approximately 50% of toddlers will exceed the upper value of the newly recommended WHO-TDI. However, we *note* that there are limitations in the methodology used to derive these estimated exposures for toddlers, which means that such estimates should be viewed with caution. We *recommend* that robust characterisation and estimates of toddler exposure, taking into account consumption of 'toddler-specific' foods, are carried out and we request that we see such information at the earliest opportunity.

15. We *note* that the WHO-IPCS/ECEH consultation recommended that continued efforts should be made to reduce exposure towards the lower end of the newly recommended WHO-TDI range. We will undertake a review of the WHO-TDI when a full report of the consultation is available and we will pay particular attention to the relevance of the WHO-TDI to toddlers.

16. We *recommend* that dietary exposure to dioxin-like compounds should continue to be monitored at regular intervals to confirm that the overall downward trend in exposure continues as a result of current and future abatement measures.

17. The available data indicate that some 50% of toddlers in the UK will exceed the upper value of the WHO-TDI but not the current UK-TDI. However, we do not consider that this exceedence necessarily poses a health risk and, in advance of a detailed review of the WHO-TDI, we do not recommend any intervention with respect to the diets of toddlers. This interim position is based upon the following considerations:

    i) it is not yet clear to what extent the WHO-TDI is particularly relevant for toddlers;

    ii) evidence that some toddlers may exceed the WHO-TDI is based upon estimations of dietary exposure that need to be treated with some caution; and

    iii) there is a continuing decline in the overall exposure to dioxin-like compounds.

Conclusions

18. Estimated exposures to dioxins and dioxin-like PCBs for adults, schoolchildren, and toddlers are all at or below the current UK-TDI. Estimated exposures for adults and schoolchildren are also below the upper value of the newly recommended WHO-TDI, although toddlers may exceed this value. However, estimated exposures for all age groups have substantially declined since 1982 and we anticipate that exposures will continue to decline in the future due to the environmental controls already in place and those planned. We conclude that the current concentrations of dioxins and dioxin-like PCBs in food are unlikely to pose a risk to health.

August 2000
COT Statement 2000/03

References

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7. Gregory J, Foster K, Tyler H and Wiseman M (1990). The Dietary and Nutritional Survey of British Adults. London, HMSO.

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11. Department of Environment (1997). United Kingdom action plan for the phasing out and destruction of polychlorinated biphenyls (PCBs) and dangerous PCB substitutes. London, Department of the Environment.

Statement on Dioxins and Dioxin-Like PCBs in Free-Range Eggs

Introduction

1. We have been informed of the results of a study conducted by the Food Standards Agency (FSA) in which free-range hen and duck eggs were analysed for the presence of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), collectively referred to as dioxins, and polychlorinated biphenyls (PCBs).[1]

Survey design

2. A total of 45 free-range hen and duck egg samples, each sample consisting of six individual eggs, were collected from farms or private houses in Kent, Essex, Norfolk, and Buckinghamshire between November 1994 and April 1996. We have been informed that the purpose of this exercise was to examine the use of free-range eggs as indicators of environmental contamination, rather than to estimate dietary exposure to these compounds through the consumption of free-range eggs. The sampling sites were selected for practical convenience rather than on the basis of concerns about local contamination. We note that these free-range egg samples may not be representative of those on sale throughout the United Kingdom.

Concentrations of dioxins and dioxin-like PCBs

3. Concentrations of dioxins and dioxin-like PCBs in the combined yolk and white of hen eggs were in the range of 1.1-22 (mean 6.3, median 3.5) ng Toxic Equivalents (TEQ)/kg fat. In the combined yolk and white of duck eggs concentrations were in the range of 1.9-49 (mean 12, median 5.2) ng TEQ/kg fat. We note that in both cases the distribution of values appeared to be skewed. We have been informed that there were no obvious major point sources of contamination in the immediate vicinity of these sampling sites to account for the higher values. We have been told that the most likely source of contamination of free-range eggs by these compounds is via the ingestion of soil and sediment by hens and ducks as they forage for food. However, environmental sampling at the sites of egg collection was not undertaken.

Estimated dietary exposures

4. We have been provided with estimated dietary exposures for toddlers, schoolchildren, and adults to dioxins and dioxin-like PCBs based on the concentrations of these compounds in free-range eggs in this survey. However, we consider that, because these free-range eggs may not be representative of those on sale throughout the UK, the data from this survey cannot be used to estimate dietary exposures to dioxins and dioxin-like PCBs with any confidence from these sources. Nor can the data from this survey be used to draw any comparisons between free-range eggs and other hen and duck eggs, for which there are few data available.

Conclusions

5. We consider that this survey of dioxins and dioxin-like PCB in free-range hen and duck eggs cannot be used to estimate the risk to health of consumers of such eggs in the UK.

6. The concentrations of dioxins and dioxin-like PCBs in free-range hen and duck eggs might be used as an indicator of environmental contamination. A larger, more rigorously designed study would be needed to investigate this.

July 2000
COT statement 2000/06

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Statement on the 1997 Total Diet Study – Fluorine, Bromine, and Iodine

Introduction

1. We have been informed of the results of a study conducted by the Food Standards Agency in which Total Diet Study (TDS) samples collected in 1997 were analysed for the presence of three halogen elements, namely fluorine, bromine, and iodine.[1]

Estimated dietary intakes

2. We have been provided with estimates of mean population, and mean and high-level (97.5th percentile) adult consumer dietary intakes of fluorine, bromine, and iodine. Mean population intakes are based on household, rather than individual, consumption data that are updated yearly and thus can be used to follow trends in dietary intakes. Mean and high-level consumer intakes are based on adult consumption data from the 1986/87 National Diet and Nutrition Survey of British Adults.[2] We note that dietary intakes for age groups other than adults have not been estimated.

3. We note that the analytical techniques used to determine the concentrations of fluorine, bromine, and iodine in the TDS samples did not distinguish between the different chemical forms in which these elements may exist in food.

Fluorine

4. The mean population dietary intake of fluorine estimated from the 1997 TDS is 1.2 mg/person per day. Estimated dietary intakes for mean and high-level adult consumers are 0.94 and 2.0 mg/person per day respectively. Dietary intakes for fluorine were last estimated in 1984 when the mean population intake, calculated from concentrations of fluorine determined in selected food samples from the 1978, 1979, and 1980 total diet studies, was estimated as 1.8 mg/person per day.[3] However, due to changes in the TDS design since 1981 and the limited number of samples that were used to estimate this intake in 1984, a direct comparison between the 1997 TDS mean population intake estimate and this earlier estimate cannot be made. There are no guidelines for fluorine against which to assess these estimated dietary intakes. However, we have been informed that the Expert Group on Vitamins and Minerals (EVM) will be considering fluorine in due course and we will await the findings of that body before considering any potential effects associated with these intakes.

Bromine

5. We have not previously considered dietary intakes of bromine. The mean population dietary intake of bromine estimated from the 1997 TDS is 3.6 mg/person per day. Estimated dietary intakes for mean and high-level adult consumers are 3.8 and 6.2 mg/person per day. We have had the opportunity to review an evaluation of bromine by the Joint Food and Agriculture Organization (FAO) and the World Health Organization (WHO) Meeting on Pesticide Residues (JMPR), which established an Acceptable Daily Intake (ADI) range of 0-1 mg/kg body weight.[4] It is not certain whether bromine is essential[5] so we consider it inappropriate to recommend a range for intakes of bromine that includes zero. However, we consider that the upper value of this range represents a bromine intake below which intakes are unlikely to pose a risk to health. In this respect the upper value of 1 mg bromine/kg body weight per day, equivalent to 60 mg/day for a 60 kg individual, can be considered to be a Tolerable Daily Intake (TDI). Estimated mean and high-level adult consumer dietary intakes of bromine are within this guideline and are therefore not a cause for concern.

Iodine

6. We have considered dietary intakes of iodine on a number of previous occasions, most recently earlier this year when we considered a survey of iodine in cows' milk.[6] We concluded that the concentrations of iodine in cows' milk were unlikely to pose a risk to health,[7] despite calculations that suggested that dietary intakes of iodine by some toddlers may exceed the Provisional Maximum Tolerable Daily Intake (PMTDI) for iodine of 0.017 milligrams per kilogram body weight (17 (g/kg body weight) as recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).[8] Dietary intakes for mean and high-level adult consumers estimated from the 1997 TDS of 240 and 420 (g/person per day are within the JECFA PMTDI, which is equivalent to 1000 (g/day for a 60 kg individual, and are therefore not a cause for concern. While dietary intakes for children aged $1^1/_2$ to $4^1/_2$ have not been calculated from the 1997 TDS, we have been informed that intakes for this age group are likely to be comparable to the intakes we considered in relation to the survey of iodine in cows' milk. There is no new information that would lead us to alter our previous advice[7] that estimated dietary intakes of iodine by toddlers are unlikely to pose a risk to health. However, we have been informed that the EVM are in the process of considering iodine and thus we may wish to reconsider these results in the light of the findings of that body.

Conclusion

7. We conclude that the estimated total dietary intakes of bromine and iodine based on data from the 1997 Total Diet Study are unlikely to pose a risk to health. However, further information on the different chemical forms of these elements in the diet would assist in risk assessment. We will await the findings of a review of fluorine by the Expert Group on Vitamin and Minerals before considering any potential effects associated with the intakes of this element.

July 2000
COT statement 2000/05

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Statement on Hexachlorobutadiene

Introduction

1. The fully chlorinated hydrocarbon hexachlorobutadiene (HCBD, C_4C_{l6}) is formed as a by-product during the manufacture of chlorinated solvents.

2. Environmental contamination with HCBD has recently been detected around a disused waste dump in a quarry at Weston in Runcorn, Cheshire. This was used by ICI for about 50 years until the early 1970s. The release of HCBD into the underlying strata and groundwater came to light as a result of a project carried out by ICI to investigate the environmental impact of its previous industrial and waste disposal activities. HCBD has been detected in the indoor air of properties close to the site.

3. We have been informed that there are 128 houses built close to the former dump which have been investigated and HCBD has been detected recently in the indoor air of 21 of these, at concentrations of under 10 parts per billion (ppb) in all houses apart from one, where a concentration of 1000 ppb was detected. The current limit of detection is 2 ppb in air. People living in most of the houses where HCBD has been detected have been moved to other accommodation. North Cheshire Health Authority has offered health checks to those residents who were, at the time, living in houses where HCBD was detected.

4. In view of public health concerns, the Committee has been asked by the Department of Health to provide advice on the toxicity of HCBD. This is given below.

Toxicology of HCBD

5. There is very little information on the toxicological effects of HCBD derived from studies on humans. Consequently, an assessment of the possible risks to human health has to be based on laboratory and animal data. However, most animal toxicity studies on HCBD have been conducted using oral exposure and there are few studies of exposure by inhalation, the prime route of exposure for residents at Weston.

6. The results of studies of repeated oral administration indicate that HCBD can cause damage to the kidneys at doses of 0.5 milligrams/kilogram body weight per day (mg/kg bw per day) and above in female mice[1] and at doses of 2 mg/kg bw per day and above in both sexes of rats.[2] Damage to other tissues (liver, nervous system) has been reported at a higher dose of 20 mg/kg bw per

day in rats.[2] In reproduction studies at this dose foetal toxicity, predominantly manifested as retardation of foetal growth, was also recorded in rats.[3] However, these effects were attributed to maternal toxicity because adverse developmental effects were not induced at doses that were not toxic to the dam. Limited information from the animal studies indicates that exposure by inhalation results in the same toxic effects, with the kidney being the prime target organ.

7. Thresholds for each of these adverse effects have been demonstrated in several studies. The Committee considered that the response in the kidneys of mice is the most sensitive indicator of the toxicity of HCBD but that the response of one female mouse dosed with 0.2 mg/kg bw per day for 13 weeks[1] was not sufficient evidence to warrant the use of a lower figure for a No Observed Adverse Effect Level (NOAEL). Therefore, the Committee considered that, for non-carcinogenic effects, the NOAEL is 0.2 mg/kg bw per day.[4,5]

8. Members of our sister committee, the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) have reviewed the mutagenicity of HCBD. There are *in vitro* data, mainly from studies using Salmonella TA100, that indicate that HCBD has mutagenic potential.[6,7] Negative results have been reported from *in vivo* assays in bone marrow [8,9] but these were inadequate to draw definite conclusions. COM members considered that further *in vivo* studies were needed, particularly in the kidney, before any definite conclusions could be drawn. On the data currently available it would be prudent to assume that HCBD is an *in vivo* somatic cell mutagen.

9. A carcinogenic response has been seen in the kidneys of rats in a study in which HCBD was administered continually in the diet for two years at a dose of 20 mg/kg bw per day (the highest dose tested). No tumours were observed in the kidneys of male or female rats administered doses of 2 mg/kg bw per day or lower.[2]

10. In view of the advice from the COM that HCBD should be regarded as an *in vivo* mutagen the COT were unable to establish a safe level in relation to cancer or to identify a tolerable daily intake (TDI) for HCBD.

Conclusions

11. From animal studies, the Committee agreed that a NOAEL of 0.2 mg/kg bw per day had been established for the **non-carcinogenic** effects of HCBD.

12. The Committee considered that, in order to estimate the concentration in air that would result in humans inhaling a dose of 0.2 mg/kg bw per day, it was necessary to make the following assumptions:

- the toxicity of HCBD following inhalation exposure is essentially the same, both qualitatively and quantitatively, as the toxicity of HCBD following oral exposure;

- there are no significant differences in the extent of absorption of HCBD by either route; and

- a 60 kg adult would inhale 20 cubic metres (m^3) of air per day.

On this basis the Committee considered that, as an approximation, a dose of 0.2 mg/kg bw per day would correspond to the continuous inhalation of air containing 0.6 mg/m^3 of HCBD, equivalent to an air concentration of about 60 ppb.

13. In view of the evidence that there are qualitative similarities between humans and animals in the way that HCBD is distributed and metabolised in the body, the Committee considered that continuous exposure to a concentration of HCBD in air of less than 0.6 ppb (ie the Margin of Exposure below 60 ppb is at least 100) can be regarded as being without appreciable adverse health effects in respect of non-carcinogenic and reproductive effects.

14. In respect of concerns about a potential **carcinogenic** effect, the Committee noted that exposures to less than 0.6 ppb HCBD were 10,000 times lower than the equivalent dose of HCBD which, when fed daily throughout a lifetime to rats, had resulted in kidney tumours. The Committee considered therefore that the carcinogenic risk at these low exposure levels was minimal and was not of appreciable health concern. However, given the uncertainties in the data, the Committee considered that exposure should be reduced to as low a level as reasonably practicable (ALARP).

15. The Committee was informed that health studies are being undertaken of exposed residents of Weston[10] and members of the ICI workforce.[11] There is also a proposal to develop a physiologically-based pharmacokinetic model for HCBD exposure.[11] In addition, the Committee was informed that a technique is being developed to allow analysis for HCBD at parts per trillion concentrations in air. The Committee welcomed this information and considered that the results of these studies should inform a further review by the Committee, in due course, of its conclusions on the health significance of low-level exposures to HCBD.

June 2000
COT Statement 2000/04

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Statement on Terephthalic and Isophthalic Acids from Can Coatings

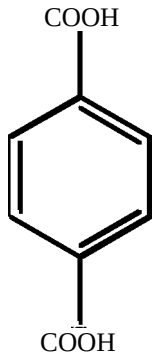
Introduction

1. The views of the Committee were sought on the health implications of the results of a survey[1] of terephthalic acid (TA) and isophthalic acid (IA) migration from can coatings into food. In particular the Committee was asked to give its views on the possibility that these compounds might have endocrine disruptor activity.

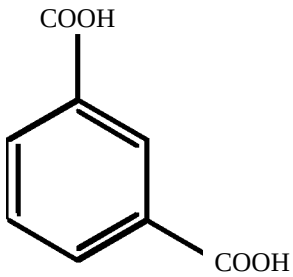
Background

2. TA and IA (see Figure) are starting materials in the manufacture of polyester resins, which are used in coatings on the internal surface of some metal cans designed to come into contact with food.

Figure 1 Terephthalic acid (TA) Isophthalic acid (IA)



Terephthalic acid (TA)



Isophthalic acid (IA)

3. As part of the Food Standards Agency's continuing programme of surveillance on the migration of chemicals from food contact materials a two-part survey for TA and IA was carried out. In the first phase of the survey various canned foods were purchased and the cans were tested for the presence of coatings made from polyester resins. In the second phase, further samples of the products in those cans which had polyester coatings were analysed to determine whether migration of TA and IA into the can contents had occurred.[1]

Survey results

4. Twenty-eight products were identified as being in cans coated with polyester resin on all, or part, of their internal surfaces. In samples of the contents of these cans, TA was found in 3 of 28 samples at or just above the limit of quantification* and in 7 samples at levels between the limit of detection† and limit of quantification. IA was detected in 4 of 28 samples at levels between the limit of detection and limit of quantification.

