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

Annual Report

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ABOUT THE COMMITTEES

This is the eleventh 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. Those seeking further information on a particular subject can obtain relevant references from the Committee's administrative secretary.

The year 2001 has seen a number of changes in the membership of the committees and these are shown in the membership lists at the end of each committee's report. The terms of reference of the Committees have also been amended to reflect changes in the Government departments, agencies and other bodies to whom the committees provide advice (see Annex 1).

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.

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.food.gov.uk/science/committees

COM: http://www.doh.gov.uk/com/com.htm

COC: http://www.doh.gov.uk/coc/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

Over the past year, the Committee's agenda has been dominated by the comprehensive review of the tolerable daily intake for dioxins and dioxin-like polychlorinated hydrocarbons. Other major items were the reviews of the reproductive effects of caffeine and the public health implications of Amnesic Shellfish Poisoning. The Committee has advised on an intense sweetener (alitame), a food contact material (bisphenol A), a number of enzymes used in food processing and polycyclic aromatic hydrocarbons. It has also received and commented on studies relating to the health outcomes of living near landfill sites, and to chlorinated water and reproductive outcome, and a paper on uncertainty factors produced by the Interdepartmental Group on Health Risks from Chemicals.

The Committee has also reviewed its working practice in light of the developing Code of Practice for Scientific Advisory Committees and the Government's response to the Phillips Enquiry. I am pleased to report that the Committee already complies with most of the recommendations and is introducing others. One of the recommendations is for committee membership to ensure both continuity and fresh perspectives. In line with this policy, four members who have each served for 6-12 years have left the Committee over the past year. Their experience and wisdom will be missed. However, it is good to welcome the four new scientific members who will introduce new ideas and approaches. I am particularly pleased, that for the first time, we have been able to appoint an expert from outside of the UK, and also a second Public Interest Representative.

In March of 2002, I will have completed the maximum ten-year term of office as Chairman, and therefore this is the last Annual Report that I will present. These ten years have seen a number of changes. Responsibility for legislation on food additives now lies with the EU, and safety assessment on most additives is conducted by the EU's Scientific Committee on Food (SCF). This has allowed the COT to consider a broader range of issues, in line with its terms of reference. Lead responsibility for the Committee's Secretariat has moved from the Department of Health to the Food Standards Agency. Perhaps the most dramatic change has related to openness, which is an increasingly important consideration for UK government, and a core aim of the Food Standards Agency. Annual reports and statements were already published. Further developments have included publication of minutes, papers and members' interests, holding open meetings on specific topics of toxicological interest and consultation on draft working group reports.

Chairing the COT has been a valuable and rewarding experience. My successor is Professor Ieuan Hughes. I wish him well and look forwarding to seeing further development of the Committee as it greets new challenges and new scientific approaches in future years.

Finally, I would like to add my sincere thanks and appreciation of the work carried out by members of the Committee during my tenure as Chairman and my great appreciation of the work of the administrative and scientific secretariat without whose excellent work the Committee would not have been able to function.

Professor H F Woods (Chairman)

CBE BSc BM DPhil FFPM FIFST HonFFOM FRCP (London & Edinburgh)

Alitame

1.1 Alitame is an intense sweetener, which 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. In 1998 the COT established an Acceptable Daily Intake (ADI) of 0.3 mg/kg bw per day, which was subsequently confirmed in 1999, following the consideration of further data submitted by the manufacturers.

1.2 The ADI was determined from data showing an increase in liver weight in dogs treated with alitame for 18 months. The COT considered it prudent to view 100 mg/kg bw per day as a LOAEL, and the NOAEL was identified as 30 mg/kg bw per day, the lowest dose tested. Uncertainty factors of 10 for inter-species variation and 10 for intra-individual variation were applied, resulting in the ADI of 0.3 mg/kg bw per day. Enzyme induction was also reported in the dog study, with a NOAEL of 100 mg/kg bw per day.

1.3 COT had also previously noted that a study in diabetics given alitame at 10 mg/kg bw per day for 90 days had reported a number of cardiovascular complications in some individuals in both the alitame and control (placebo) groups. 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 over the one-year period of the study. On this basis the manufacturers asked for the ADI to be increased. However, COT noted that the new study did not include an assessment of enzyme induction and the results of the statistical analysis were questioned (see 2000 Annual Report, page 10).

1.4 In 2001, COT considered additional data submitted by the company, including the results of further statistical analysis and analytical data on clinical chemistry parameters of the diabetic study. No biological samples were available for further analysis. COT considered that the data did not address the issue of enzyme induction. This was important, as the ADI had been established on the basis of increased liver weight in dogs, it would therefore be helpful to know the sensitivity of human liver enzyme induction compared with that in dogs. As a result the COT considered that the new data did not warrant a review of the ADI for alitame.

Amnesic shellfish poisoning

1.5 COT was asked to review the chemistry, toxicology and biology of amnesic shellfish poisoning (ASP) and to comment on the public health implications of a proposed tiered approach to scallop harvesting. ASP is caused by domoic acid, a water-soluble amino acid produced by species of *Pseudonitzschia* phytoplankton and accumulated by shellfish, and is characterised by a range of clinical symptoms including gastrointestinal effects and neurotoxic effects such as hallucinations, memory loss and coma. An action limit of 20 µg domoic acid/g tissue for mussels was established by

Canadian authorities following an outbreak of ASP in 1987. The EU adopted this action limit for mussels and other bivalve shellfish, including scallops and clams in 1997 (EC Directive 97/61/EC). The 20 µg/g tissue action limit was based on the LOAEL identified in one individual, incorporating a ten-fold safety margin. COT was informed that although no major outbreaks of ASP had been reported since the limit had been adopted, it was not possible to evaluate if this was due to the efficacy of the action limit.

1.6 The COT considered the action limit to be a pragmatic guideline, which had not been derived from robust toxicological data. At present the toxicological data were insufficient to establish a NOAEL. COT therefore recommended that further long-term toxicological studies, conducted using appropriate animal models, are required to allow the derivation of a TDI and also that additional information on shellfish consumption is needed.

1.7 The proposed tiered approach would allow the sale of individual organs from whole scallops that exceeded the action limit, provided that those individual organs contained less than or equal to 20 µg domoic acid/g tissue.

1.8 The COT noted the high variability of domoic acid concentrations between scallops, and in different organs of a scallop, and expressed concern about the potential for cross-contamination of tissues during processing. It noted the important and severe public health implications of ASP and strongly recommended that if a tiered approach were introduced, rigorous monitoring and enforcement at the point of sale would be required to ensure protection of public health. The statement is included at the end of this report.

Bisphenol A in canned foods

1.9 Bisphenol A (BPA) is a component of epoxy resins used in the manufacture of internal coatings for some food cans. BPA is also used in PVC coatings on the inside of some cans and in polycarbonate plastics used in consumer products. Bisphenol F (BPF) is also used to make epoxy resins, but these resins are rarely used in food contact materials. The committee was asked to comment on the health implications of the results of a survey (FSA Food Surveillance Information Sheet No. 13/01) which had shown that BPA migrates from can coatings into food at low levels. BPF was not found to migrate at detectable levels.

1.10 The COT initially considered BPA in 1997, when it was asked to comment on the implications of a study on its estrogenic activity (see 1997 Annual report, page 6). In 1986, the EC's Scientific Committee for Food (SCF) had recommended a Tolerable Daily Intake (TDI) of 0.05 milligram/kilogram (mg/kg) body weight (bw) per day for BPA in the context of its uses in food contact plastics. The TDI for BPA was set on the basis of long-term animal studies before the potential endocrine modulating activity of this compound was proposed and is currently under review by the SCF.

1.11 The COT was provided with an up-to-date review, being prepared under the Existing Substances Regulations, of the large body of toxicological data on BPA, including the apparent low-dose effects. The COT considered that the effects on reproductive organs reported at low doses represented very sensitive biomarkers that indicated the need for further studies, but could not be defined as “adverse”. In view of the uncertainty

relating to the functional significance of the effects at low doses, as well as the lack of reproducibility, it was considered that it would not be appropriate, at this time, to base human health risk assessment on these effects.

1.12 BPA intakes for adults and infants had been estimated from the mean contaminant level and high level consumption of the canned foods in the FSA survey. The estimated intakes were in the region of two orders of magnitude below the TDI established by the SCF. Recent multi-generation studies on BPA demonstrated effects on fertility in rats at high doses. The COT noted the presence of a substantial margin of safety (about 100,000 fold) between human exposure to BPA via canned food and doses that cause effects on fertility in animal studies.

1.13 Overall, the COT acknowledged the uncertainties that exist in the scientific understanding of potential endocrine effects of BPA. However, on the basis of the available evidence, it concluded that the levels of BPA identified in canned foods in the FSA survey were unlikely to be of concern to health, and that there was no reason for consumers to change their source of foodstuffs as a result of the survey findings. The COT noted that it would be prudent to review this toxicological advice as further scientific evidence on possible low-dose effects of endocrine modulating substances unfolds. The COT statement is included at the end of this report.

Code of Practice for Scientific Advisory Committees - second round of consultation

1.14 The COT had discussed the first consultation document on the Code of Practice for Scientific Advisory Committees in October 2000, and was now invited to take part in the second round of consultation.

1.15 The COT welcomed the draft code of practice, noting that it is a broad-ranging document and that not all aspects are applicable to all scientific advisory committees. Members considered that the COT already complies with the majority of the provisions, and have made recommendations to the Secretariat on additional aspects to be included in future practice.

1.16 Two main points were raised. The COT considered that, whilst committees should be aware of social and ethical issues and public and stakeholder concerns, these do not influence the scientific judgement. Secondly the COT considered that the current practice, in which a Government department sets a committee's agenda, has the potential to compromise the committee's independence. A formal requirement for the committee to take a broad view of the topics to be discussed, or not discussed, would help to avoid this situation.