5. Estimates were made of the potential intakes of TA and IA from canned foods studied in the second phase of the survey. Intakes were estimated for different age groups according to the types of foods in which these substances were found. The estimates used the analytical results for samples in which TA and/or IA were found. Intakes were calculated by summing the intakes of 97.5th percentile consumers‡ for each food in which the given substance was detected, giving greatest weight in this summation to the two highest estimates of intake. The intake estimate was divided by bodyweight to derive contaminant exposure in milligrams per kilogram of bodyweight (mg/kg bw) per day, bodyweights used were: 8.8 kg for infants, 14.5 kg for toddlers ($1^1/_2$-$4^1/_2$ years old) and 60 kg for adults.

6. The potential intake of TA by infants between 6 and 12 months old who were 97.5th percentile consumers was estimated as 0.0074 mg/kg bw per day. For toddlers who were 97.5th percentile consumers the potential intake of TA was estimated as 0.083 mg/kg bw per day. For adults who were 97.5th percentile consumers the potential intake of TA was estimated as 0.0025 mg/kg bw per day.

7. The intake of IA by adult 97.5th percentile consumers was estimated as 0.0013 mg/kg bw per day. There are no estimates of intake by infants as no IA was detected in baby foods.

* Limit of quantification: the lowest level at which the amount of a substance can be stated with confidence.
† Limit of detection: the lowest level at which a substance can be detected with confidence.

‡ 97.5th percentile consumers are those whose consumption of a specific food or group of foodstuffs corresponds to the 97.5th percentile point on a distribution curve for consumption of the given food or foods.

Toxicology of TA and IA

8. The European Commission's Scientific Committee for Food (SCF) reviewed studies of the toxicity and migration of both TA and IA.

9. In view of the availability of data from long-term studies the SCF was able, pending submission of full reports, to set a temporary Tolerable Daily Intake (TDI) for TA of 0.125 mg/kg bw, which was based on 3-month and 2-year dietary studies in rats.[2] The major finding in the long-term study with TA was the occurrence of malignant and benign tumours of the urinary tract at high doses.[3,4] These were documented as being associated with the formation of stones in the urinary bladder which represents a potential non-genotoxic mechanism for the formation of such tumours.

10. On the basis of the available data from migration and toxicity studies submitted by industry the SCF has also set a restriction (for migration from plastics) of 5 mg/kg food for IA.[2] This limit was based on negative genotoxicity data and a 90-day dietary study in rats, from which a No Observed Effect Level of 250 mg/kg bw per day was established.

11. The manufacturers of TA and IA submitted a commentary on the available reproductive and developmental toxicity data for both compounds. In this it was proposed that the weight of the evidence from these studies does not support a role for these acids in modulating the endocrine system.[5]

12. The Committee noted that the toxicity studies on TA and IA were not carried out to modern standards. It was recognised that the limited nature of the published work would not allow them to address fully the questions that they had been asked.

13. It was requested that, in the light of the urinary tumours occurring in rats fed the highest dietary concentration of TA, the view of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment be sought on the potential *in vivo* genotoxicity of this compound.

14. It was noted that the estimated intakes of TA by infants, toddlers and adults who were 97.5th percentile consumers were below the temporary TDI established by the SCF. In addition, it was noted that the concentrations of IA found in samples of canned food in the survey were below the migration limit set by that committee.

Conclusions

i) The Committee *concluded* that the concentrations of TA and IA that had been determined in foods analysed in the survey were not of concern for public health on the basis of available information.

ii) The Committee *noted* the commentary of the manufacturers on possible endocrine disruptor activity of TA or IA. However, it was considered that the toxicity studies were inadequate to exclude this possibility. It was therefore *recommended* that appropriate studies should be carried out to determine whether TA or IA possess endocrine disruptor activity.

September 2000
COT Statement 2000/08

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2000 Membership of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

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K V Butler (*Administrative Secretary* from November)
A Sewart BSc PhD (to September)
C Tahourdin BSc PhD
D Gott BSc PhD (from November)
C A Mulholland BSc
N Thatcher BSc PhD
J Shavila BSc MSc PhD
B Maycock BSc MSc

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 HSBC	Shares 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
Profesor I Kimber	British Airways British Petroleum-Amoco ICI Halifax AstraZeneca	Share Holder Share Holder Share Holder Share Holder Share Holder Employee	Unilever plc	Grant for Research

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
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
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 food companies
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 provides advice on the potential mutagenic activity of specific chemicals at the request of UK Government departments and agencies. Such requests generally relate to chemicals for which there are incomplete, non-standard or controversial data sets for which the expertise of the independent committee members is required to provide recommendations on potential hazards and risks and frequently suggestions for further studies.

During 2000, advice was provided on a diverse range of chemicals including food packaging contaminants, industrial chemicals and alcoholic beverages. Amongst the most complex evaluations of mutagenic potential are estimates of how conclusions may be extrapolated from *in vitro* to *in vivo* tests, and from somatic to germ cells. As part of an ongoing development of expertise in such extrapolations, the committee published statements on data extrapolation from somatic to germ cells for chemicals which disturb the fidelity of chromosome segregation which may thus induce aneuploidy.

The year 2000 saw the completion of a major committee project; the preparation of a guidance document on a strategy for the testing of chemicals for mutagenicity. This document provides guidance on both test method selection and their appropriate use in the assessment of the potential mutagenic activity of chemicals. The updated guidance provides advice on the application of a range of methods (such as the use of transgenic animals) that have been developed over the past 10 years and highlighted the importance of measuring the potential of aneuploidy induction by chemicals in mutagenic screening programmes. The development of the guidance document involved extensive discussions and meetings with a wide range of interested parties, including the Industrial Genotoxicology Group, the UK and the European Environmental Mutagen Societies. The completed guidance document is being widely distributed and current indications are that the strategy recommended will have a major influence on future European policy on chemical testing.

Professor James M Parry (Chairman)
BSc PhD DSc

Hydroquinone and phenol

2.1 At the request of HSE the Committee had provided advice on the interpretation of mutagenicity data on hydroquinone and phenol in 1994 and 1995. The COM had agreed that both hydroquinone and phenol should be regarded as somatic cell *in-vivo* mutagens. The Committee had been persuaded for these two compounds that by the oral route there was a potential for a threshold of activity. This conclusion was based on good evidence of protective mechanisms (namely rapid conjugation and detoxification via the glutathione pathway) that would substantially reduce systemic exposure to any active metabolites formed. However, members agreed that there was insufficient data regarding activity following inhalation and dermal exposures and it was not possible to assume a threshold existed for mutagenic activity when exposure was via the respiratory tract or via the skin. The Committee reviewed some additional data provided by industry in 1995 on the metabolism of hydroquinone and phenol in animals and humans. These data were useful but did not allow for an assessment of pre-systemic metabolism following either inhalation or dermal exposure. The Committee recommended that further studies were required which should include early sampling for free and conjugated hydroquinone or phenol in blood following administration of test substances to rats or dogs via a bronchoscope.

2.2 In 1999, further published data was provided to the Committee on the kinetics of hydroquinone metabolism in rats following intratracheal instillation and its percutaneous absorption, together with some *in-vitro* studies using rat skin and human stratum corneum. A number of additional *in-vivo* mutagenicity studies including an investigation of site of contact mutagenicity in skin and respiratory tract of Muta^{TM} mice using the LacZ transgene were also considered. The Committee considered these new data during 1999 and agreed a statement which was published on the COM Website in January 2000.

2.3 In summary the new toxicokinetic study involved giving rats a single intra-tracheal dose of ^{14}C-hydroquinone. Free hydroquinone in arterial blood was detected 5-10 seconds after dosing. This suggested a potential hazard of site-of- contact and systemic mutagenic effects following exposure by inhalation to hydroquinone. The Committee thus reaffirmed its previous conclusions on hydroquinone (and phenol) which are given in full in the statement at the end of this report.

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

2.4 3-Monochloropropane-1,2-diol (3-MCPD) is a member of a group of chemicals present as contaminants known as chloropropanols. 3-MCPD can be present as a contaminant in epichlorhydrin/amine copolymers used as flocculants or coagulant 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 arising from their use. 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). The COC was asked to evaluate and advise on the carcinogenicity of 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, Transport and Regions on the approval of chemical substances in contact with public water supplies.

2.5 The COM had reviewed the available mutagenicity data on 3-MCPD in 1999 which suggested that 3-MCPD had mutagenic activity *in-vitro*. 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*. The Committee considered at its October 2000 meeting two new *in vivo* mutagenicity studies commissioned by the UK Drinking Water Inspectorate. These comprised a rat bone-marrow micronucleus test and a rat liver UDS assay, both of which are widely used to assess genotoxicity *in-vivo*.

2.6 The Committee concluded that both the rat bone-marrow micronucleus test and the rat liver UDS test had been carried out to acceptable standards and were negative. Thus, the additional information recommended by the COM as being necessary to provide adequate reassurance that the mutagenic activity seen *in-vitro* was not expressed *in-vivo* had now been provided. The Committee agreed that ß-chlorolactic acid was the major urinary metabolite in rats formed by the oxidation of 3-MCPD and that the two new mutagenicity studies supported the view that reactive metabolites if formed did not produce genotoxicity *in-vivo* in the tissues assessed.

2.7 The Committee concluded that 3-MCPD can be regarded as having no significant genotoxic potential *in-vivo*. A copy of the revised COM statement on 3-MCPD can be found at the end of this report.

Mutagenicity of ethanol, acetaldehyde and alcoholic beverages

2.8 In 1995 the COM gave detailed consideration to the potential mutagenicity of ethanol, acetaldehyde and alcoholic beverages. This was to provide input to the Interdepartmental Working Group reviewing current advice on this topic. The Committee on Carcinogenicity (COC) also carried out a detailed review of the available data, mainly from epidemiology studies, on the carcinogenicity of alcoholic beverages. The advice from these Committees was considered by the Interdepartmental Working Group when drawing up their Report on Sensible Drinking published in December 1995. The main conclusions reached by COC and COM at that time were:

i) The COM agreed that the consumption of alcoholic beverages does not present any significant concern with respect to their mutagenic potential.

ii) The COC concluded that the epidemiological evidence supported the view that drinking alcohol causes a dose-related increase in the risk of squamous carcinomas of the upper aerodigestive tract as a whole, and for cancers of the oral cavity, pharynx, larynx and oesophagus.

2.9 The COM was asked to update its statement on the mutagenicity of ethanol, acetaldehyde and alcoholic beverages by the COC in order to provide additional information as part of a review of the evidence on the association between drinking alcohol and breast cancer.

2.10 The Committee reaffirmed its 1995 conclusion that consumption of alcoholic beverages does not present any significant concern with respect to their mutagenic potential. A copy of the statement published on the COM Website can be found at the end of this report.

Di-isopropylnaphthalene(s) in food packaging made from recycled paper and board: Conclusion on mutagenicity studies using the mouse lymphoma assay (MLA)

2.11 The Committee was asked by the COT to provide advice on the conduct and interpretation of *in-vitro* mutagenicity tests with di-isopropylnaphthalenes using the mouse lymphoma assay (MLA). The COM reviewed two separate tests, one at its May 1999 meeting and a further test at its February 2000 meeting. The COM concluded that the results of the two mouse lymphoma

assays were similar. The evidence suggested equivocal mutagenicity in the mouse lymphoma assay, therefore no conclusion based on the MLA studies could be drawn. A more detailed summary of the results from these two tests has been published on the COM Website.

2.12 However, the Committee noted that di-isopropylnaphthalene(s) contained no chemical groups which would be structurally alerting for potential mutagenicity. In addition there was no evidence for a mutagenic effect in other *in-vitro* mutagenicity tests or in an adequately performed *in-vivo* micronucleus assay in mice. The Committee agreed that no further mutagenicity testing was required.

Testing strategies and evaluation

2.13 The Committee completed a major piece of work during this year, namely the revision of its guidelines on an appropriate strategy for the testing of chemicals for mutagenicity. This involved contributions from members during 1999 whose terms of appointment ended in April 2000 and then from members of the new committee. The Committee's deliberations concentrated on the strategy itself, and members did not undertake any updating of the other aspects covered in the earlier guidelines. A draft document was issued for public consultation in March 2000. This was discussed at a meeting of the UKEMS's Industrial Genotoxicity Group in May 2000. It was agreed that the final document should be referred to as "Guidance on a strategy for testing of chemicals for mutagenicity" to emphasise the advisory nature of these recommendations.

2.14 The Committee also provided advice to the Advisory Committee on Pesticides on the evaluation of chemically induced aneuploidy and in particular the extrapolation of data from somatic cells to germ cells. This latter piece of work involved a detailed consideration of the conclusions reached by the European Commission's Group of Specialised Experts who were considering the classification and labelling of benomyl, carbendazim and thiophanate-methyl under the Dangerous Substances Directive 67/548/EEC. A report outlining the Committee's consideration of each of these items is given below and the statement by the Committee on the extrapolation of data on chemical induced aneuploidy is given at the end of this report.

Guidance on a strategy for testing of chemicals for mutagenicity.

2.15 The COM first published guidelines for testing of chemicals for mutagenicity in 1981. These provided guidance to the relevant government departments/agencies on the state of the art approach to testing at that time. The need for these to be periodically updated, to reflect advances in development and validation of methods, was recognised and revised guidelines were published in 1989. The new guidance which has been published as a separate document and on the COM Website continues this updating process. The strategy outlined is believed to be the most appropriate with regard to available methods and recognising the need to avoid the use of live animals where practical and validated alternative methods where available. It is recognised that, as with earlier guidelines, it will be some time before this strategy is reflected in the mandatory, regulatory guidelines of the various agencies, and it is not intended for this guidance to be applied retrospectively. The Committee believes that the approach outlined will remain valid for several years and will encourage steps to obtain international recognition of the newer tests being recommended for which there are, currently, no international harmonised guidelines.

2.16 An outline of the overall strategy in the revised guidance is given below. It is not possible to adequately cover all of the issues covered in the revised guidance document and the reader is encouraged to obtain a copy from the secretariat or to view the document on the COM Website.

2.17 The strategy being recommended, as in the Committee's earlier guidance, is based on three progressive stages.

2.18 Stage 1 (initial screening) is based on *in-vitro* tests. For most chemicals three tests are recommended, but for those where little or no human exposure is expected (eg industrial intermediates, some low production volume industrial chemicals) two tests may be appropriate, namely a bacterial assay for gene mutation and an *in-vitro* mammalian cell assay for clastogenicity and aneugenicity. The Committee believes that screening for both clastogenicity and aneugenicity is now possible in the initial (Stage 1) tests. The second test may be metaphase analysis, with consideration of hyperdiploidy, polyploidy and effects on mitotic indices as indicators of possible aneugenicity; if these suggest potential aneugenicity this needs to be confirmed by use of appropriate staining procedures, such as FISH and chromosome painting. Alternatively an *in-vitro* micronucleus test may be used. If a positive result is obtained, kinetochore or centromeric staining should be employed to ascertain the nature of the micronuclei induced (ie whether induction is due to clastogenicity or aneugenicity). The third recommended assay is an

additional gene mutation assay in mammalian cells, the mouse lymphoma assay being the current best choice. The three assays, if negative, will provide sufficient information for the assessment of most chemicals. However where high, or moderate and prolonged, levels of exposure are expected (eg most human medicines) an *in-vivo* assay is recommended to provide additional reassurance regarding lack of mutagenic activity. Decisions on the extent of testing appropriate for given exposure levels of specific chemicals need to be taken by the relevant regulatory authority on a case-by-case basis.

2.19 Stage 2 involves an assessment of whether genotoxic activity seen in any of the *in vitro* tests can be expressed in somatic cells *in vivo*. In addition, one appropriate *in-vivo* test is needed for all chemicals (which are negative in *in-vitro* assays) for which human exposure is expected to be high, or moderate and prolonged. A flexible approach is needed with consideration of the nature of the chemical, its metabolism and results obtained in the initial *in-vitro* tests. The most appropriate initial test will be a bone marrow micronucleus assay unless the initial considerations give an indication to the contrary. Techniques for the assessment of whole chromosomes are appropriate if there is evidence of aneugenicity. If negative results are obtained in this assay additional testing in other tissue(s) will be required for all compounds that are positive *in-vitro*, to provide adequate reassurance for the absence of activity *in vivo*. The type of study (or studies) needs to be considered on a case-by-case basis having regard to the available information on the compound including the results from earlier tests. Studies that may be appropriate include liver UDS assay, comet assay, ^{32}P-postlabelling assay, covalent binding to DNA and assays using transgenic animals; the reasons for the choice of assay in a specific given situation should be justified.

2.20 Stage 3 consists of assays in germ cells. The need for such studies requires careful consideration. In most cases chemicals that are recognised as *in-vivo* somatic cell mutagens will be assumed to be both potential genotoxic carcinogens and potential germ cell mutagens, and no further genotoxicity testing is necessary. However, in some cases germ cell studies may need to be undertaken to demonstrate that a somatic cell mutagen is not a germ cell mutagen. Information on whether a compound is genotoxic in germ cells may be obtained from a number of assays (eg metaphase analysis in spermatogonia or micronuclei induction in spermatocytes, the dominant lethal assay and mutation assays in transgenic animals). Information on the induction of DNA lesions in germ cells may be obtained using the various approaches listed for phase 2. Consideration of the type of mutation produced in earlier studies is important when selecting the appropriate assay. None of these assays provide conclusive information as to whether effects will be seen in future generations, and the only methods on which risk

estimates for the effects can currently be based are the heritable translocation test and the mouse specific locus test. These are not practical options in view of the very large number of animals needed. Currently there are no routine methods available for investigating the induction of aneuploidy in offspring following exposure of parental animals.

Thresholds for aneugens: Extrapolation of data from somatic cells to germ cells.

2.21 The safety evaluation of aneuploidy inducing chemicals (aneugens) acting by inhibition of microtubule formation is based on the identification of a threshold dose or NOEL below which aneuploidy is not induced. Benomyl, carbendazim and thiophanate-methyl belong to the methyl benzimadazole carbamate (MBCs) class of chemicals. The MBC class of chemicals are widely used in approved pesticide products as fungicides and also in veterinary medicines including anthelmintics in both food producing and companion animals. These chemicals act by interfering with microtubule formation during mitosis. The Committee was asked by the Advisory Committee on Pesticides (ACP) to advise on the applicability of extrapolating to germ cells evidence for thresholds for induced aneuploidy obtained in studies on somatic cells, and the relevance of these conclusions for the approach used by PSD to evaluate aneuploidy data in the risk assessment of agricultural pesticides, specifically in respect of MBCs.

2.22 The Specialised Experts concluded that "..current knowledge does not allow extrapolation to meiotic cells of the *in-vitro* finding of a threshold [for induced aneuploidy in somatic cells]. Meiosis I is fundamentally different from mitosis in the structures and processes involved in chromosome segregation. Due to the current lack of knowledge on the interaction of aneugens with these possible targets, the concept of a threshold for induced aneuploidy in germ cells is as yet a hypothetical one." The Committee undertook a detailed review of a study published by de Stoppelaar JM, *et al*, (1999) Mutagenesis, **14**, 621-631, which had been identified as the critical piece of evidence used by the Specialised Experts in reaching their conclusion.

2.23 The Committee concluded that the aneuploidy induced by methyl benzimadazole carbamates (specifically benomyl, carbendazim and thiophanate-methyl) which act by inhibiting spindle formation is a threshold-related effect. There is a sound scientific basis to assume that these chemicals have a threshold of action in both somatic and germ cells. The Committee did not agree with the interpretation reached by the European Commission's Group of Specialised Experts in fields of carcinogenicity, mutagenicity and

reprotoxicity at its meeting of the 1-2 September 1999 particularly with regard to the finding by de Stoppelaar *et al* (1999) of the induction of diploid sperm in rats in the absence of induction of micronuclei in peripheral erythrocytes. The Committee considered the finding of diploid sperm to be an expected effect of carbendazim on male germ cells undergoing meiosis and entirely consistent with the known effects of this chemical on microtubule formation.

2.24 A copy of the statement providing the detailed evaluation of the relevant data is given at the end of this report.

Ongoing Work

2.25 The Committee has agreed to consider Risk Assessment of mutagens other than aneugens with regard to thresholds during 2001.

Statements of the COM

Statement on the Mutagenicity of Hydroquinone and Phenol

Statement on Mutagenicity of 3-Monochloropropane 1,2- Diol (3-MCPD)

Statement on Alcoholic Beverages : Update on Information Published Between 1995-2000.

Statement on Thresholds for Aneugens : Extrapolation of Data from Somatic Cells to Germ Cells

STATEMENT ON THE MUTAGENICITY OF HYDROQUINONE AND PHENOL

Introduction

1. The Health and Safety Executive (HSE) asked for advice from the Committee during 1994 and 1995 and most recently in 1999 on the interpretation of the mutagenicity data on hydroquinone and phenol. The advice from COM was required by HSE as part of its regulatory reviews of occupational exposure limits to hydroquinone and phenol.

2. The principal use for hydroquinone is in the manufacture of black and white film developers. Other uses include the manufacture of antioxidants and polymerisation inhibitors; as a chemical intermediate in the manufacture of pharmaceutical, agrochemicals and dyes; in the production of cosmetics and topical creams; and as a laboratory reagent. Occupational exposure to hydroquinone in the UK is mainly via inhalation of airborne concentrations usually below 1 mg m^{-3} and averaging about 0.15 $mg.m^{3}$ [8 hour time weighted average (TWA)]. Dermal exposure to hydroquinone in the occupational setting is low. [The current UK occupational inhalation exposure limits for hydroquinone are 2 $mg.m^{-3}$ as an 8-hour TWA and 4 $mg.m^{-3}$ as a 15 minute short-term exposure limit (STEL).[1]]

3. Phenol is mostly used in the manufacture of phenolic resins, and is also used in the manufacture of disinfectants, some shampoos and in the preparation of soaps. The highest occupational exposures would be expected to occur in the paint stripping of aircraft, where exposures are controlled to below 8 $mg.m^{-3}$ (8 hour TWA). The other possible circumstances where high exposures may occur is in the use of phenolic resins in foundries. The resins contain small amounts of free phenol and whilst most exposures are very low, in some special cases exposures of up to 12 $mg.m^{-3}$ (8-hour TWA) may occur. There are no data available for occupational dermal exposure to phenol. However, as personal protective equipment is known to be extensively used, it is considered that exposure via the skin will be very low. [The current UK occupational inhalation exposure limits for phenol are 20 $mg.m^{-3}$ as an 8-hour TWA and 39 $mg.m^{-3}$ as a 15 minute STEL.[2]]

Overview of COM considerations.

4. A brief overview of the Committee's discussions held in 1994, 1995 and 1999 is given below. Full details of the Committee's considerations in 1994 and 1995 have been published in the Annual reports.[2,3]

5. In 1994, the COM agreed that both hydroquinone and phenol should be regarded as somatic cell *in-vivo* mutagens.[4-11] The Committee agreed that for exposure to these two compounds by the oral route there was potential for a threshold of activity as there was good evidence that two protective mechanisms (namely rapid conjugation and detoxification via the glutathione pathway) would substantially reduce systemic exposure to any active metabolites formed. However, Members agreed that there were insufficient data on inhalation and dermal exposure and it was not possible to assume that a threshold existed for activity when exposure was via the respiratory tract or the skin. The Committee noted the information from one published paper that when radiolabelled phenol was given intratracheally, initially all the radiolabel in the plasma was present as phenol.[12] These data suggested that there was little conjugation of phenol on the "first-pass" from airways to the circulation. The Committee recommended that appropriate toxicokinetic studies were needed.

6. In 1995, a submission from industry to the HSE provided some additional studies on the metabolism of hydroquinone and phenolic derivatives in the lung and skin, HSE requested the Committee's assessment of these new data.[13-20]

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

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

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

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

8. In 1999, further data from published papers on the kinetics of hydroquinone in rats following intratracheal instillation and on its percutaneous absorption in *in-vitro* studies using rat skin and human stratum corneum were provided to the Committee.[21,22] A number of additional *in-vivo* mutagenicity studies

including an investigation of site of contact mutagenicity in skin and respiratory tract of Muta^{TM} mice using the LacZ transgene were also considered.[23-25]

9. Regarding the new data on hydroquinone,[23] the Committee agreed that a positive result had been obtained in a new bone-marrow micronucleus assay and that these results were consistent with previous studies considered in 1994. The new toxicokinetic study in which rats were given a single intratracheal dose of ^{14}C-hydroquinone showed detectable free hydroquinone in arterial blood within 5-10 seconds after dosing.[21] This new information suggested a potential risk of site-of- contact and systemic mutagenic effects following inhalation exposure to hydroquinone.

10. Regarding the new data on phenol, the new bone-marrow micronucleus studies showed that a small but consistent positive result with phenol could be identified in studies conducted according to OECD guidelines at intraperitoneal dose levels of around 100-160 mg/kg.[23,24]

11. The Committee considered the new transgenic mutagenicity test with phenol using the LacZ transgene in Muta^{TM} mice.[25] Animals were given either dermal doses of 100 mg/kg bw or exposed for a period of 2 hours to a vapour containing 100 ppm phenol (390 mg.m^{-3}) on five consecutive days. Samples of tissues (liver, bone marrow, and blood, and also, for inhalation exposure, nasal epithelia and lung) were taken at a number of time points after dosing and the DNA extracted and packaged for analysis of LacZ mutants. Members noted that a positive control chemical (benzo(a)pyrene) had been used for the dermal studies but no positive control had been used for the inhalation studies presumably because of the potential hazards involved in handling and controlling exposures to test animals. Members acknowledged that there would be an observable degree of inter-animal variation in results for *in-vivo* mutation assays such as LacZ, which complicates the assessment of data but agreed that the results reported for the study concerned could not be assessed in view of the failure to obtain acceptable levels of DNA packaging in many of the trials. The Committee considered that inhalation exposure to phenol followed by assessment of mutation frequency in nasal tissue were critical to the identification of site-of-contact mutagenicity and felt that a further study with acceptable levels of DNA packaging would be needed before any conclusions on site-of-contact mutagenicity could be reached.

Overall conclusions

12. The Committee reached the following conclusions based on all the available information.

Hydroquinone

a. Hydroquinone is an *in-vivo* mutagen in somatic cells,[4-11] but there is no convincing evidence for effects in germ cells *in vivo*.[26-28] Any risk to human health by ingestion would be likely to be greatly reduced by rapid conjugation and detoxification via the glutathione pathway. Furthermore, mutagenicity appeared to be positively related to peroxidase activity while catalase could also have a protective role.[29] Actual systemic exposure levels in humans would be very much lower than levels at which positive results had been achieved in studies in animals.

b. The Committee concluded that by the oral route there was potential for a threshold of activity based on the protective mechanisms outlined at (a).

c. However, there is insufficient evidence to support a threshold approach to risk assessment for inhalation or dermal exposure to hydroquinone.

d. The Committee concluded that the available data showed that occupational exposure to hydroquinone was associated with a mutagenic hazard but it was not possible to quantify the risk.

Phenol

a. *In-vitro* mutagenicity data on phenol were of poor quality and results difficult to interpret, but *in-vivo* data show phenol to be a somatic cell mutagen following intraperitoneal doses of approximately 100-160 mg/kg [5,8,23,24] No conclusions can be drawn from the one available study in transgenic animals (Muta^{TM} mice) on site-of-contact mutagenicity following dermal or inhalation exposure.[25] The Committee felt that a further study in transgenic animals, with acceptable levels of DNA packaging, would be helpful before any conclusions on site-of-contact mutagenicity could be reached. Data from germ cell studies *in vivo* were inadequate to allow any definite conclusions to be drawn.[30,31]

b. Any risk to human health by ingestion would be likely to be greatly reduced by rapid conjugation and detoxification via the glutathione pathway. Furthermore mutagenicity also appeared to be positively related to peroxidase activity while catalase could also have a protective role. Actual systemic exposure levels in humans would be very much lower than levels at which positive results had been achieved in studies in animals.

c. The Committee concluded that by the oral route there was potential for a threshold of activity based on the protective mechanism outlined at (b).

d. However, there is insufficient evidence to support a threshold approach to risk assessment for inhalation or dermal exposure to phenol.

e. The Committee concluded that the available data showed that occupational exposure to phenol was associated with a mutagenic hazard but it was not possible to quantify the risk.

January 2000
COM/00/S1

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MUTAGENICITY OF 3-MONOCHLORO PROPANE-1,2-DIOL (3-MCPD)

Introduction

1. 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 COM was asked in 1999 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. The COM 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.[1]

2. In 1999 the COC noted that in a carcinogenicity study undertaken by Sunahara *et al* (1993) 1,3-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.[2] Statistically significant increases in leydig cell adenomas (intermediate and high dose level) and mammary gland fibroadenomas (high dose level) had been noted in males and a statistically significant increase in kidney tumours had been noted in females at the high dose level. The COC had noted in 1999 that the high dose level had exceeded the Maximum Tolerated Dose. The COC therefore asked the COM for an assessment of the mutagenicity data on 3-MCPD as part of its evaluation of the mechanism for the carcinogenic effects seen in rats.

Evaluation: 1999

3. 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.[1] 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,[3] a review

document published by the Institute of Toxicology, National Food Agency of Denmark,[4] and one *in-vivo* mutagenicity submitted in an in-confidence basis.[5] 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 oral mass balance investigation available. 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.

4. The Committee reached the following conclusions on the mutagenicity data available in 1999.

   i) 3-MCPD was mutagenic in Salmonella typhimurium in the absence of exogenous metabolic activation.[6-9] 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,[4] but the full report of the study was not available to the Committee. Positive results were also reported in tests in yeast (Schizosaccharomyces pombe)[10] and in tests for Sister Chromatid Exchange in mammalian cells.[4]

   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 to 120 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 (ie ratio of PCE/NCE) and thus there was no evidence to show exposure of the bone marrow to the test material and its metabolites had occurred.[5]

   v) The Committee agreed that no conclusions could be drawn from the investigation of colonic micronuclei in mice[5] in view of the limited database available for this assay or from the inadequately reported dominant lethal assays.[11,12]

   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*.

Evaluation: 2000

5. The Committee considered two new *in-vivo* mutagenicity studies commissioned by the UK Drinking Water Inspectorate at its October 2000 meeting. These comprised a rat bone-marrow micronucleus test and a rat liver UDS assay, both of which are widely used to assess genotoxicity *in vivo*.

Rat in-vivo bone-marrow micronucleus test[13]

6. The assay protocol conformed to OECD 474. A single sex [male (Crl: HanWist BR): group size 6] was used as there was no substantial sex differences in toxicity. The top dose was selected from a range-finding study in which single oral doses of between 20-100 mg/kg bw were administered once daily for two consecutive days to groups of male and female rats. Dose levels of 60 mg/kg bw resulted in severe toxicity and some deaths. In the main study, doses of 15, 30 and 60 mg/kg bw were given for two consecutive days. Signs of toxicity were seen at the top dose level (piloerection) which was also associated with a clear reduction in polychromatic erythrocytes to normochromatic erythrocytes, indicating bone marrow cytotoxicity (and hence that 3-MCPD and/ or its metabolites reached the bone-marrow).

7. There was no increase in the number of micronucleated PCEs at any dose level in 3-MCPD treated animals (2000 polychromatic erythrocytes scored/animal). The positive control, cyclophosphamide produced a clear increase in micronuclei.

Rat Liver UDS assay[14]

8. The UDS assay protocol conformed to OECD protocol 486. The top dose, a single oral dose of 100 mg/kg bw, was chosen on the basis of a sighting toxicity study which had shown severe toxicity at oral doses of 150 mg/kg bw. In the main study single oral doses of 40 mg/kg bw or 100 mg/kg bw were administered to male rats (Han Wistar) and hepatocytes recovered for UDS analysis using autoradiography after 12-24 hours (4 animals/dose level) and 2-4 hours (5 animals/dose level). No signs of toxicity were seen at either dose level. There was no evidence for any increase in UDS at either dose level or time point. The two positive control substances (2-AAF and DMN) both gave clear positive results.

Discussion

9. Members agreed that 3-MCPD has a chemical structure which suggests that it may be metabolised to genotoxic intermediates (particularly glycidol). 3-MCPD was clearly mutagenic *in vitro* in the salmonella assay and in the mouse lymphoma assay in the presence of metabolic activation.

10. The committee noted that the predominant urinary metabolite in rats fed or given intraperitoneal doses of 3-MCPD was ß-chlorolactic acid[15] ie by a pathway not producing glycidol or other genotoxic intermediates. A degradation product of ß-chlorolactic acid, namely oxalic acid, has been documented to induce the nephrotoxic effects seen with 3-MCPD.[5,16] One study has also shown that 3-MCPD may be metabolised by a minor pathway and undergo conjugation with glutathione to ultimately form a mercapturic acid in urine of rats [N-acetyl-S-(2,3-dihydroxypropyl) cysteine],[17] suggesting the formation of a reactive metabolite, glycidol, at low levels that are subsequently inactivated. The COM considered that the metabolism of the 3-MCPD in rats had not been fully examined, but agreed that evidence from the two new *in-vivo* mutagenicity studies supported the view that reactive metabolites were not produced in the tissues where genotoxicity was assessed. Thus the Committee reached the following conclusions.

Conclusion

11. The Committee concluded that both the rat bone-marrow micronucleus test and the rat liver UDS test had been carried out to an acceptable standard and were negative. Thus the additional information recommended by the COM as being necessary to provide adequate reassurance that the mutagenic activity seen *in vitro* was not expressed *in vivo* had now been provided.

12. The Committee agreed that the major urinary metabolite ß-chlorolactic acid in rats was formed by oxidation of 3-MCPD and that the two new mutagenicity studies supported the view that reactive metabolites if formed did not produce genotoxicity *in vivo* in the tissues assessed.