1.17 The third and final draft of the Code of Practice was distributed to members in December 2001.

Contaminants in soil

1.18 The COT was informed that the Department for Environment, Food and Rural Affairs (DEFRA) and the Environment Agency (EA) are developing a framework for

assessing the possible risks to human health associated with exposure to contaminants in soil, based upon the Contaminated Land Exposure Assessment model (CLEA). The EA had requested a COT opinion on the toxicological approach used within the framework, which involved a step-wise approach to identification of appropriate TDIs or Index Doses.

1.19 Within the EA model, a TDI is established for substances considered to act via a threshold mechanism and is compared with background intake of the contaminant from other sources (the Mean Daily Intake) in order to allocate a portion of the TDI to potential intake from soil – the Tolerable Daily Soil Intake (TDSI). An Index Dose is derived for genotoxic carcinogens, which is not compared to background exposure, since it is assumed that the "As Low As Reasonably Practicable" (ALARP) principle is applied to all sources.

1.20 The COT approved the general approach and raised a number of specific points to be taken into account by EA in finalising the report. The COT agreed that the "Index Dose" should not be viewed as a precedent for establishing guidance values for genotoxic carcinogens and that use of quantitative risk assessments, as conducted in the USA, was not accepted by UK expert committees. Where UK guideline values are available, such as the drinking water standards, these would be used in deriving appropriate Index Doses.

Dioxins and dioxin-like polychlorinated biphenyls (PCBs): Consideration of the Tolerable Daily Intake (TDI)

1.21 The COT has completed its evaluation of whether the existing UK TDI for dioxins and dioxin-like PCBs was sufficiently protective of consumer health. The review included the available data and approaches taken by the other expert committees in recent and on-going assessments, including those of the World Health Organization (WHO), the SCF, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the US Environmental Protection Agency (EPA).

1.22 The COC was consulted on the carcinogenicity of dioxins and dioxin-like PCBs (see paragraphs 3.1 to 3.7). Based on the COC advice, the COT agreed there was sufficient information to assume a threshold existed for the effects of dioxins and hence a TDI could be established. There were two critical components to this decision:

i) There is considerable evidence that dioxins do not directly damage the genetic material.

ii) There is considerable understanding of the biological reactions by which dioxins cause harmful effects, and evidence that these reactions will not occur at sufficiently low levels of exposure.

1.23 Because the COT decided to use a threshold approach, it needed to review the evidence for all possible effects of dioxins, and not just cancer. A TDI should be set to protect the most vulnerable population against the harmful effect that could occur at the lowest level of exposure.

1.24 The human studies are mainly on workers in chemical plants or following accidental contamination of the environment (e.g. Seveso in Italy) or edible oils (e.g. Yusho in Taiwan). These studies provided evidence that dioxins are associated with increased

risk of cancer, at much higher levels of dioxin exposure than the general public receive via the diet. Weaker evidence suggests increased risks of cardiovascular disease. A number of other effects have been associated with dioxin exposure in people, but there is insufficient information to draw conclusions.

1.25 The COT calculated the total amounts of dioxins that could have been present in the bodies of the people in these studies, referred to as their body burden (these evaluations are on the COT website at http://www.food.gov.uk/multimedia/pdfs/cot-diox-epi.pdf). If a body burden could have been identified at which there was no increased risk of harmful effects, then this would have been used to set a TDI. However, the human data did not provide an adequate basis for the TDI because:

i) the exposure data were rough estimations and did not include all the dioxins and dioxin-like substances of concern;

ii) the studies did not adequately consider other possible causes of the observed effects;

iii) apart from a series of developmental studies conducted in the Netherlands, the patterns of exposure included periods of high level exposure rather than continual low level exposure from food, as occurs in the general population;

iv) in the occupational studies, exposed workers were mostly male and therefore the wrong population to study the critical effect seen in animal studies (effects on the developing fetus).

1.26 A wide range of toxic effects has been observed in animal studies, including cancer and effects on the immune and reproductive systems. The effects occurring at the lowest dose levels were observed when dioxins were given to pregnant animals, the most sensitive and consistent effect being on the developing reproductive system of the male offspring, particularly changes in sperm production and quality. These changes indicate that exposure of the male fetus to high levels of dioxins whilst in the uterus could lead to decreased fertility of the adult male.

1.27 In the evaluation of dioxins, the COT decided that the data allowed the use of chemical-specific adjustment factors based on knowledge of the fate and toxicity of dioxins, in preference to the default uncertainty factors that are sometimes used. Applying these adjustment factors to the lowest dose level at which effects were observed in the developing male fetus, resulted in recommendation of a TDI of 2 pg TEQ/kg bw per day. As this TDI is based on the most sensitive end-point, it will also protect against the risk of other adverse effects, including cancer. Some other scientific advisory committees have recently recommended tolerable intakes related to periods of one week or one month. The COT considered that because intakes are usually expressed on a daily basis, a tolerable daily intake was more appropriate and transparent, but that it is long term exposure that is important.