13. The Committee concluded that 3-MCPD can be regarded as having no significant genotoxic potential *in vivo*.

October 2000
COM/00/S4

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STATEMENT ON ALCOHOLIC BEVERAGES: UPDATE ON INFORMATION PUBLISHED BETWEEN 1995-2000

Introduction

1. In 1995 the COM gave detailed consideration to the mutagenicity of ethanol, acetaldehyde and alcoholic beverages. This was to provide input to the Government Interdepartmental Working Group reviewing overall advice on this topic. The Committee on Carcinogenicity (COC) also carried out a detailed review of the available data, mainly from epidemiology studies on the carcinogenicity of alcoholic beverages. The advice from these Committees was considered by the Interdepartmental Working Group when drawing up their Report on Sensible Drinking published in December 1995.[1] The main conclusions reached were:

   i) The COC concluded that the epidemiological evidence supported the view that drinking alcohol causes a dose-related increase in the risk of squamous carcinomas of the upper aerodigestive tract as a whole, and for cancers of the oral cavity, pharynx, larynx and oesophagus.

   ii) The COM agreed that the consumption of alcoholic beverages does not present any significant concern with respect to their mutagenic potential.

2. With regard to the COC conclusions on the particularly important topic of the association between alcohol and breast cancer, the Committee felt that while there was no decisive evidence that breast cancer is causally related to drinking alcohol, the potential significance for public health of a weak causal association between alcohol and breast cancer was such that they recommended that this matter be kept under review.

3. The COC has recently finalised its review of the published literature from 1995-1999 on alcohol and breast cancer (*www.doh.gov.uk/coc.htm*). The COC concluded:

   i) There is an association between drinking alcoholic beverages and increased risk of breast cancer. It is difficult to resolve whether this is causal. The magnitude of the observed association is small (ie the relative risk is modest and, even for heavy drinkers, rarely in excess of 3) and within the range where it is difficult to exclude bias and/or confounding as explanations for the observed results in epidemiological studies. It is difficult to derive a quantitative relationship from the dose-response data available in the literature.

ii) Further epidemiological studies have been published since 1995. There is a need for further systematic review of the epidemiological literature to assess fully the influence of bias, confounding and effect modification. This will contribute to a conclusion on causality and population attributable risk associated with drinking alcoholic beverages.

iii) Studies of possible mechanisms provide evidence for a plausible basis for the causation of breast cancer by consumption of alcohol. Alcohol increases blood levels of oestrogens and in particular oestradiol in both premenopausal and postmenopausal women. These data suggested a similar mechanism to other known breast cancer risk factors.

iv) The COM should be asked to update its opinion of 1995 on the mutagenicity data on alcohol.

4. This statement details the conclusions reached by the COM with regard to the published information on ethanol, acetaldehyde and alcoholic beverages from 1995 to February 2000, and whether there was any need to modify the conclusions drawn in 1995. The Committee recalled that alcoholic beverages contain small amounts of a significant number of volatile and non-volatile organic compounds formed during production, storage and maturation. The Committee reaffirmed its view that it was not essential nor practical to review these constituents individually for their mutagenic potential.

5. The conclusions reached with regard to the mutagenic potential of ethanol, acetaldehyde and alcoholic beverages are given below. A discussion of one recent hypothesis[1] that alcohol might induce breast cancer via the production of reactive oxygen species (ROS) is also included.[2]

Mutagenicity of ethanol

6. The Committee noted that there were no new *in-vitro* mutagenicity studies with ethanol. No conclusions could be drawn regarding the *in-vitro* investigations of effects of ethanol in the pre-implantation development of mouse oocytes injected with spermatozoa stored in 70% ethanol.[3]

7. The Committee reaffirmed its previous conclusions with regard to the mutagenicity data on ethanol, namely: negative results have been obtained in a wide range of *in-vitro* tests and in *in-vivo* tests including those for effects on germ cells; it was concluded that there was no evidence that ethanol induces germ cell mutation *in vivo.*

Mutagenicity of acetaldehyde

8. The committee agreed that the most recent experiments using human lymphoma cells had confirmed earlier studies that acetaldehyde induces protein-DNA cross links, but only at concentrations which resulted in cell death. In addition acetaldehyde induced HPRT mutations in human T cells.[5] Members agreed that no conclusions could be drawn from the finding of acetaldehyde DNA adducts in peripheral white blood cells of alcoholics in view of lack of control for the effects of smoking by alcoholics in the study group and the well known abnormalities in metabolism in alcoholics.[6]

9. The Committee reaffirmed its previous conclusions with regard to acetaldehyde. The available data show that acetaldehyde induces chromosome aberrations in mammalian cells in the absence of an exogenous metabolising fraction. There is some evidence to show that covalent binding (DNA-protein cross links) in the nasal mucosa of rats exposed to high levels of acetaldehyde by inhalation.

10. The mutagenic profile of acetaldehyde is very similar to that of formaldehyde. The compound has direct acting mutagenic potential *in vitro*, but would only be expected to have the potential of *in-vivo* activity at sites where it is not rapidly metabolised to acetic acid. The COC has concluded that the observation of tumours in animals exposed to high inhalation doses of acetaldehyde is not relevant to drinking alcohol.[1]

Mutagenicity of Alcoholic Beverages

11. The Committee recalled that in 1995, considerable weight had been attached to one study from the Medical Research Council's Cell Mutation Unit, who has examined hprt mutant frequency in circulating T-lymphocytes of normal adults and the relationship with alcohol intake.[7] The study showed that alcohol intake in 143 people over the range of 0-56 units/week (1 unit – 8g ethanol) had no effects on hprt mutant frequency. Less weight had been placed on studies which examined the mutagenicity of concentrated extracts of wines and sprits in bacteria,[1] and the significance of such data was felt to be questionable. There were no adequate *in-vivo* mutagenicity studies of alcoholic beverages available in 1995 or for the current review.

12. Since 1995 two further studies of the relationship between hprt mutant frequency in lymphocytes obtained from individuals for whom information on drinking patterns were available.[8,9] There was no association between hprt mutant frequency and alcohol ingestion in these studies, thus confirming the results of the earlier MRC investigation.

The Reactive Oxygen Species hypothesis of alcohol induced breast cancer

13. Members reviewed the hypothesis published by Wright *et al*.[2] Wright and colleagues had noted the finding that alcohol metabolism is known to produce reactive oxygen species (ROS) and mammary tissue contains the necessary metabolising enzymes to produce ROS from alcohol. In addition, Wright and colleagues noted two further observations which supported their hypothesis, namely that breast cancer is associated with higher levels of hydroxyl modified DNA and iron, which has been proposed to catalyse the formation of ROS, accumulated with time in breast tissue. Members agreed that there was evidence that ethanol and its metabolites induced the formation of free radicals *in vitro*, but the evidence *in vivo* was conflicting. Members commented that the observations reported by Wright and colleagues might be a result of tumour progression rather than an initiator of cancer. In addition it was noted that co-administration of iron and alcohol to rats in the initiation phase of a two stage model for hepatocarcinogenesis[10] did not result in any genotoxic effects. Overall, the COM concluded that there was insufficient evidence to support the Wright *et al* hypothesis regarding breast cancer.

Overall Conclusion

13. The Committee reaffirmed its 1995 conclusion that consumption of alcoholic beverages does not present any significant concern with respect to their mutagenic potential.

November 2000
COM/00/S5

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STATEMENT ON THRESHOLDS FOR ANEUGENS: EXTRAPOLATION OF DATA FROM SOMATIC CELLS TO GERM CELLS

Consideration of Summary record of European Commission group of specialised experts in fields of carcinogenicity, mutagenicity and reprotoxicity meeting 1-2 September 1999

Introduction

Risk Assessment of benomyl, carbendazim and thiophanate-methyl

1. Benomyl, carbendazim and thiophanate-methyl belong to the methyl benzimadazole carbamate (MBCs) class of chemicals. These are widely used in approved pesticide products as fungicides and also in veterinary medicines, in particular as anthelmintics, in both food producing and companion animals. These chemicals act by interfering with microtubule formation during mitosis. The COM has provided advice to the UK regulatory Authorities namely the Pesticides Safety Directorate (PSD) and the Veterinary Medicines Directorate (VMD) of the Ministry of Agriculture, Fisheries and Food on the most appropriate approach for the risk assessment of MBCs. [1-3]

2. In 1993 the COM agreed that it was reasonable to assume that aneuploidy inducing chemicals (particularly those that function by interfering with the spindle apparatus of cell division) have a threshold of action.[1] The safety evaluation of aneuploidy inducing chemicals (aneugens) acting by inhibition of microtubule formation is based on the identification of a threshold dose below which aneuploidy does not occur. The Committee provided advice on methodology for identifying thresholds in 1993, namely appropriate *in-vitro* experiments in human lymphocytes using the detection and quantification of non-disjunction, chromosome loss and centromere positive micronuclei using FISH (Fluorescent in-situ hybridisation) analysis of selected chromosomes for centromeric DNA. This advice was used by PSD and VMD when requesting data from approval/licence holders of products containing MBCs. In 1996, the Committee considered the results of experiments undertaken with benomyl and carbendazim and concluded that the studies had been satisfactorily conducted and the data indicated No Observed Effect Levels (NOELs) for these two chemicals.[4-6] It was noted that that it would be difficult to define

precise thresholds for activity from these data and the mathematical models that had been used for their analysis. Appropriate studies which provided evidence for a threshold effect have also been undertaken with thiophanate-methyl.[7]

3. The UK Advisory Committee on Pesticides (ACP) considered that the available *in vitro* aneuploidy data were consistent with a threshold for MBC-induced aneuploidy. The ACP considered that *in vitro* aneuploidy threshold studies should be regarded as providing data that underpinned the regulatory decision rather than providing critical NOELS for direct use in setting Acceptable Daily Intakes (ADIs) and Acceptable Operator Exposure Level (AOEL) values. The conclusions of the UK review have been passed to the rapporteur for the ongoing EC review (under Directive 91/414/EEC) of the use of MBCs as agricultural pesticides.

4. In the case of consumer safety for veterinary medicines, ADIs and Maximum Residue Limits (MRLs) in edible tissues are set on a substance specific basis by the EU Committee for Veterinary Medicinal Products (CVMP). All currently authorised veterinary medicines have been assessed on the basis of the concept that aneuploidy induced by spindle inhibitors is a threshold effect.

Background to current review

5. The Committee was asked by the ACP to consider the conclusions reached by the European Commission's Group of Specialised Experts who were considering the classification and labelling of benomyl, carbendazim and thiophanate-methyl under the Dangerous Substances Directive 67/548/EEC. The Committee considered a draft summary record of the meeting of the Specialised Experts held on the 1-2 September 1999.[8] The Committee was asked by the ACP to advice on the applicability of extrapolating to germ cells, evidence for thresholds for induced aneuploidy obtained in studies on somatic cells, and the relevance of those conclusions for the approach used by PSD to evaluate aneuploidy data in the risk assessment of agricultural pesticides. The Committee has not been asked to comment on the proposals for classification and labelling of these MBCs.

Conclusion reached by Specialised Experts

6. The Specialised Experts concluded that "..current knowledge does not allow extrapolation to meiotic cells of the *in-vitro* finding of a threshold [for induced aneuploidy in somatic cells]. Meiosis I is fundamentally different from mitosis in the structures and processes involved in chromosome

segregation. Due to the current lack of knowledge on the interaction of aneugens with these possible targets, the concept of a threshold for induced aneuploidy in germ cells is as yet a hypothetical one."

7. The COM considered that the critical piece of evidence used to reach this conclusion came from the publication by de Stoppelaar *et al* (1999) which reported diploidy (ie polyploidy but not aneuploidy) in sperm of rats exposed to carbendazim.[9] de Stoppelaar *et al* concluded that their findings suggested that diploidy in sperm is induced at a lower dose level than micronuclei in peripheral blood erythrocytes (no micronuclei were seen in this study). The Committee reaffirmed that there was adequate information available on the mechanism of interaction of MBCs with microtubules to assess the effects of these chemicals in both somatic and germ cells. The Committee agreed it therefore important to review the results obtained by de Stoppelaar *et al* in detail in order to comment on the conclusions reached by the Specialised Experts.

Consideration of de Stoppelaar *et al* Mutagenesis, 14, 621-631, 1999

8. Groups of 5 Wistar (Unilever) rats aged 13-14 week were given a single oral dose of carbendazim (50, 150, 450 or 800 mg/kg bw) in corn oil. The control group received corn oil only. A further group of 5 rats were given an intraperitoneal dose of 150 mg/kg bw carbendazim in corn oil. The animals were killed at thirty-one or 50 days (at 450 mg/kg bw only) and epididymal sperm isolated. In a second experiment, groups of three rats received a single oral dose of carbendazim (2.5, 5, 10, 20, 30, 40, 50, 100, 150, 450, 800 mg/kg bw) in corn oil. A group of 4 rats received 3 mg/kg bw mitomycin C (intraperitoneal) and the negative control received corn oil only. One day before and 48 hours and 72 hours after treatment peripheral blood samples were collected from the tail vein for assessment of micronucleated erythrocytes. The animals in the second experiment were killed thirty-one days after treatment and epididymal sperm isolated. Fluorescence in situ hybridisation was carried out using DNA probes specific for rat chromosomes 4 and Y (and 19 in the second experiment). Additional analyses were undertaken using some animals from the first experiment for chromosome 4 and 19 to confirm the presence of diploid sperm. Five thousand sperm were scored on two slides per animal (ie 10,000 sperm per animal). Only one slide was scored for rats treated intraperitioneally or in trials where rats were killed at 50 days after treatment.

9. The main finding of experiment one was a small but dose-related increase in the absolute frequency of diploid sperm (0.03% to 0.22%) following oral dosing and analysis of sperm at 31 days after treatment. An increase in sperm classified as diploid was only seen in one of five animals following intraperitoneal treatment with carbendazim at 150 mg/kg bw. No increase in 'diploid' sperm was seen in animals killed 50 days after treatment with an oral dose of 450 mg/kg bw carbendazim. The Committee noted that a smaller increased frequency of diploid sperm was reported in the second experiment in animals given an oral dose of 800 mg/kg bw carbendazim which may have resulted from sub-optimal exposure conditions in the experiment. No micronuclei were induced in peripheral blood erythrocytes following oral treatment of up to 800 mg/kg bw after sampling peripheral blood at 24 or 48 hours post treatment.

10. The Committee noted that the mechanism of action of carbendazim involved interference with the formation of polar microtubules. This effect combined with differences in the type of nuclear organising centres (NOC's) for germ cells in first meiotic division in males (a single pole) and in females (multiple poles) would result in carbendazim inducing polyploidy in sperm and aneuploidy in oocytes. The Committee agreed that the finding of diploid but not aneuploid sperm by de Stoppelaar *et al* was to be expected. The Committee noted the finding of aneuploid oocytes in hamsters given a single oral dose of 1000 mg/kg bw carbendazim was also an expected finding.[10] The Committee considered that in the case of MBCs such as benomyl, carbendazim and thiophanate-methyl that affect the formation of spindles it was scientifically plausible for such compounds to be aneugenic in both somatic and germ cells. There is currently no evidence to suggest that MBCs are capable of modifying meiosis I specific events such as chromosome pairing. The Committee agreed that it would be expected that MBCs had a threshold of activity in somatic and germ cells.

11. The Committee considered that the results obtained by de Stoppelaar *et al* did not provide evidence for a lower threshold for aneuploidy in germ cells compared to somatic cells. The Committee felt that the analysis of peripheral blood samples for micronuclei in rats undertaken by de Stoppelaar *et al* was suboptimal in that a 24 hour sampling time point should have been used as micronucleated erythrocytes may have been efficiently removed by the spleen in rats and, as noted in paragraph 9 above, exposure conditions used may not have been optimal for the production of micronuclei. The Committee agreed that a more appropriate study in somatic cells for comparison with germ cells in the rat would be an investigation of the dose-response for the formation of micronuclei containing aneuploid chromosomes in polychromatic erythrocytes obtained in bone marrow smears from rats treated using a similar protocol to that used by de Stoppelaar *et al*. The Committee noted that such

data would be informative with regard to any differences between aneuploidy in somatic and germ cells and agreed to review the subject when appropriate studies had been undertaken. Even if it could be established that the effects on sperm occur at lower doses than for somatic cells, this would not invalidate the concept of a threshold effect.

Conclusions

12. The Committee agreed the following conclusions:

i) The aneuploidy induced by methyl benzimadazole carbamates (specifically benomyl, carbendazim and thiophanate-methyl) which act by inhibiting spindle formation is a threshold related effect. There is a sound scientific basis to assume that these chemicals have a threshold of action in both somatic and germ cells. The Committee did not agree with the interpretation reached by the European Commission's Group of Specialised Experts in fields of carcinogenicity, mutagenicity and reprotoxicity at its meeting of the 1-2 September 1999 particularly with regard to the finding by de Stoppelaar *et al*[8] of diploid sperm in rats. The Committee considered the finding of diploid sperm to be an expected effect of carbendazim on male germ cells undergoing meiosis and entirely consistent with the known effects of this chemical on microtubule formation.

ii) The Committee concluded that de Stoppelaar *et al*[9] had not adequately demonstrated a lower threshold for aneuploidy in male germ cells of the rat compared to somatic cells. The Committee agreed that a more appropriate study in somatic cells for comparison with germ cells in the rat would be an investigation of the dose-response for the formation of micronuclei containing aneuploid chromosomes in polychromatic erythrocytes obtained in bone marrow smears from rats using a similar treatment protocol to that used by de Stoppelaar *et al*. The Committee agreed to review the subject when appropriate studies had been undertaken.

iii) The Committee agreed that the approach used for risk assessment of MBCs by regulatory authorities for pesticides and veterinary medicines and the strategy outlined in the Pesticide Safety Directorate position paper on the role of aneuploidy in the risk assessment of agricultural pesticides were acceptable but would need to be reviewed should a marked difference in sensitivity to aneuploidy induced by these chemicals be reported between germ cells and somatic cells.

iv) The Committee agreed that these conclusions were only relevant to aneuploidy inducing chemicals acting by spindle inhibition. The risk assessment (ie consideration of thresholds in somatic and germ cells) of aneuploidy inducing chemicals acting via other mechanisms needed to be considered on a case by case basis.

June 2000
COM/00/S2

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2000 Membership of the Committee on the Mutagenicity 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 J M Parry (Chairman)	Compass Catering JIB Insurance National Power SmithKline Beecham Quintiles	Share Holder Share Holder Share Holder Consultant Consultant	Astra BAT Boehringer Glaxo/Wellcome Pfizer Welsh Water	Grant Grant Grant Grant Grant Grant
Prof J Ashby	Zeneca ML Labs Phytopharm	Employee, Salary, Share option Share Holder Share Holder	NONE	NONE
Dr J Clements	Covance	Employee, Salary, Share Option, Share Holder	NONE	NONE
Prof C Cooper	Halifax Norwich Union	Share Holder Share Holder	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
Dr N Gooderham	Abbey National plc Albert Fisher Bula resources ML Laboratories plc Northern Rock Protherics Sunderland AFC	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	SmithKline Beecham	BBSRC Collaborative Studentship
Ms M Langley	NONE	NONE	NONE	NONE
Prof D Phillips	Abbey National plc BG Group Bradford & Bingley Centrica CGNU Lattice Group National Grid	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	NONE	NONE

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Dr I Mitchell	Kelvin Associates	Associate/ Consultant	NONE	NONE
	IM Enterprises	Director/ Creditor		
	Glaxo Smithkline	Pensioner, Option and Share Holder, Consultant		
	Bass	Share Holder		
	Cable & Wireless	Share Holder		
	Cadbury Schweppes	Share Holder		
	Marconi	Share Holder		
	Nokia	Share Holder		
	Pfizer	Share Holder		
	RTZ	Share Holder		
	Shell	Share Holder		
	Unilever	Share Holder		
	Vodafone	Share Holder		
	Whitbread	Share Holder		
	British Telecom	PEP Holder		
	Centrica	PEP Holder		
	Scottish Power	PEP Holder		
	Shire	PEP Holder		
Prof D J Tweats	Glaxo Wellcome	Salary Employee Share Option Holder Share Holder	NONE	NONE
	Halifax	Share Holder		
	Co-operative Bank plc	PEP Holder		

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 & Food Standards Agency and the other Government Departments including the Regulatory Authorities. A new Committee has been recently appointed via open advertisement procedures established by the Commissioner for Public Appointments. The new appointments were made on 1 April 2000 to run for 3 years. I would like to welcome the new members (Professor D Harrison, Ms D Howel, Professor D Phillips, Dr R Roberts and Professor D Shuker) to the Committee. The details of the Membership are published on the Internet and the Agenda, Minutes and Statements are published on a regular basis.

During the year 2000 the COC has published 3 statements; alcohol and breast cancer, carcinogenicity of 3-MCPD, cancer incidence near municipal solid waste incinerators in Great Britain. The advice on alcohol and breast cancer was an update of data published between 1995 – 1999. The Committee made a recommendation for further research, namely a systematic review of epidemiology, which is being funded by the Department of Health. The Committee also provided advice on research to be sponsored by the Department of Health on the early identification of non-genotoxic carcinogens. A preliminary assessment of a pre-publication paper on the trends in the incidence of intrahepatic cholangiocarcinoma was undertaken. The Committee looks forward to assessing the final published version of this particular research. The Committee also provided advice on test strategies including the problems associated with poor survival in certain strains of rat.

During 2000, the Food Standards Agency was established and the COC now has a joint Secretariat with DH/FSA. The DH Toxicology Unit at the Imperial College of Science, Technology and Medicine now prepare some of the papers for the COC.

Professor P G Blain (Chairman)
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3-Monochloropropane-1,2-diol (3-MCPD)

3.1 3-Monochloropropane-1,2-diol (3-MCPD) can be present as a contaminant in epichlorhydrin/amine copolymers used as flocculants or coagulant 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 as a contaminant in drinking water arising from this use. 3-MCPD is a member of a group of contaminants known as chloropropanols. 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) The COC was asked to evaluate and advise on the carcinogenicity of 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, Transport and the Regions on the approval of chemical substances in contact with public water supplies.

3.2 The COC had reviewed the available carcinogenicity data on 3-MCPD in 1999 and had 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, the COM conclusions were noted. These were that 3-MCPD was an *in vitro* mutagen and that further *in vivo* data was needed to provide reassurances that this activity could not be expressed *in vivo*. The COC concluded that it would be prudent to assume that the compound was an *in vivo* mutagen. In view of these COM conclusions the COC agreed that it would be prudent to reduce exposures to as low as technologically practicable.

3.3 Another review of the carcinogenicity data was undertaken by the COC at its November 2000 meeting following further advice from the COM which had reviewed new *in-vivo* mutagenicity studies conducted using 3-MCPD. These provided evidence that reactive (mutagenic) metabolites of 3-MCPD are not produced *in vivo* in the tissues examined in these studies. In the light of the new data the COM was able to conclude that 3-MCPD is an *in-vitro* mutagen but has no significant genotoxic potential *in-vivo*. The COC was asked to consider the implications of these revised conclusions on the mutagenicity data for carcinogen risk assessment.

3.4 The Committee considered the proposal that all of the increases in tumours noted in rats were mediated by non-genotoxic mechanisms involving either cytotoxicity (in respect of the findings in the kidney) or hormonal disturbances. The possible influence of the stereoisomerism of 3-MCPD was also discussed. Members agreed that it was now probable that 3-MCPD induced tumours by non-genotoxic mechanisms.

3.5 The Statement on 3-MCPD can be found at the end of this report.

Accelerator Mass Spectrometry - An aid to carcinogen risk assessment

3.6 Accelerator Mass Spectrometry (AMS) is the most sensitive technique available for measuring the formation of adducts with DNA. AMS technology allows the accurate measurement of very low levels of radiolabelled chemicals (particularly ^{14}C) in biological samples at around 10^{-21} to 10^{-18} mole. The Committee was asked to consider the value of AMS for the assessment of chemically induced carcinogenicity.

3.7 The Committee noted that high levels of sensitivity and reproducibility in the analysis of biological samples were reported with AMS. The sensitivity of the technique was related to the background level of radioactivity in the sample and, if individual adducts are being investigated, the quality and effectiveness of the HPLC separation used in the sample preparations.

3.8 The Committee concluded that AMS is a highly sensitive and reproducible technique. Its main uses in the area of chemical carcinogenicity are in hazard characterisation, measurement of tissue levels of administered radiolabelled compounds and mechanistic investigations. However, the biological significance of the very low levels of binding that may be observed is difficult to assess. Furthermore, the very high cost of the technology currently limits the use of AMS.

The association between alcohol and breast cancer

3.9 Breast cancer is the most common cancer in women. In England, approximately 30,000 cases are registered each year and there are roughly 11,000 deaths from breast cancer. It is clearly important to identify preventative measures to reduce the incidence of breast cancer.

3.10 The COC last reviewed the extensive literature on the association between alcohol and breast cancer in 1995, at the request of the Interdepartmental Working Group on Sensible Drinking (IDWG), as part of the review of medical and scientific evidence on alcohol and health and interpretation of the long term effects of drinking alcoholic beverages. The Committee advised the IDWG that drinking alcoholic beverages causes a dose-related increase in the risk of squamous carcinomas of the upper aerodigestive tract as a whole, and of cancers of the oral cavity, pharynx, larynx, and oesophagus which is independent of the effect of smoking tobacco.

3.11 With respect to breast cancer, the Committee concluded "... while there is no decisive evidence that breast cancer is causally related to drinking alcoholic beverages, the potential significance, for public health, of even a weak association between alcohol and breast cancer is such that we recommend, in particular, that this matter be kept under review." The IDWG endorsed the COC's conclusions and the recommendation that the relationship between alcohol and breast cancer should be kept under review.

3.12 The Committee undertook a detailed review of the literature published since 1995 at three meetings in 1999 and finalised a statement at its March 2000 meeting. It was evident that a large number of epidemiology studies had been published since the first review. The Committee's finalised statement is appended to this report. The detailed secretariat papers considered during the review have been published on the COC Website (www.doh.gov.uk/coc.htm)

3.13 Following publication of the COC statement, the Department of Health is funding a systematic review of the epidemiological literature at Imperial College of Science, Technology and Medicine. The study is due to be completed by the end of 2001. The Committee discussed further the potential mechanism by which alcohol might induce breast cancer at its November 2000 meeting where members considered a draft scoping study by Dr Tim Keys (ICRF, Oxford) on the investigation of the effects of alcohol on oestrogen metabolism. The Committee will consider this aspect in more detail when the report of the systematic review becomes available.

3.14 The Statement on Alcohol and Breast Cancer can be found at the end of this report.

Cancer incidence near municipal solid waste incinerators on Great Britain

3.15 According to the Department of the Environment, Transport and the Regions (DETR), currently around 26 million tonnes of municipal waste is produced in the UK each year; around 10% of this is disposed via incineration. In the UK all municipal waste incinerators (MWIs) are regulated by the Environment Agency or local authorities. Since 1 December 1996, all MWIs have been required to meet the standards in the Municipal Waste Incineration Directives 89/369/EEC and 89/429/EEC and this resulted in the closure of the majority of the existing incinerators and the upgrading of the remainder. A dioxin emission limit of 1 nanogram per cubic metre ($ng.m^{-3}$) was imposed at the same time although, in practice, most existing plants already achieve dioxin emissions close to 0.1 $ng.m^{-3}$. The Committee was informed that there is expected to be a significant increase in UK incinerator capacity over the next

10-20 years to meet the requirements of the EC Landfill Directive which sets limits for the percentage of biodegradable waste which may be landfilled.

3.16 There have been very few epidemiological studies published which investigated cancer incidence or mortality amongst individuals living in proximity to incinerators in Great Britain. The COC was asked during 1993-4 to comment on a study undertaken by the Small Area Health Statistics Unit (SAHSU) which investigated the cancer incidence of over 14 million people living near to 72 MWIs. SAHSU is a research unit based at the Department of Epidemiology and Public Health, Imperial College School of Science, Technology and Medicine. The study was subsequently published [Elliott P, *et al* (1996) Cancer incidence near municipal solid waste incinerators in Great Britain. *British Journal of Cancer*, **73**, 702-710]. The study reported an increased incidence of liver cancer in people living near to MWIs. However, it was difficult to interpret this finding because it is known that there is often misdiagnosis of liver cancer, with secondary tumours originating in other organs being wrongly recorded as primary liver cancer. The COC recommended that there should be a histological review of the liver cancer cases identified in the first study, to determine whether there really was an increase in primary liver cancer in people living near MWIs. The report of this review was considered at the June 1999 meeting and a statement subsequently agreed in March 2000 to coincide with publication of the follow-up investigation [Elliott P *et al* (2000) Cancer Incidence near Municipal Solid Waste Incinerators in Great Britain 2 : Histopathological and Case Note Review of primary liver cancer cases. *British Journal of Cancer*, **82(5)**,1103-1106].

3.17 The Committee concluded that any potential risk of cancer due to living near to MWIs (for periods in excess of 10 years) was exceedingly low and probably not measurable by the most modern epidemiological techniques. The Committee agreed that, at the present time, there was no need for any further epidemiological investigations of cancer incidence near MWIs.

3.18 A copy of the full statement is given at the end of this section.

Code of Practice for Scientific Advisory Committees

3.19 In July 2000, the Office of Science and Technology published a consultation paper, inviting comments on a proposed code of practice for Scientific Advisory Committees. The paper outlined proposed guidelines for Scientific Advisory Committees and complemented a second document on "Review Of Risk Procedures Used By The Government's Advisory Committees Dealing with Food Safety", which was published in September 2000. The consultation's paper described the duties, rights and responsibilities of Committee Members and their independence from the Committee's secretariat, stressing the need for inclusivity, transparency and proportionality and raising the issue of the manner in which confidential information is handled. It stressed the need for clear explanation of levels and types of uncertainty, and how this information is incorporated into advice, and called for training of Committee Members in communication skills.

3.20 Members felt that the current arrangements for openness (publication of agenda, minutes, statements) were adequate. The Committee discussed the holding of open meetings. Some members felt that there would be no impact on Committee work whereas others felt that the role of specialist advisory Committees was to produce advice which could be subject to public scrutiny. It was agreed that any further proposals for greater openness needed careful planning in consultation with members and would place additional resource requirements on the secretariat.

Early identification of non-genotoxic carcinogens

3.21 The development of rapid methods for the identification of chemicals that induce cancer by non-genotoxic mechanisms would be beneficial for public health, because it would enable more compounds to be tested. It would also reduce the need for long term studies which use large numbers of animals. For these reasons the COC identified this as an important research area when considering research priorities in 1996. The COC reviewed a paper on this topic, prepared by the DH Toxicology Unit, at the November 1999 meeting. 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) hormomimetic activity and (iv) ligand binding with xenobiotic induction receptors. Members considered that increased cellular proliferation (mechanism (i)) was of particular significance.

Members also noted that no one test would be suitable for the detection of all non-genotoxic carcinogens.

3.22 The COC recommended that further research to develop such tests was desirable and a project was commissioned from Professor Kevin Chipman. Professor Chipman made a presentation to the COC on the outline of the proposed research project. The studies were to involve daily administration of selected chemicals to rats over a period of 28 days, using three dose levels selected from the available information from carcinogenicity bioassays. Molecular markers indicative of disturbance of cell cycle control, intercellular communication and inhibition of apoptosis would be studied at two time points (after 3 days and 28 days of dosing). The evaluation of mediators would involve the use of immuno-histochemical methods including analysis of phosphorylated gene products.

3.23 The Committee was asked to advise on priority chemicals for inclusion in the research project. Members agreed that priority should be given to carcinogens that pose the greatest hazard to humans and were also carcinogenic to the rat. For pragmatic reasons the work would only consider the oral route of exposure. Members stressed the need for appropriate negative controls. It was noted that inclusion of a further positive control (such as phenobarbitone or a peroxisome proliferator) and d-limonene as a negative control in the female rat would be valuable. Members agreed that TCDD, oestrodiol, hexachlorobenzene and tetrachloroethylene should be considered as high priority. It was suggested that chloroform, nitrobenzene, alachlor, methapyriline, pyrilamine (a non-carcinogenic structural analogue of methapyriline), dichlorobenzene and paracetamol could be considered to be of interest.

Evidence for an increase in mortality rates from intrahepatic cholangiocarcinoma in England and Wales 1968-1996.

3.24 An increase in the age-standardised mortality rate (ASMR) for all causes of liver cancer has been documented in England and Wales over the period 1979-1994. A preliminary investigation suggested that the increase in mortality from liver cancer may in part be due to an increase in the incidence of intrahepatic cholangiocarcinoma. Intrahepatic cholangiocarcinoma is a relatively rare tumour in the UK, and the prognosis for patients with this tumour is poor. The COC was asked to review the evidence for an increase in the incidence of this tumour.

3.25 The Committee was provided with a prepublication copy of a draft report which presented a detailed analysis of mortality data provided by the Office of National Statistics (ONS). At the November 1999 meeting of the Committee members heard a presentation from Professor Howard Thomas and colleagues at the Department of Medicine, Imperial College of Science, Technology and Medicine.

3.26 The total number of deaths attributed to particular tumours [using the International Classification of Disease (ICD) 8th and 9th revisions] were analysed by year and sex. Professor Thomas noted that in 1978 there was a total of 95 deaths reported from intrahepatic cholangiocarcinoma in England and Wales, whereas there were 736 cancer deaths reported in 1996. Preliminary data from 1998 indicated a total of 835 deaths from intrahepatic cholangiocarcinoma. The increase in the ASMR over the period 1968 to 1996 was from 0.1/100,000 to 1.22/100,000 in males and from 0.05/100,000 to 0.92/100,000 in females. The age-specific mortality rates (ASpMR) in both males and females aged 45+ for intrahepatic cholangiocarcinoma increased by approximately 15 fold over the study period.