1.28 COT noted that the most recent intake estimates for the UK population are 1.8 pg/kg bw/day for the average consumer and 3.1 pg/kg bw/day for the 97.5 percentile consumer and that dietary intakes are decreasing. There are no short-term measures that can be used to decrease the body burden of dioxins and dioxin-like PCBs in humans because of their long half-lives and widespread presence at low levels in food. The full technical statement is included at the end of this report, and a lay summary is also available on the COT website (http://www.food.gov.uk/science/ouradvisors/toxicity/statements/dioxinsstate).

Enzyme submission - Amano 90

1.29 Amano 90 is a hemicellulase enzyme added to wheat flour prior to baking. In 2000, the COT had considered 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 on this enzyme. The COT had requested additional information on the assay methodology and inactivation of the enzyme during baking. The company had submitted new information intended to answer these points.

1.30 The COT considered that the amount of detail in the new information was limited and not all the requested information had been provided. Members noted that the new information probably demonstrated that inactivation of the enzyme was occurring. However, the COT remained concerned that there was still inadequate information on the methodology. The COT was unable to recommend full approval of the hemicellulase enzyme Amano 90 in the absence of details of the assay and performance of the assay on duplicate samples, both of which it had previously requested.

Enzyme submission - Lipase D

1.31 In 2000, the COT had considered a submission seeking approval of an immobilised enzyme preparation, Lipase D, to be used in manufacture of yellow fat spreads. The COT had agreed that the level of detail submitted on the manufacturing processes was appropriate and agreed to recommend a two-year temporary clearance for the use of immobilised Lipase D in production of yellow fat spreads, pending submission of specified analytical data.

1.32 The company provided results of rhizoxin analysis on 5 production batches of lipase D, all of which were below the limit of detection. The supplier has agreed to test production batches for rhizoxin as a matter of routine, but the anticipated scale of production would only provide limited additional results. The COT considered the results from 5 production batches combined with testing of all production batches were sufficient to answer its concerns.

1.33 The company had provided further information on the support material on which the enzyme is immobilised, showing that its composition is consistent with food grade material of this polymer. Evidence was provided that the material met the overall migration limits as specified in an appropriate European standard and the specific migration of each of the three substances restricted under Dutch regulations were below their limits of detection. COT considered that this information provided reassurance that the material was suitable for use as the support material. The company also provided a new 90-day study performed as part of their submission to US regulatory authorities, required as a result of a change in production method resulting in higher yields and higher purity material.

1.34 The COT noted that there appeared to be some evidence of increased liver weight in the absence of histopathological changes. COT considered that further clarification of

the assessment of these findings was necessary before deciding whether they should be taken into account in identifying a NOAEL.

1.35 The company subsequently provided a detailed statistical analysis of the reported findings and information on the rationale for their interpretation in the report. The COT considered that, although there was still some uncertainty, the reported NOAEL was valid since the changes in liver weight were relatively small and both histopathology and clinical biochemistry were normal.

1.36 The COT concluded that full clearance could now be granted to this enzyme preparation.

Enzyme submission - Xylanase preparation from GM *Aspergillus niger*

1.37 This was a new submission of a xylanase preparation from a genetically modified *Aspergillus niger* strain, which the Advisory Committee on Novel Foods and Processes (ACNFP) had already considered.

1.38 The COT noted that the toxicity studies were well conducted and no dose-related findings were reported. There was a very large margin of safety between the NOAEL in the toxicity studies and the estimated intake values at the maximum application rates. The COT noted that there were difficulties in demonstrating the lack of allergenicity requested by ACNFP and that methodology had not been specified in the Framework for the Assessment of Enzymes. However, several experimental methods are available.

1.39 The COT asked the company to provide the additional information, relating to testing for mycotoxins and other contaminants, compliance with the specification and laboratory data to demonstrate that there is no residual enzyme activity/allergenic problem in the final food.

1.40 The COT agreed temporary approval for 1 year for the use of the powdered and micro-granulated products in the formulation of enzyme preparations used in the production of baked goods using yeast, including bread and biscuits such as wafers and crackers.

Government's Response to the Phillips Enquiry: Consideration of Committee procedures

1.41 The COT considered the Government's final response to the Phillips Committee Enquiry which was published in September 2001, and incorporated comments received following a public consultation. COT focused its response on the enquiry's findings relating to the role of Scientific Advisory Committees and the Government's assessment and use of scientific advice and considered whether it needed to make further changes to its way of working in the light of the response. The COT noted that the government response acknowledged moves that had been made to meet the Phillips Enquiry recommendations including a number of measures initiated by the Food Standards Agency.

1.42 In considering possible involvement in "horizon scanning", Members noted that the COT already considers generic issues in order to keep abreast of current issues both in toxicology and other areas that impinge on the risk assessment process. The recent joint COT/COM/COC meeting on the uses of proteomics and genomics in toxicology was highlighted as an example of the consideration of a generic issue. The COT working groups also consider generic issues and future research needs.

1.43 The COT agreed the need for wider consultation, allowing consideration of views outside the mainstream. A list of future agenda items should be published on the website so that interested parties could submit their comments before papers are finalised. The Committee also agreed to play a more active role in setting its agenda.

1.44 COT noted that over the past five years it has already greatly increased its openness by holding open meetings, usually on an annual basis, and publication of minutes and statements on its website. Certain data considered by the committee are commercially sensitive and cannot be released. The option of a two-tier meeting (partly open) was considered, but concern was noted that the ability to ascribe specific comments to individual Members might compromise their personal safety.

1.45 In order to make COT conclusions more accessible to the public it may be necessary to provide lay summaries. This was recognised as being particularly important when the topic was highly technical such as the statement on dioxins and dioxin-like PCBs (see statement at the end of the COT report).

1.46 Members noted that the COT is already meeting the recommendation for providing quick and efficient action on new issues. The Chairman or individual Members have provided urgent opinions when requested and the issue was then noted under "Matters Arising" at the subsequent full committee meeting. Members confirmed they were also willing to be involved in contingency planning if requested.

1.47 The COT agreed that a formal mechanism for identifying when COT opinions need to be revised is inappropriate and that it is the Secretariat's responsibility to ensure that new information is presented to the Committee. Additionally it was agreed that the Secretariat would keep members abreast of developments in the COC and COM to increase opportunities for cross working between the committees.

Health outcomes in populations living close to landfill sites

1.48 In 1998, members considered a study by the EUROHAZCON group, which reported an association between living close to hazardous waste landfill sites in Europe and an increased risk of congenital anomalies. Members also considered a study around the Nant-y-Gwyddon site in South Wales, which reported an excess risk of malformations in close to the site, both before and after opening. A statement of the COT opinion on these studies, and other relevant data in the scientific literature at the time was published in the 1998 Annual Report (page 12).

1.49 In November 2000, the COT commented on a revised proposal from the Small Area Health Statistics Unit (SAHSU) for a study of health outcomes in populations living around all known landfill sites in Great Britain. SAHSU had revised the protocol when it became clear that there are around 19,000 open or closed landfill sites in Great Britain and therefore that most of the population (about 80%) lives near to a

landfill site. After discussion of aspects of the study design, the COT considered that it was appropriate to proceed with the study by urged caution in interpretation of the results (2000 Annual Report, page 19).

1.50 The SAHSU study had now been completed and the results were presented to the COT for consideration. The SAHSU study was the largest investigation of landfills and health effects to have been carried out and, unlike the EUROHAZCON study, it was not selective in terms of the sites studied. The COT noted that the SAHSU study was a very careful and sound investigation but there were some limitations in the interpretation of the data. Ideally, a geographical study would incorporate a "dose-response" analysis by calculating relative risks in populations living within different distance bands from the hazard under investigation. This was not possible in the SAHSU study as further subdivisions of distance less than 2 km from the site would not be meaningful in view of the uncertainties in the geographical data both on landfill sites and on postcode locations. A further limitation of ecological studies of this kind is that they do not indicate whether any reported association is causal or not.

1.51 The SAHSU study analysed data on several congenital anomalies and the prevalence of stillbirths and low and very low birthweights. The under-ascertainment of malformation data in the UK was highlighted, noting that the more subtle malformations may often have been missed. It is possible that residual socio-economic confounding might account, at least in part, for the differences between the study and reference populations. In view of the uncertainties associated with the sub-analysis of the congenital anomalies data, it was not possible to draw firm conclusions on the possible health effects of landfill sites from the results of the SAHSU study.

1.52 Another sub-analysis undertaken by the SAHSU study focussed on special landfill sites, which take predominantly the same type of waste as most landfill sites, but are also licensed to take certain specified types of special waste. Without good information on emissions and controls at these and other landfill sites, it is not possible to assess whether special landfill sites may present a greater potential risk to health than other landfill sites.

1.53 A COT view was also sought on any further studies that might be useful to investigate the potential health risks of landfill sites. The COT noted an alternative approach to an ecological design would be to carry out case-control or cohort studies, with estimates of exposure and more detailed consideration of confounding factors. More information on the characteristics of the sites under investigation, as well as the type of hazardous waste received by such sites may also aid the interpretation of future studies. The COT statement is included at the end of this report.

Hyperactivity and Food Additives – additional analyses on research project results

1.54 COT considered the results of a research project entitled "Do food additives cause hyperactivity and behaviour problems in a geographically defined population of three-year-olds?" in 2000. In 2001, COT considered the results of additional analyses of the data that the researchers had provided at the request of the COT. Published data suggest exclusion of specific dietary components can affect some measures of behaviour in some children. The researchers suggested that their research provided evidence that food additives had statistically significant effects on some measures of

behaviour, irrespective of whether the children were atopic, hyperactive or neither. The COT acknowledged that the study was consistent with published reports of behavioural changes occurring in some children following consumption of particular food additives. No statistically significant changes in the children's behaviour were apparent when assessed in a clinical setting. However parental observations suggested that small but statistically significant changes in some aspects of behaviour were apparent after challenge with the additives. The COT had reservations about the generalisation and interpretation of these findings in view of some aspects of the study design and noted that the reported effects on behaviour were small when compared to previous research. As reported, the data did not allow the COT to determine whether there was an adverse effect of the additives in all children or a possible idiosyncratic effect in a susceptible sub-group. The COT therefore considered that it was not possible to reach firm conclusions about the clinical significance of the observed effects.