3.27 It is possible that the recorded increase in intrahepatic cholangiocarcinoma was an artefact due to confounding factors. The Committee noted that a number of potential confounding factors had been considered by the authors, Professor Thomas and colleagues, such as changes in criteria for International Classification of Disease codes (ICD revisions 8 and 9), the introduction of endoscopic retrograde cholangiopancreatography in the mid to late 1970s as a new diagnostic technique for facilitating precision in the location of site for several cancers of the hepatobiliary system and the consequent possibility that tumours which had previously been classified as tumours of the pancreas, gallbladder and extrahepatic biliary tree were now being classified as cholangiocarcinoma. The Committee agreed that changes in diagnostic standards over time could account for the reported increase in age-standardised mortality rate for intrahepatic cholangiocarcinoma over the period 1968 to 1996. It was therefore important to undertake additional investigations before a definite conclusion could be reached about the apparent increase in the incidence of intrahepatic cholangiocarcinoma.

3.28 The Committee agreed that it was important to keep this topic under review, and to assess further the work of Professor Thomas and colleagues when published.

Longevity of carcinogenicity studies: consideration of a database prepared by Pesticides Safety Directorate (PSD)

3.29 The proper conduct of carcinogenicity studies in rats is an important part of the evaluation and prediction of potential human carcinogens. For a rat carcinogenicity bioassay to be considered acceptable, survival at 24 months should be 50% or greater in all groups (see OECD, EPA and EC guidelines). Significant reductions in the number of control rats surviving to the scheduled end of the study linked to obesity have been widely reported in the scientific literature. This is a matter of concern since inadequate carcinogenicity studies could result in failure to identify potential human carcinogens. In addition, inadequate studies could be rejected by regulatory agencies with the consequent need to repeat the study using more animals to obtain a valid result.

3.30 PSD reviewed survival in control animals from 26 rat carcinogenicity studies which had been submitted over the period 1993-1998. These carcinogenicity tests had been undertaken between 1983 and 1995. Of these studies, 18 had used Sprague-Dawley rats (from various sources), six used Wistar rats and two used Fischer 344 rats. Adequate survival was reported for 3/18 studies in Sprague-Dawley rats, all of the studies in Wistar rats, and one study undertaken in Fischer 344 rats. Most inadequate studies had been undertaken using Charles River Sprague-Dawley rats. There was no evidence to support a previous suggestion that virus antibody status had an effect on survival. Improved survival has been shown to occur in Sprague-Dawley rats which have been fed diets containing fewer calories or smaller portions. The US Food and Drugs Administration has been considering ways to improve survival by reducing the amount of food available to individual animals.

3.31 The Committee reached the following conclusions:

i) Information from the database of rat carcinogenicity studies reviewed by PSD supports the view that unacceptable survival at termination (<50%) in carcinogenicity tests is predominantly confined to Charles River Sprague-Dawley rats. Survival in long-term carcinogenicity bioassays should be compliant with current UK and EC guidelines for a negative result from such studies to be acceptable.

ii) The available information supports the view reached by the COC in its guidelines published in 1991 that dietary restriction in carcinogenicity studies should be applied with caution and is the responsibility of the toxicologist undertaking the study. This subject may be reviewed when more information is available.

3.32 The Committee agreed to consider this topic further when the US Food and Drugs Administration publishes its revised proposals for dietary restriction. A short statement was published on the COC website in April 2000.

Ongoing work

3.33 Full details of ongoing work can be found in the minutes of meetings published on the COC website. This has been substantially upgraded and now contains a "What's new" section. A brief review of the discussions about the influence of genetic susceptibility on chemical induced carcinogenesis is given below.

Genetic susceptibility

3.34 The Committee was aware of increasing recognition of the modulating role of genetics in human disease and that there was evidence in some published epidemiological studies that genetic variation had a role in the metabolism of chemical carcinogens in determining risk of cancer. The Committee was also aware that potential genetic susceptibility to environmental chemicals had been the subject of media attention. Members held an initial discussion at the July 2000 meeting and agreed to a further detailed review. Members asked for papers on three topics to be prepared for discussion. These are:

i) Criteria for the design of gene-environment epidemiology studies

ii) A review of potential target genes for susceptibility to carcinogenesis

iii) A review of how gene-environment studies should be used in risk assessment process

3.35 These papers will be presented to the Committee for discussion in 2001.

Risk procedures used by the Government's Advisory Committees dealing with food safety

3.36 COC was informed that, at the Prime Minister's request, Sir Robert May (then Chief Scientific Advisor to the Government) together with the Chief Medical Officer, Professor Liam Donaldson, and the Chairman of the Food Standards Agency, Sir John Krebs, had carried out a review of risk procedures in scientific committees that deal with food safety. The review group also included representatives of the devolved administrations and Dr Jim McQuaid, former Health and Safety Executive (HSE) Chief Scientist and

Chairman of the Interdepartmental Liaison Group on Risk Assessment (ILGRA). The completed review outlined how the committees approached risk analysis and provided recommendations for best practice.

3.37 Members agreed that the role of COC predominately concerned hazard identification and risk assessment but not risk management. The Committee agreed that it should focus its work on scientific considerations and it was not the role of the Committee to consider policy options.

Statements of the COC

Statement on Evidence for Association Between Consumption of Alcoholic Beverages and Breast Cancer : Update of Information Published Between 1995 –1999

Statement on Carcinogenicity of 3-Monochloropropane-1,2-diol (3-MCPD)

Statement on Cancer Incidence Near Municipal Solid Waste Incinerators in Great Britain

STATEMENT ON EVIDENCE FOR ASSOCIATION BETWEEN CONSUMPTION OF ALCOHOLIC BEVERAGES AND BREAST CANCER: UPDATE OF INFORMATION PUBLISHED BETWEEN 1995-1999

Introduction

1. Breast cancer is the most common cancer in women and the most common cause of cancer mortality in women. Each year there are approximately 30,000 cases registered in England and approximately 11,000 deaths from breast cancer.[1] The aetiology of breast cancer is very complex (see paragraph 5 below). The most clearly established risk factors which are reproductive (eg age at first full term pregnancy, parity, age at menarche) offer limited scope for prevention. The reason for the interest in further consideration of the association between alcohol and breast cancer is that even a small risk, if causally associated with alcohol, could have serious public health implications in terms of the number of breast cancer cases attributable to drinking alcoholic beverages. An extensive literature on the association between alcohol and breast cancer was reviewed by the World Health Organisation's International Agency for Research on Cancer in 1988[2] and by this Committee in 1995[3] but both groups were unable to establish a causal association between drinking alcoholic beverages and breast cancer. The factors which prevented definite conclusions from being drawn are considered in detail in a section of this statement. As a large number of research publications have become available since 1995, including some recent studies investigating the potential mechanism by which alcohol could induce breast cancer, it is now timely for the Committee to update its assessment.

Background to COC consideration

Statement for the Interdepartmental Working Group on Alcohol (1995)

2. The Committee first considered the epidemiological evidence for an association between alcohol and breast cancer in 1995 at the request of the Interdepartmental Working Group (IDWG) on Sensible Drinking[4] as part of the review of medical and scientific evidence and its interpretation of the long term effects of drinking alcoholic beverages. The Committee provided a

statement to the IDWG on the evidence for alcohol and cancer at all sites and concluded that drinking alcoholic beverages causes a dose-related increase in the risk of squamous carcinomas of the upper aerodigestive tract as a whole, and for cancers of the oral cavity, pharynx, larynx, and oesophagus which was independent of the effect of smoking tobacco. There was a substantial amount of information available to members who were able to draw conclusions on dosimetry, duration and frequency of drinking alcoholic beverages and the effect of abstinence and of smoking.[3]

3. A substantial amount of research was available to the Committee on drinking alcoholic beverages and breast cancer in 1995. Members reviewed the 1988 IARC monograph, which provides an evaluation of four large prospective and 13 case-control studies. The Committee also reviewed seven additional prospective studies,[5-11] 17 new case control studies[12-28] and two meta-analyses.[29,30] In addition a number of reviews of the available information were also considered.[31-33] The Committee agreed that the adequacy of control for confounding by known and/or alleged risk factors for breast cancer varied in the different accounts. A dose-related association was reported in most cohort studies and in some hospital-based case-control studies. The results of population-based case-control studies did not generally support an association. A statistically significant dose-related increase in relative risk (RR) was reported in the two meta-analyses [RR at 3 drinks/day 1.38 (95% CI 1.23-1.55)]. The Committee noted that the small increases in relative risk documented in epidemiological studies ranging between approximately 1.2-3 were associated with highly variable estimates of consumption (ca 1-60g ethanol/day). It was agreed that clear evidence of causality had not been demonstrated.[3,4]

4. The Committee concluded "...that while there is no decisive evidence that breast cancer is causally related to drinking alcoholic beverages, the potential significance, for public health, of even a weak association between alcohol and breast cancer is such that we recommend, in particular, that this matter be kept under review."[3] The Interdepartmental Working Group endorsed the COC's conclusions and the recommendation that the relationship between alcohol and breast cancer should be kept under review.[4]

Evaluation of epidemiological data on alcohol and breast cancer

5. The factors which may affect the adequacy and interpretation of any epidemiological studies, such as bias, confounding and errors of measurement have been discussed in detail in the Committee's guidelines for the evaluation of chemicals for carcinogenicity.[34] The assessment of the available epidemiological literature on drinking alcoholic beverages and breast cancer

is particularly difficult as the size of the relative risk estimates reported in the literature (ca 1-3) are within the range where it is difficult to exclude bias and/or confounding as explanations for the results. It is therefore important to highlight the relevant factors of particular concern in interpreting studies of drinking alcoholic beverages.

Estimating alcohol consumption data

6. The difficulty in obtaining an accurate drinking history is an important cause of the observed variation in estimates of the consumption of alcohol and of relative risks for breast cancer at particular levels of drinking alcoholic beverages. Factors which affect the collection and interpretation of alcohol consumption data include inaccurate recall of drinking alcoholic beverages, leading to under reporting, changes in drinking patterns over time, cultural and regional variations in drinking habits, and differences in quantifying alcohol intakes between studies. The inadequate and inconsistent stratification of exposure groups further complicates the assessment of epidemiological data.

Confounding

7. Adequate measurement or control for confounding breast cancer risk factors is also difficult to achieve. Known risk factors for breast cancer include age, ethnic group, family history of the disease, age at birth of first child, at menarche and at menopause, history of biopsy for benign breast disease, socio-economic status, obesity and, in premenopausal breast cancer, history of lactation.[1] Other proposed risk factors have been cited, such as parity (in addition to age at birth of first child), use of oral contraceptives and hormone replacement therapy.

Introduction to current review

8. The Department of Health commissioned three discussion papers from its Toxicology Unit based at Imperial College of Science, Technology and Medicine to assist the Committee in its review. The first paper considered an update of the epidemiological literature from 1995 to March 1999[35] and the second paper was a review of the evidence (up to June 1999) on possible mechanism(s) by which drinking alcoholic beverages could induce breast cancer.[36] The third paper was requested by the Committee following an initial consideration of the evidence on possible mechanisms, and presented a tabulation of data on plasma and urinary sex hormones following consumption of alcohol.[37] The full evaluation of confounding and the

demonstration of a plausible mechanism between drinking alcoholic beverages and breast cancer would be significant steps towards establishing a causal relationship. A summary of the literature reviewed in these papers is given below.

9. All of the information was evaluated in accordance with the Committee's guidelines[34] and also with regard to the criteria proposed by Sir Austin Bradford-Hill.[38] These latter criteria, which are listed below, are generally regarded as being valuable in the consideration as to whether or not an association between an outcome (in this case breast cancer) and a putative risk factor (drinking alcoholic beverages) is causal.[39]

Bradford-Hill criteria

Strength
Consistency
Specificity
Temporality
Biological gradient
Plausibility
Coherence
Experiment
Analogy

Objectives of current review

10. The primary objectives of the current COC review were:

i) To update the assessment of breast cancer in relation to alcohol consumption; to assess this risk in relation to the level and type of alcohol consumption; to examine any differences in risk between premenopausal and postmenopausal women and/or between women using or not using exogenous hormones [oral contraceptives (OCs) and hormone replacement therapy (HRT)].

ii) To review the evidence relating to the mechanistic basis for an association between alcohol consumption and breast cancer.

iii) To assess whether any association between alcohol consumption and the risk of breast cancer can be considered as causal.

iv) If a conclusion regarding causality cannot be reached, to identify the nature of any additional research required to reach a definite conclusion.

Review of new information

Update on epidemiological evidence[35]

11. Three new prospective studies were identified in the DH Toxicology Unit discussion paper.[40-42] These investigations found a small but statistically significant association between drinking alcoholic beverages and increased risk of breast cancer and thus confirmed the findings of prospective studies reviewed by the COC in 1995. A further 22 case-control studies were reported.[43-64] A statistically significant association between drinking alcoholic beverages and increased risk of breast cancer was reported in 17 of these studies with relative risks in drinkers estimated to be between 1.2 and 2.5. A dose-related trend for the association between drinking alcoholic beverages and breast cancer was reported in the two cohort studies where this aspect was considered[40,42] and in the majority of the case-control studies reviewed.[45,47,50,53,57,64] A significant trend between increasing alcohol consumption and relative risk of breast cancer was documented in a pooled analysis of six prospective studies.[65] The extent of correction for potential confounding risk factors varied between the different studies and a number of different methods for estimating alcohol consumption were used. An analysis of risks in pre menopausal and post menopausal women separately was undertaken in nine case-control studies[43,45,46,48-50,59,62,64] and in one pooled analysis of six prospective studies[65] but no conclusions could be drawn regarding these data in view of the variation in quality and results between the individual investigations. Other important variables, such as beverage type and duration and frequency of drinking alcoholic beverages were considered in a number of the epidemiology studies but no clear conclusions could be drawn from the narrative review provided.

Consideration of epidemiological data

12. The Committee noted that the DH Toxicology Unit had considered dose-response and duration of drinking alcoholic beverages and had come to similar conclusions to that reached by the COC in its 1995 review; namely that there was evidence for an association between drinking alcoholic beverages and breast cancer. Overall, there were no definitive data on an effect of beverage type on relative risk and thus the authors had concluded that most information pointed to an effect of alcohol itself rather than any congeners or other ingredients. The Committee agreed that a more comprehensive review of all the epidemiological data was required, particularly with respect to the quality assessment of the individual investigations, and suggested that the epidemiological papers should be

assessed for quality using a scoring method and that a formal systematic review, and where appropriate, meta-analyses of all the epidemiological data should be undertaken. Two further epidemiological studies published after the DH Toxicology Unit report considered the evidence for a risk of breast cancer in premenopausal women.[66,67] The Committee agreed that the results of these studies needed further consideration as part of the systematic review.

Possible mechanisms for association between drinking alcoholic beverages and Breast Cancer[36,37]

13. The discussion paper drafted by the Department of Health Toxicology Unit identified sparse evidence for a number of potential mechanisms by which alcohol could induce breast cancer including enhanced metabolism of carcinogens,[68-70] increased cellular permeability to potential carcinogens,[71] impaired immune responsiveness,[72] and abnormal differentiation of mammary tissue.[73] A further published paper presented a hypothesis that alcohol could induce tissue and DNA damage via the formation of reactive oxygen species in breast tissue.[74] However, most of the available studies on mechanism examined the effects of drinking alcoholic beverages on oestrogen metabolism in humans. There was evidence from both cross-sectional and intervention studies that alcohol consumption affected oestrogen metabolism in premenopausal[75,76] and postmenopausal[77-83] women. The mechanism by which alcohol affected oestrogen metabolism was not readily apparent from these studies particularly in view of the evidence for confounding and interaction by other possible breast cancer risk factors such as obesity,[77] the use of oral contraceptives[84] and hormone replacement therapy.[82] One small study published after the DH Toxicology Unit review[86] provided evidence suggesting that among premenopausal women there may be a group which is more susceptible to the effect of alcohol consumption on breast cancer, because of genetic differences in alcohol metabolism. The results obtained in this latter study need to be confirmed before any definite conclusions can be reached.

Consideration of potential mechanisms

14. The Committee agreed that there was now substantially more information on the potential effects of alcohol on oestrogen metabolism than was available in 1995. However the interpretation was complex and it was requested that the data be reviewed by an independent expert endocrinologist who would advise on what effects alcohol might have on the metabolism of oestrogens in premenopausal and postmenopausal women. A further discussion paper[37] prepared by the Department of Health Toxicology Unit was considered

together with a submission from Professor H S Jacobs (Emeritus Professor of Reproductive Endocrinology, University College Medical School, London) who provided an oral assessment of the data to the Committee. The Committee agreed with Professor Jacobs that there was sufficient evidence from the available studies in humans to conclude that drinking alcoholic beverages can elevate blood concentrations of oestrogens (particularly oestradiol) and that the data concerning oestrogen-receptor status in breast cancer suggested a plausible link between alcohol consumption and an increased risk of breast cancer.[85] Overall the available data suggested a plausible mechanistic link between consumption of alcohol and breast cancer mediated via an effect of alcohol on hormones. The interpretation of these data was particularly complicated and difficult; for example, the influence of confounding effects of other possible breast cancer risk factors such as obesity, use of oral contraceptives and hormone replacement therapy and their potential interaction with drinking alcoholic beverages needed to be considered carefully.