IGHRC paper on uncertainty factors

1.55 The COT considered the draft version of a guidance paper on uncertainty factors, prepared by the Interdepartmental Group on Health Risks from Chemicals (IGHRC). IGHRC, which functions as a subgroup of the Interdepartmental Liaison Group on Risk Assessment (ILGRA) is the successor to the Risk Assessment and Toxicology Steering Group (RATSC) established in response to the UK government's Forward Look set up in 1995[1]. IGHRC aims to improve and increase the coherence of risk assessment across government departments.

1.56 IGHRC's First Report and Forward Plan[2] published in 2000 identified one of its goals as the production of two guidance documents, this one on uncertainty factors and a second on exposure assessment. As these documents should be geared to serve UK government departments and agencies, it was considered essential that individual departments, and the expert committees that serve them, were consulted regarding the content. Following revisions resulting from this round of consultations the revised paper will be brought back to the COT and other key committees for final approval in late 2002.

1.57 The draft document outlined the risk assessment process highlighting areas of toxicological uncertainty, describing current approaches to dealing with uncertainties, those used in UK government regulatory decision-making and in other countries. It also included a brief historical perspective and listed some of the recent advances in dealing with toxicological uncertainty.

1.58 The COT considered the draft and made a number of constructive suggestions for revision. Amongst the points noted was the need for the document to focus on the way uncertainty is handled in UK government departments. Where there are differences in approach by different departments the paper should provide a clear explanation of

[1] HMSO (1995) Forward Look of Government-funded Science, Engineering and Technology (Volume 1), London, UK, HMSO

[2] IGHRC (2000) *Interdepartmental Group on Health Risks from Chemicals:* First report and forward plan to 2002 (cr7A), Leicester, UK, Institute for Environment and Health

where (and why) these arise. In addition it was considered important to emphasise that the document focuses on the use of uncertainty factors in risk assessment, and so other methods such as probabilistic modelling are not described in detail.

1.59 Among the issues considered by the committee were the advantages and disadvantages of using the Benchmark Dose (BMD) as opposed to the no-observed adverse effect level (NOAEL), uncertainty factors applied for children and infants, and deficiencies in the toxicological database for some types of chemicals. In addition members sought further clarification of the distinction and overlap between UK and EU procedures. Comments on the layout, content and additional areas for discussion were also considered and noted by the IGHRC drafting group members present at the meeting.

1.60 After revision in the light of comments received from the COT and other expert committees, the paper will be brought back to the COT and other key committees for endorsement.

PAH concentrations in food: Interim pragmatic guideline limits for use in emergencies

1.61 In 2000, the 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. The COT had noted that the COC had identified benzo(a)pyrene, benz(a)anthracene and dibenz(a,h)anthracene as being of greatest concern in respect of carcinogenic hazard on the basis of *in vivo* mutagenicity and/or multi-site carcinogenicity.

1.61 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. Members agreed that an interim pragmatic guideline limit could be applied to any one of the three PAHs of greatest concern, but should only be used in emergency circumstances where comparable (control) data were not available to indicate whether the level of food contamination resulted from the emergency.

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

PAHs in shellfish

1.63 In December 2000, the COT had considered a draft CEFAS report and concluded that, in order to support risk assessment, additional information was needed on consumption patterns for the general population and for potential high level consumers residing around coastal resorts.

1.64 Information on consumption patterns and estimations of intake of polycyclic aromatic hydrocarbons (PAHs) from bivalve molluscs had now been provided. No data was available on whether PAH concentrations remained constant over time.Concentrations

were therefore assumed representative except in the single example of a high level thought to be associated with a specific pollution incident.

1.65 Members were also provided with a JECFA review of benzo(a)pyrene (BAP) (WHO Food Additive Series 28: 301-363, 1991), noting the several orders of magnitude difference between human dietary intake and the level of BaP needed to induce tumours in experimental animals. The data indicated that intakes of PAHs from bivalve molluscs are likely to be within the normal range for the general population, but may result in a higher level of intake for high level consumers in coastal regions with persistent PAH contamination. Members noted that in this CEFAS survey, samples had been collected on the basis of microbiological concern, and thus were not necessarily representative of worst case PAH contamination.

1.66 The data indicated no major difference in consumption between coastal areas and the general population, but were based upon too few consumers to be reliable. It was not possible to determine the proportion of total exposure to PAHs from oysters and other bivalve molluscs as total diet studies of PAHs have grouped shellfish with other fish and fish products. In view of the limitations of the database on consumption patterns for consumers living around coastal areas, and given that the total exposures to individual PAHs were estimated from old data, members agreed that it was not possible to comment on the health implications of the levels determined in the CEFAS study.

1.67 The Committee was informed that a Total Diet Study on PAHs is due to be commissioned later this year. The Secretariat agreed to give consideration to the design and sampling methodology of future surveys in order to assist with the Committee's interpretation of relevance to public health.

PCBs in breast milk

1.68 COT discussed the recently published article by Walkowiak and colleagues (Lancet, 358: 1602-7, 2001), describing studies on the effects of PCB exposure on development in a cohort of 171 mother infant pairs. Members were reminded that they had previously discussed two series of Dutch studies on the effects of exposure to dioxins and dioxin-like PCBs on development during their recent evaluation of dioxins, and had sought additional advice on the relevance of the methodology and the results of the Dutch studies.

1.69 Walkowiak *et al*. estimated exposure from the sum of the concentrations of three PCB congeners in cord blood, blood obtained from infants at 42 months of age and milk samples obtained two weeks postpartum. The authors measured psychodevelopment using the same tests as in the Dutch studies, which COT had concluded were "the best available in the absence of an *a priori* hypothesis of specific effects". The authors concluded that the overall positive trend for quality of the home environment was stronger than the negative effect of neonatal PCB exposure. The authors found a significant negative effect of postnatal PCB exposure on development at 42 months, but not at the earlier times.

1.70 Members noted that many of the comments made on the Dutch studies were applicable to the work of Walkowiak. In particular "it was not possible to determine

whether any cognitive changes represented temporarily delayed milestones of development or a persistent decrement and that follow-up studies were needed".

1.71 There was a lack of information in the paper on potentially serious confounders, such as data on the physical health of the children, and limited information on the statistical analyses, which prevented adequate assessment of the models used. Members also noted several concerns regarding the exposure data. Members considered that, on the basis of the data available, it was not possible to reach conclusions on whether or not effects occurred.

1.72 The COT concluded that these data were insufficient to contradict the current advice on breast-feeding, namely that "although intakes of some chemicals by breast-fed babies are higher than is desirable, encouragement of breast-feeding should continue on the basis of convincing evidence of the benefits of human milk to the overall health and development of the infant".

1.73 Members noted that the compounds measured non-dioxin-like PCBs, but were representative exposure markers and any reported findings could not be ascribed to specific effects of these compounds. Evaluation of non-dioxin-like PCBs had recently been added to the 2002 work programme of the EU Scientific Committee on Food. They also requested information on the levels of non-dioxin-like PCBs in the environment and whether these were decreasing in line with dioxins and dioxin-like PCBs. This information should also consider whether the distribution of PCB exposure from the environment is changing.

RCEP study of long term effects of chemicals

1.74 The COT was informed that the Royal Commission on Environmental Pollution (RCEP) had announced its intention to study the long-term effects of chemicals in the environment, and was currently inviting submission of evidence. The COT focused on the issues raised in respect of risk assessment.

1.75 Members noted that the RCEP documents did not provide a definition of "chemical" or the "worrying trends in human health" and therefore considered that the scope of the study was unclear. Many of the issues raised are extremely complex, there are a large number of objectives and it is not clear whether all of these are attainable. Members questioned whether the assumptions were sufficiently balanced. Although the issues described may reflect concerns of the public, the scientific evidence in our experience often does not support the assumption that these are contributing to ill health. Members noted that, rather than assuming that health problems could be prevented by conducting risk assessments on the many chemicals present in the environment, it would be preferable to direct attention to results of health monitoring. Thus the RCEP study may benefit from interrogating various registries (e.g. cancer and malformation registries) rather than focusing on the databases for chemicals.

1.76 The main emphasis of the RCEP study is on environmental effects, whereas the COT is concerned with human health. Members noted that it may be beneficial for the RCEP to align its work with that of the European Commission's White Paper strategy for a future chemical policy. However, they stressed that not all chemicals present in the environment have been synthesised and marketed by chemical industries.

1.77 COT noted that expression of uncertainty is an important aspect of risk assessment, but that the relative importance of false positive and false negative results is a risk management issue, which will depend on the specific circumstances of chemical use.

1.78 COT stressed that there is no evidence that man-made chemicals introduce a qualitatively different risk to that posed by exposure to naturally occurring chemicals, and this distinction may be artificial. Dioxins (formed as a result of industrial activity, but also from "natural" combustion sources such as forest fires) and domoic acid (the toxin considered to be responsible for Amnesic Shellfish Poisoning) were cited as examples. Data on industrial chemicals are generally provided by manufacturers for consideration by expert committees, who check the robustness of the submitted information. The EU White Paper defines the responsibilities of manufacturers and down-stream users. However for chemicals that are not produced industrially, there may be no organisation with responsibility for providing the data. Risk assessment of both natural and synthetic compounds should take into account all potential sources of exposure.