15. Some recent research has noted that the effects of alcohol on serum oestradiol concentrations occur in premenopausal women using oral contraceptives.[87] The Committee agreed that further epidemiological work should consider a number of sub-groups, ie premenopausal women who either used or did not use oral contraceptives and postmenopausal women who had or had not taken HRT. The Committee agreed that there were insufficient data available to describe a threshold of action for alcohol-induced elevation in oestrogens.

16. The Committee agreed that it was important to consider carefully all the available evidence relating to potential mechanisms and therefore asked the COM to update its conclusions, reached in 1995, on any new and relevant mutagenicity studies.

Consideration of causality

17. The Committee felt it helpful to consider all the available evidence under the Bradford-Hill criteria which were outlined above in paragraph 9, in order to assess whether a definite conclusion on causality between drinking alcoholic beverages and breast cancer can be reached and, if not, to use the criteria to identify key areas where further work is required. An assessment of the available evidence has been tabulated as shown below.

Criterion	Evidence regarding alcohol and breast cancer	Comments
Strength	Limited. Magnitude of association is small	The RR in alcohol drinkers is modest and, even for heavy drinkers, rarely exceeds 3. However the RR for most other identified breast cancer risk factors also rarely exceed this value.
Consistency	Limited. Under review.	The available published meta-analysis by Longnecker MP[30] reported significant heterogeneity. A reason for marked variation in results across studies was not found. The pooled analysis of prospective studies published by Smith-Warner SA et al[65] found evidence of heterogeneity in results for premenopausal women but not postmenopausal women. There is a need for a further systematic review, using all studies available to date, to evaluate heterogeneity more fully. (A DH funded study is in progress).
Specificity	Not relevant.	Cancer risk attributed to alcohol is not specific for breast cancer (e.g. prolonged alcohol consumption can induce cancers of the head and neck and oesophagus and liver).[3] The mechanism for alcohol induced causation of these cancers is unknown but is unlikely to be related to that for breast cancer.
Temporality	Yes	Association demonstrated in prospective studies where alcohol consumption can be studied before the occurrence of disease.
Biological gradient	Limited. Some evidence available	There is some evidence for a dose-response effect but the RR rarely exceeds 3 even in heavy drinkers. Assessment of potential confounding and bias required to reach a conclusion on this criterion.
Plausibility	Yes	Evidence for effect of alcohol consumption and elevations in blood levels of oestrogen metabolites (in particular oestradiol) documented.[36,37] Raised oestradiol is a risk factor for breast cancer.[39] The evidence therefore suggests a plausible mechanism in both premenopausal and postmenopausal women.
Coherence	Limited	Evidence for an increased risk of breast cancer in alcoholics[88] and for a relatively low rate of breast cancer incidence among populations abstaining from alcohol (e.g. Mormons).[89] Difficult to assess this criterion on these data.
Experiment	Limited. Some evidence available.	No evidence that alcohol is carcinogenic in experimental animals.[3] Some evidence that alcohol affects breast tissue differentiation in animals.[90]
Analogy	Yes	Other causes of significantly increased oestradiol levels in exposed populations are suggested risk factors for breast cancer (e.g. use of oral contraceptives and HRT).[39]

18. Taking all the available data into account there is evidence to satisfy three of the criteria (temporality, plausibility, and analogy) and some limited evidence to satisfy a further four of the criteria (consistency, biological gradient, coherence, and experiment). The Committee agreed that there was no evidence that alcohol is carcinogenic from experimental studies in animals. The Committee considered that the criterion of specificity was not relevant to the assessment of breast cancer risk. The Committee agreed that there was considerable evidence to support an association between drinking alcoholic beverages and increased risk of breast cancer but the magnitude of the association was small (ie the relative risk is modest and, even for heavy drinkers, rarely exceeds 3) and it was difficult to ascertain the nature of the dose-response relationship from the available information. The small magnitude of the association between drinking alcoholic beverages and risk of breast cancer and the complex aetiology (ie it is not specific to a single risk factor) of breast cancer are the main reasons for the difficulty in reaching a definite conclusion based on the Bradford-Hill criteria. The association could be due to biases in the studies or to confounding by other breast cancer risk factors.

19. The Committee conclude that, in view of the difficulty in assessing the data on drinking alcoholic beverages and breast cancer, there is need for a rigorous systematic review of the epidemiological literature using appropriate methods (ie meta-analysis) to identify and evaluate potential biases, confounding and heterogeneity so that an assessment of causality and risk associated with drinking alcoholic beverages can be facilitated. The Committee agreed that it would be important for any further analyses of the data to provide a population-attributable risk estimate for the UK. The Committee subsequently agreed an outline proposal for a meta-analysis study prepared by a research team from Imperial College of Science, Technology and Medicine. The study has been commissioned by the Department of Health and was initiated in December 1999. A draft report should be available for scrutiny by the Committee in approximately 18 months time. The Committee was also aware that additional relevant data on alcohol consumption and risk of breast cancer from the Oxford Collaborative Group on Hormonal factors in Breast Cancer would be forthcoming and should be reviewed when available.

Conclusions of current review

20. The Committee reached the following interim conclusions based on its updated review of the published literature since 1995.

i) There is an association between drinking alcoholic beverages and increased risk of breast cancer. It is difficult to resolve whether this is causal. The magnitude of the observed association is small (ie the relative risk is modest and, even for heavy drinkers, rarely exceeds 3) and within the range where it is difficult to exclude bias and/or confounding as explanations for the observed results in epidemiological studies. It is difficult to derive a quantitative relationship from the dose-response data available in the literature.

ii) Further epidemiological studies have been published since 1995. There is a need for further systematic review of the epidemiological literature to assess fully the influence of bias, confounding and effect modification. This will contribute to a conclusion on causality and population attributable risk associated with drinking alcoholic beverages.

iii) Studies of possible mechanisms provide evidence for a plausible basis for the causation of breast cancer by consumption of alcohol. Alcohol increases blood levels of oestrogens and in particular oestradiol in both premenopausal and postmenopausal women. These data suggest a similar mechanism to other known breast cancer risk factors.

iv) The COM should be asked to update its opinion of 1995 on the mutagenicity data on alcohol.

April 2000
COC/00/S4

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CARCINOGENICITY OF 3-MONOCHLOROPROPANE-1,2-DIOL (3-MCPD)

Introduction

1. 3-Monochloropropane-1,2-diol (3-MCPD) can be present as a contaminant in epichlorhydrin/amine copolymers used as flocculants or coagulant 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.

2. 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.[1] 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,[2] and a review document published by the Institute of Toxicology, National Food Agency of Denmark.[3] 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 in 1999, members commented that the available metabolism data on 3-MCPD were relatively old and focused on metabolic pathways following intraperitoneal administration. There was no oral mass balance investigation available. 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. However, additional *in-vivo* mutagenicity data became available to the COM in 2000, namely a bone marrow micronucleus test and a rat liver UDS assay. Both studies were conducted to appropriate protocols and 3-MCPD was negative in both studies.

Conclusions

3. The Committee has now reached the following conclusions on all the available mutagenicity and carcinogenicity 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 but has no significant genotoxic potential *in-vivo*. (The COM statement on mutagenicity of 3-monochloropropane-1,2-diol has also been revised). The COM also noted that the predominant urinary metabolite in rats following dietary or intraperitoneal doses of 3-MCPD was beta-chlorolactic acid[4],(ie resulting from a pathway not producing glycidol or other genotoxic intermediates). A study has also shown that 3-MCPD may be also metabolised by a minor pathway and undergo conjugation with glutathione ultimately to form a mercapturic acid in urine of rats [N-acetyl-S-(2,3-dihydroxypropyl) cysteine].[5]

iii) 3-MCPD has been tested in four long-term animal carcinogenicity experiments, two in mice and two in rats.[6-8] However, three of these studies [6,7] were conducted between 1970 and 1981 to inadequate protocols. The conclusions reached by the COC therefore refer to the one study conducted to contemporary standards.[8] The Committee had access to the full study report [8] and to published reviews of this study.[2,3] 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)[8] 3-MCPD was administered via drinking water to groups of 50 male and 50 female F344 rats (aged 6 weeks at study initiation) 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. The high dose group exceeded the Maximum Tolerated Dose as evidenced by a decrease in body weights relative to controls of 33% and 35% in males and females respectively. There was no evidence of any treatment-related increase in mortality in this study. Survival to termination was acceptable (ie >50%) in all dose groups with the exception of the male high 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 tumours were reported in both sexes in the kidney and in males only at hormonally responsive sites (ie the testes, mammary gland and preputial gland) at dose levels which exceeded the maximum tolerated dose. Evidence from previously conducted investigations with 3-MCPD was considered in evaluating possible explanations for these findings.

viii) In the kidney, the Committee noted that tumours in both sexes were benign (renal tubular adenoma) and that these were accompanied by a chronic progressive nephropathy. In considering possible mechanisms, the Committee were aware of earlier findings that metabolism to beta-chlorolactic acid is a major pathway in the rat [4] and that this metabolite is further broken down to yield oxalate and CO_2. Oxalate is known to induce severe renal cytoxicity.[3,9] Other evidence, including a study which reported crystals of oxalate in the urine of rats treated with 3-MCPD (single dose of 100mg/kg ip),[4] supported a role for sustained cytotoxicity as a possible mechanism for the induction of kidney tumours. The renal adenoma recorded in one female animal at the lowest dose was not considered to be biologically significant, and the

Committee agreed that a dose of 1.1mg/kg bw/day was a no observed effect level for the induction of kidney tumours. The Committee, however, noted some evidence of a toxic effect upon the kidney at this dose level (ie increased tubular hyperplasia and statistically significant increase in absolute kidney weight).

ix) With regard to the sex-specific tumours in male rats (in the testes, mammary gland and preputial gland), the Committee noted that the testicular tumours needed to be viewed against the high spontaneous incidence of Leydig-cell tumours common in ageing F344 rats, which may be up to 100% in control groups.[10,11] The high proportion of Leydig cell adenoma (between 86% and 100% in treated animal groups, compared to 76% in controls) was particularly noted in this study. However, Leydig-cell carcinoma developed only at the highest dose in 3/50 treated animals. As 3-MCPD has been shown to induce a prolonged increase in circulating hormone levels [a single intraperitoneal dose of 80mg/kg bw causing increased serum levels of follicle stimulating hormone (FSH), luteinising hormone (LH) and prolactin],[12] it is possible that increases in the spontaneous rate of Leydig-cell tumours may have been promoted by hormonal imbalance caused by 3-MCPD. Subsequently, the increase in tumours at other hormonally responsive sites (ie in the male mammary gland and the preputial gland) may be secondary to further hormonal disturbances known to be induced by proliferating Leydig cells.[2] Overall, the Committee noted that there was no evidence of a significant increase in tumourigenic response at any of these sites at a dose of 1.1 mg/kg bw/day.

x) The Committee considered 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.[2,3,8] The possible influence of the stereoisomerism of 3-MCPD was also discussed. Members agreed that the proposed non-genotoxic mechanisms advanced were plausible, now that specific evidence was available that reactive metabolites were not produced *in-vivo* in tissues where genotoxicity was assessed.

xi) The Committee concluded that the no observed effect level (NOEL) for tumourigenic effects of 3-MCPD in rats was approximately 1.1mg/kg bw/day.

xii) The Committee agreed that an approach utilising the NOEL with appropriate uncertainty factors would be acceptable for carcinogenic risk assessment for 3-MCPD. An overall uncertainty factor of 1000

was considered appropriate in view of the uncertainties identified in the data, particularly in respect of the quality and incompleteness of the metabolic data on 3-MCPD.

xiii) The Committee concluded that 3-MCPD was unlikely to present a carcinogenic risk to man, provided the exposure was 1000 times lower than the NOEL of 1.1mg/kg bw/d for tumourigenicity.

December 2000
COC/00/S5

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CANCER INCIDENCE NEAR MUNICIPAL SOLID WASTE INCINERATORS IN GREAT BRITAIN

Introduction

1. There have been very few epidemiological studies published which investigated cancer incidence or mortality amongst individuals living in proximity to incinerators in Great Britain.[1,2] The COC was asked during 1993-4 to comment on a study undertaken by the Small Area Health Statistics Unit (SAHSU) which investigated the cancer incidence of over 14 million people living near to 72 solid waste incinerators. This investigation had been initiated following the publication of several reviews of the potential health risks associated with incineration which highlighted the lack of appropriate epidemiological investigations of cancer risk.[1, 3,4] and was published in the scientific literature in 1996.[5] However, before drawing any conclusions on the SAHSU study, the Committee requested further information in respect of the data on liver cancer; namely a histopathological and case-note review of primary liver cancer cases. The Committee considered the report of this latter investigation during 1998 and at its March 1999 meeting. This statement presents some background information on municipal solid waste incineration in the UK, a review of the SAHSU investigations of cancer incidence near to municipal solid waste incinerators and conclusions reached by the Committee regarding the risk of cancer associated with living near to municipal incinerators.

Municipal solid waste incineration in the UK

2. According to the Department of the Environment, Transport and the Regions (DETR), currently around 26 million tonnes of municipal waste is produced in the UK each year; around 10% of which is disposed via incineration. In the UK all municipal waste incinerators (MWIs) are regulated by the Environment Agency or local authorities. Since 1 December 1996, all MWIs have been required to meet the standards in the Municipal Waste Incineration Directives 89/369/EEC and 89/429/EEC and this resulted in the closure of the majority of the existing incinerators and the upgrading of the remainder. A dioxin emission limit of 1 nanogram per cubic metre (ng m^{-3}) was imposed at the same time although, in practice, most existing plants already achieve dioxin emissions close to 0.1 ng m^{-3}. There are currently 11 MWIs in operation in the UK, with another due to start operating in 2000. The Committee was informed that there is expected to be a significant increase in UK incinerator capacity over the next 10-20 years to meet the requirements of the proposed EC Landfill Directive which sets limits for the percentage of biodegradable waste which may be

landfilled (it has been estimated that a further 16 MWIs may be required by 2006).[6] However, the draft Waste Incineration Directive currently being discussed within the EU seeks to reduce further emissions of key pollutants from incineration processes, including particulates, dioxins, and heavy metals.

SAHSU studies of municipal solid waste incinerators.

A. 1996 Investigation of health statistics

3. The cancer incidence of over 14 million people living near to 72 municipal solid waste incinerators in Great Britain was examined from 1974-1986 (England), 1974-1984 (Wales), and 1975-1987 (Scotland).[1] The study was conducted in two stages: the first involved a stratified sample of 20 incinerators and the second considered the remaining 52 incinerators. Overall there was a statistically significant decline in risk with distance from incinerators for all cancers combined and for stomach, colorectal, liver and lung cancers. The excess risk in people living within 1 km of a MWI for these cancers after allowing for a 10 year lag period, was estimated from the second stage investigation to vary from 5% (colorectal) to 37% (liver; 0.95 excess cases 10^{-5} $year^{-1}$). SAHSU estimated a total of 23 excess cases of liver cancer in the 0-1 km zone from the second stage of the analysis. There was evidence of residual confounding which the authors suggested was a likely explanation for the findings for all cancers, stomach and lung, and also to explain at least part of the excess of liver cancer. For this reason and because of the substantial level of misdiagnosis (mainly secondary tumours) believed to occur among registrations and death certificates for liver cancer, the COC asked for a further investigation. This was to comprise a histological review of the liver cancer cases identified in the first study, in order to determine whether or not an increase in primary liver cancer had occurred.

B. Histological and case-note review of primary liver cancer cases

4. This diagnostic histopathological and case-note review considered 235 cases (155 males, 80 females) registered with primary liver cancer and included all 87 cases within 1km of a MWI, and random samples of 74 cases from 1-7.5 km and 74 from the rest of Great Britain. Diagnostic material was available for 94 cases (of which 26 also had clinical notes available) and medical records only were available for 25 additional cases. Histopathological slides were reviewed independently by three pathologists and any discrepancies resolved at case conferences. The medical records were reviewed independently by one senior clinician.

5. Primary liver cancer was confirmed in 66/119 cases (55%, 95% CI 46-64%) while 21 cases (18%; 95% CI 11-24%) were considered to be definite secondary cancers. The remaining cases could not be distinguished between primary and secondary cancers (26 cases) or no malignant tissue was found in the specimens available (6 cases). There was no evidence to suggest that the proportion of cases confirmed as having primary liver cancer, nor of those with evidence of cirrhosis and associated risk factors, differed with distance from incinerators. The Committee agreed that the confirmation of 55% of registered primary liver cancer cases following diagnostic review, is in accordance with a previous study in Great Britain.[7] The Committee agreed that the finding of a high concordance between cancer registration and death certificate data for the confirmed primary liver cancer cases (80%) was unexpected but important new information which suggested that the use of death certificates was acceptable in epidemiological investigations of liver cancer.

6. Two cases of angiosarcoma were diagnosed on histopathological review within 7.5 km of a MWI (cf 0.26 expected based on a national register ($p<0.05$)), but there was no evidence more generally of clustering near incinerators of cases ascribed to angiosarcoma in a national register. Neither of these two cases had been diagnosed previously, both being registered as hepatocellular carcinoma, and neither was an industrial case. The Committee noted that there was no background information on the extent to which angiosarcoma was misdiagnosed routinely as hepatocellular carcinoma or carcinoma (not otherwise specified) in the general population. The Committee agreed that SAHSU had adopted an acceptable approach to the evaluation of the significance of the two cases of angiosarcoma given the limitations in the national register data used.

7. The histopathology diagnostic review allows a range of estimates to be made of possible (absolute) excess of “true” primary liver cancer near incinerators, based on relative risk estimates from the previous study. Assuming that primary liver cancer was the correct diagnosis in 55% of all registered cases then the excess number of cases among the population living within 1 km of an incinerator is reduced from 23 to 12.6, i.e. an excess of 0.53 excess cases 10^{-5} $year^{-1}$. With only definite secondary cancer cases excluded (18%) then the excess within 1km is reduced to 18.8 cases, ie 0.78 excess cases 10^{-5} $year^{-1}$. [8]

COC evaluation of SAHSU studies

8. The Committee was informed that there have been considerable reductions in the levels of emissions of pollutants from incinerators in recent years. The Royal Commission on Environmental Pollution recognised that epidemiological studies are much less likely to reveal any health effects in relation to current standards of controls on emission of pollutants from MWIs.[1] Thus estimates of the relative risk derived from the SAHSU investigations would, if causally associated with exposure to emissions, be related to accumulated exposures prior to the introduction of the controls implemented through the 1989 Municipal Waste Incineration Directives.

9. The Committee agreed that there were a number of factors that should be considered in deriving conclusions on the SAHSU studies of MWIs: i) accuracy of health statistics, ii) accuracy of cancer diagnosis, iii) potential confounding factors for individual cancers, and iv) a number of environmental variables particular to incineration such as type of waste burnt, geographical and meteorological conditions, and controls placed on the emission of pollutants.

10. With regard to the 1996 study of cancer incidence, the Committee agreed that the excess of all cancers, stomach, lung and colorectal cancers were due to socio-economic confounding as has been reported by the SAHSU group following adjustment of the data by use of a deprivation index. Post-hoc analyses which compared cancer incidence prior to establishment of an incinerator with cancer incidence following a 10 year lag period since first exposure was consistent with this conclusion.

11. With regard to the diagnostic histopathology study of liver cancer, the Committee agreed that whilst the excess of primary liver cancer near incinerators was not readily explained by known confounding or other factors, residual confounding by socio-economic factors could not be excluded in view of the strong association of deprivation with liver cancer incidence.

Conclusions

12. The Committee agreed the following overall conclusions with respect to the SAHSU investigations of cancer incidence near MWIs:

    i) The SAHSU studies found a small excess of primary liver cancer near municipal solid waste incinerators (estimated to be between 0.53-0.78 excess cases 10^{-5} $year^{-1}$). It is not possible to conclude that this small increase in primary liver cancer is due to emissions of pollutants from incinerators, as residual socio-economic confounding cannot be excluded. The Committee agreed that an excess of all cancers, stomach, lung and colorectal cancers was due to socio-economic confounding and was not associated with emissions from incinerators.

    ii) The finding of two cases of angiosarcoma during the histopathology review in individuals who were resident within 7.5 km of a municipal solid waste incinerator was unexpected. The Committee considered that the evaluation of this finding was difficult given the limitations in the registration of angiosarcoma and lack of information regarding accuracy of diagnosis in the general population. The Committee, however, agreed that there was no evidence more generally of clustering near incinerators of cases ascribed to angiosarcoma in a national register.

    iii) The Committee was reassured that any potential risk of cancer due to residency (for periods in excess of 10 years) near to municipal solid waste incinerators was exceedingly low and probably not measurable by the most modern epidemiological techniques. The Committee agreed that, at the present time, there was no need for any further epidemiological investigations of cancer incidence near municipal solid waste incinerators.

March 2000
COC/00/S1

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2000 Membership of the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

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Declaration of COC members' 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 Cooper	Halifax Norwich Union	Share Holder Share Holder	NONE	NONE
Prof D Forman	Halifax Woolwich	Share Holder Share Holder	NONE	NONE
Prof D Harrison	Medical Solutions AstraZeneca GenoVar Diagnostics	Share Holder Consultant Consultant	Fairfield Imaging	Research Support
Ms D Howel	NONE	NONE	NONE	NONE
Dr S J Kennedy	Unilever	Share Holder	NONE	NONE
Ms M Langley	NONE	NONE	NONE	NONE
Prof J M Parry	Compass Catering JIB Insurance National Power SmithKline Beecham Quintiles	Share Holder Share Holder Share Holder Consultant Consultant	Astra BAT Boehringer Glaxo/Wellcome Pfizer Welsh Water	Grant Grant Grant Grant Grant Grant
Prof D Phillips	Abbey National plc BG Group Bradford & Bingley Centrica CGNU Lattice Group National Grid	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	NONE	NONE
Prof A G Renwick OBE	International Sweeteners Association	Consultant	Hoffmann-La Roche SmithKline Beecham Unilever FEMA Pfizer	Research Support Research Support Research Support Grant Grant

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Dr R Roberts	AstraZeneca Eli-Lilly P & O Zeneca	Share Holder Share Holder Share Holder Salary	Baxter Healthcare European Council for Plastics and Intermediates (ECPI) Halogenated Solvents Industry Alliance (HSIA) Novartis	Departmental Support Departmental Support Departmental Support Departmental Support
Prof D Shuker	NONE	NONE	Glaxo-Wellcome Unilever	Postgraduate Studentship Project Support
Prof G T Williams	Abbey National plc AMP Ltd Bradford & Bingley CGNU Aphton Corporation	Share Holder Share Holder Share Holder Share Holder Consultant	NONE	NONE

ANNEX 1

TERMS OF REFERENCE

To advise at the request of:

Food Standards Agency
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
Scientific Advisory Committee on Nutrition
Home Office
Scottish Executive
National Assembly for Wales
Northern Ireland Executive
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

CODE OF CONDUCT FOR MEMBERS OF ADVISORY COMMITTEES

Public service values

Members must at all times:

- observe the highest standards of impartiality, integrity and objectivity in relation to the advice they provide and the management of this Committee;

- be accountable, through the Chairman of the Food Standards Agency, the Chief Medical Officer, to Ministers, Parliament and the public for its activities and for the standard of advice it provides.

The Ministers of the sponsoring departments are answerable to Parliament for the policies and performance of this Committee, including the policy framework within which it operates.

Standards in Public Life

All Committee members must:

- follow the Seven Principles of Public Life set out by the Committee on Standards in Public Life (see below);

- comply with this Code, and ensure they understand their duties, rights and responsibilities, and that they are familiar with the function and role of this Committee and any relevant statements of Government policy. If necessary members should consider undertaking relevant training to assist them in carrying out their role;

- not misuse information gained in the course of their public service for personal gain or for political purpose, nor seek to use the opportunity of public service to promote their private interests or those of connected persons, firms, businesses or other organisations; and

- not hold any paid or high profile unpaid posts in a political party, and not engage in specific political activities on matters directly affecting the work of this Committee. When engaging in other political activities, Committee members should be conscious of their public role and exercise proper discretion. These restrictions do not apply to MPs (in those cases where MPs are eligible to be appointed), to local councillors, or to Peers in relation to their conduct in the House of Lords.

Role of Committee members

Members have collective responsibility for the operation of this Committee. They must:

- engage fully in collective consideration of the issues, taking account of the full range of relevant factors, including any guidance issued by the Food Standards Agency; the Department of Health and sponsor departments or the responsible Minister;

- in accordance with Government policy on openness, ensure that they adhere to the Code of Practice on Access to Government Information (including prompt responses to public requests for information); agree an Annual Report; and, where practicable and appropriate, provide suitable opportunities to open up the work of the Committee to public scrutiny;

- not divulge any information which is provided to the Committee in confidence;

- ensure that an appropriate response is provided to complaints and other correspondence, if necessary with reference to the sponsor department; and

- ensure that the Committee does not exceed its powers or functions.

Individual members should inform the Chairman (or the Secretariat on his or her behalf) if they are invited to speak in public in their capacity as a Committee member.

Communications between the Committee and the Food Standards Agency (FSA) Board and /or Ministers will generally be through the Chairman except where the Committee has agreed that an individual member should act on its behalf. Nevertheless, any member has the right of access to the FSA Board and/or Ministers

on any matter that he or she believes raises important issues relating to his or her duties as a Committee member. In such cases the agreement of the rest of the Committee should normally be sought.

Individual members can be removed from office by the FSA Board if they fail to perform the duties required of them in line with the standards expected in public office.

The role of the Chairman

The Chairman has particular responsibility for providing effective leadership on the issues above. In addition, the Chairman is responsible for:

- ensuring that the Committee meets at appropriate intervals, and that the minutes of meetings and any reports to the FSA Board accurately record the decisions taken and, where appropriate, the views of individual members;
- representing the views of the Committee to the general public; and
- ensuring that new members are briefed on appointment (and their training needs considered), and providing an assessment of their performance, on request, when members are considered for re-appointment to the Committee or for appointment to the board of some other public body.

Handling conflicts of interests

The purpose of these provisions is to avoid any danger of Committee members being influenced, or appearing to be influenced, by their private interests in the exercise of their public duties. All members should declare any personal or business interest which may, or may be *perceived* (by a reasonable member of the public) to, influence their judgement. A guide to the types of interest that should be declared is below.

(i) Declaration of Interests to the Secretariat

Members of the Committee should inform the Secretariat in writing of their current personal and non-personal interests, when they are appointed, including the principal position(s) held. Only the name of the company and the nature of the interest are required; the amount of any salary etc. need not be disclosed. An

interest is current if the member has an on-going financial involvement with industry, eg if he or she holds shares in 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 industry. Members are asked to inform the Secretariat at any time of any change of their personal interests and will be invited to complete a declaration form once a year. It is sufficient if changes in non-personal interests are reported in the annual declaration form following the change. (Non-personal interests involving less than £1,000 from a particular company in the previous year need not be declared to the Secretariat).

The register of interests should be kept up-to-date and be open to the public.

(ii) Declaration of Interest and Participation at Meetings

Members of the Committee are required to declare any direct interests relating to salaried employment or consultancies, or those of close family members,[1] in matters under discussion at each meeting. Having fully explained the nature of their interest the Chairman will, having consulted the other members present, decide whether and to what extent the member should participate in the discussion and determination of the issue. If it is decided that the member should leave the meeting, the Chairman may first allow them to make a statement on the item under discussion.

Personal liability of Committee members

A Committee member may be personally liable if he or she makes a fraudulent or negligent statement which results in a loss to a third party; or may commit a breach of confidence under common law or a criminal offence under insider dealing legislation, if he or she misuses information gained through their position. However, the Government has indicated that individual members who have acted honestly, reasonably, in good faith and without negligence will not have to meet out of their own personal resources any personal civil liability which is incurred in execution or purported execution of their Committee functions save where the person has acted recklessly. To this effect a formal statement of indemnity has been drawn up.

[1] Close family members include personal partners, parents, children, brothers, sisters and the personal partners of any of these.

Annex 1

THE SEVEN PRINCIPLES OF PUBLIC LIFE

Selflessness
Holders of public office should take decisions solely in terms of the public interest. They should not do so in order to gain financial or other material benefits for themselves, their family, or their friends.

Integrity
Holders of public office should not place themselves under any financial or other obligation to outside individuals or organisations that might influence them in the performance of their official duties.

Objectivity
In carrying out public business, including making public appointments, awarding contracts, or recommending individuals for rewards and benefits, holders of public office should make choices on merit.

Accountability
Holders of public office are accountable for their decisions and actions to the public and must submit themselves to whatever scrutiny is appropriate to their office.

Openness
Holders of public office should be as open as possible about all the decisions and actions that they take. They should give reasons for their decisions and restrict information only when the wider public interest clearly demands.

Honesty
Holders of public office have a duty to declare any private interests relating to their public duties and to take steps to resolve any conflicts arising in a way that protects the public interests.

Leadership
Holders of public office should promote and support these principles by leadership and example.

Annex 2

DIFFERENT TYPES OF INTEREST

The following is intended as a guide to the kinds of interests that should be declared. Where members are uncertain as to whether an interest should be declared they should seek guidance from the Secretariat or, where it may concern a particular product which is to be considered at a meeting, from the Chairman at that meeting. **If members have interests not specified in these notes but which they believe could be regarded as influencing their advice they should declare them.** However, neither the members nor the Secretariat are under any obligation to search out links of which they might *reasonably* not be aware. For example, either through not being aware of all the interests of family members, or of not being aware of links between one company and another.

Personal Interests

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

- **Consultancies and/or direct employment** any consultancy, directorship, position in or work for industry which attracts regular or occasional payments in cash or kind;

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

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

Non-Personal Interests

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 industry;

- **Support by Industry**: any payment, other support or sponsorship which does not convey any pecuniary or material benefit to a member personally, but which does benefit their position or department eg:

i) a grant 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 or a post graduate research programme 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.

Members are under no obligation to seek out knowledge of work done for, or on behalf of, the industry or other relevant bodies by departments for which they are responsible, if they would not normally expect to be informed.

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

DEFINITIONS

In this Code, 'the industry' means:

- Companies, partnerships or individuals who are involved with the production, 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

- Trade associations representing companies involved with such products;

- 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.

In this Code 'the Secretariat' means the Secretariat of the COT/COM/COC.

ANNEX 3

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 Chief Medical Officer and the Chairman of the Food Standards Agency and, through them, 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 (during the course of 1999) 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 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 genetic issues in chemical toxicology, carcinogenicity, mutagenicity and risk assessment. There would be no discussion of individual commercial products.
Agenda	Agreed	Made publicly available via Internet site prior to meeting.
Papers	Agreed	Finalised papers 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 paras 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. 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.

ANNEX 4

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.
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 chemicals such as chlorinated dibenzodioxins and polychlorinated biphenyls bind to Ah receptor. The available research suggests that binding to the Ah receptor is an integral 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.
BRADFORD - HILL CRITERIA	Sir Bradford-Hill established criteria that have been universally used to assist in the interpretation of associations reported from studies:-

STRENGTH – The stronger the association the more likely it is causal. The COC has previously noted that the relative risks of<3 need careful assessment for effects of bias or confounding.

CONSISTENCY – The association has been consistently identified by studies Using different approaches and is also seen in different populations with exposure to the chemical under consideration.

SPECIFICITY – Limitation of the association to specific exposure groups or to specific types of cancers increases likelihood that the association is causal.

TEMPORALITY – The association must demonstrate that exposure leads to cancer. The relationship of time since first exposure, duration of exposure and time since last exposure are all important in assessing casuality.

BIOLOGICAL GRADIENT – If an association reveals a biological gradient or dose-reponse curve, then this evidence is of particular importance in assessing causality.

PLAUSIBILITY – Is there appropriate data to suggest a mechanism by which exposure could lead to concern? However, even if an observed association may be new to science or medicine it should not be dismissed.

COHERENCE – Cause and effect interpretation of data should not seriously conflict with generally known facts.

EXPERIMENT – Can the association be demonstrated. Evidence from experimental animals may assist in some cases. Evidence that removal of the exposure leads to a decrease in risk may be relevant.

ANALOGY – Have other closely related chemicals been associated with cancer.

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 to the disease (such as occupational exposure to a carcinogen) is examined by comparing the disease and nondiseased with regard to how frequently the attribute is implicated in each of the groups.

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 fore stomach and the glandular stomach. The glandular stomach is the only area directly comparable to human situations.
HEPATIC	Pertaining to the liver
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 it's 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 fetus 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 maintain 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 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 infested 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 (lacZ 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 (qv) -, 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.
XENOBIOTIC	A chemical foreign to the biologic system.
XENOESTROGEN	A 'foreign' compound, ie not natural to the body, with oestrogenic activity.

ANNEX 5

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 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.

1993 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321808-7 Price £11.95.

1994 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321912-1 Price £12.50.

1995 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321988-1 Price £18.50.

1996 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. The Stationery Office ISBN 0 11 322115-0 Price £19.50.

1997 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Department of Health.

1998 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Department of Health.

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

Guidelines for the Testing of Chemicals for Toxicity DHSS Report on Health and Social Subjects 27 HMSO ISBN 0 11 320815 4 Price £4.30.

Guidelines for the Evaluation of Chemicals for Carcinogenicity DH Report on Health and Social Subjects 42 HMSO ISBN 0 11 321453 7 Price £7.30.

Guidelines for the Testing of Chemicals for Mutagenicity DH Report on Health and Social Subjects 35 HMSO ISBN 0 11 321222 4 Price £6.80.

Guidelines for the Preparation of Summaries of Data on Chemicals in Food, Consumer Products and the Environment submitted to DHSS Report on Health and Social Subjects 30 HMSO ISBN 0 11 321063 9 Price £2.70.

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment: Peanut Allergy, Department of Health (1998).

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment: Organophosphates, Department of Health (1998).

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment: Adverse Reactions to Food and Food Ingredients, Food Standards Agency (2000).

COM Guidance on a Strategy for Testing of Chemicals for Mutagenicity, Department of Health (2000).

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First Published: June 2001

Produced by the Department of Health
& the Food Standards Agency

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