1.79 The monitoring of biological systems and the measurement of concentrations of chemicals continued to be important facets for informing the risk assessment process. The COT would prefer to base its risk assessments on good quality human data, but these are rarely available. The value of *in vivo* animal data is well-established in respect of risk assessment for humans. If data are available relating to the fate of a chemical in the human body, then these can be used to derive chemical-specific adjustment factors to decrease the uncertainty in extrapolating from animals to humans. The value of *in vivo* data on a limited number of species for the prediction of the effects of chemicals on the environment and ecosystems is less clear.

1.80 The COT evaluates all relevant data in its risk assessments and considers that results from *in vitro* studies may be particularly helpful in elucidating mechanisms of action. However, the available alternatives to *in vivo* test systems are in need of further validation before being used routinely, and there is currently little prospect of replacement of *in vivo* tests for regulatory purposes.

1.81 The COT noted that the IGHRC reports (discussed in paragraphs 1.54 to 1.59) would be helpful in informing the issues that have been raised by the RCEP study. Members also noted that the British Toxicology Society and Directorate General SANCO of the European Commission were not amongst the organisations that had been invited to comment on the RCEP consultation. A response was prepared for submission by the Chairman to the RCEP.

Reproductive effects of caffeine

1.82 COT last considered the safety of caffeine, including possible effects on reproduction, during 1980-1984. At that time, many animal data but few human data were available. A number of human studies have since been published, many, but not all, of which have implicated maternal caffeine consumption in adverse effects on pregnancy, including risk of low birth weight and miscarriage. COT therefore re-considered possible adverse effects of caffeine on reproduction.

1.83 COT concluded that it was plausible to assume that there was an association between caffeine intakes of more than 300 mg/day and spontaneous abortion or low birth

weight. However, it was not possible to define this association as causal. COT noted that the human data were limited by inaccurate estimates of caffeine intake by women during pregnancy and and by inter-individual variation in the rate of metabolism, and that research using biomarkers to measure caffeine concentration more accurately would help to demonstrate whether or not the observed association is causal. In addition, further information on the comparative activity of individual metabolites of caffeine may aid the extrapolation of animal data to humans.

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

Surveys: Guidelines for project officers

1.85 The FSA carries out a substantial programme of food surveys and is developing detailed guidelines to assist project officers in the commissioning, management and reporting of surveys. In view of the COT input in advising on the health implications of the results of surveys of food chemicals, it was presented with an opportunity to comment on a revised version of the current survey guidelines.

1.86 The COT proposed that the current survey guidelines should accurately note the rationale and the primary objectives. It was also important to discriminate between descriptive surveys which are to be used for qualitative purposes and those where quantitative data is being carried out to underpin further work and evaluations. Other forms of advice (e.g. epidemiological) should be sought in addition to mandatory statistical and analytical input. In terms of the sampling plan and collation and retention of samples, the guidelines should emphasise the importance of having samples that are representative of the group(s) under consideration. In addition, the guidelines should be explicit about the identity of samples purchased. Routine archiving of samples would be advisable. Archived samples would provide an opportunity for the analysis of time trends and/or for analysis by subsequent more sophisticated methodology.

1.87 The survey guidelines will be revised and finalised by the Food Standards Agency in due course.

Ongoing work

Chlorinated drinking water and reproductive outcomes

1.88 During 1998, the COT reviewed epidemiological evidence for an association between disinfection by-products in drinking water and adverse birth outcomes. It agreed that there was insufficient evidence to conclude that the presence of chlorination by-products in tap water increased the risk of adverse reproductive outcomes, and recommended that the claimed associations between patterns of exposure from drinking-water and the incidence of adverse reproductive outcomes be investigated (see 1998 Annual Report, page 7).

1.89 Following this recommendation for further investigations, the Small Area Health Statistics Unit (SAHSU), in collaboration with, and with the support of UK water supply companies, undertook a major study to investigate possible associations between birth outcomes and levels of disinfection by-products in drinking water. The study used routinely collected trihalomethane (THM) measurements in drinking water and routinely available health statistics on stillbirths and birth weight. The COT reviewed the results of this work and also took into account 5 relevant epidemiological studies, published since the previous evaluation, and the available animal reproductive toxicity studies with chlorinated water or individual disinfection by-products.

1.90 The initial results of the SAHSU study differed in the three water supply areas. There was evidence of confounding by social deprivation, adjustment for which may not have been completely successful in this analysis. Further analysis is currently being undertaken to explore this. The COT reaffirmed its previous conclusions and agreed that further research was needed, in particular, prospective studies with appropriate assessment of exposure, allowing for seasonal variation and confounding factors. There was also a need for further animal studies, in order to study the effect of interactions with other chlorinated compounds present in water.

1.91 The COT statement will be released to coincide with the publication of the study results.

Fluorine: 1997 Total Diet Study

1.92 In 2000, the COT considered the results of the 1997 Total Diet Study. At that time, consideration of any potential effects of dietary fluorine was deferred until the Expert Group on Vitamins and Minerals (EVM) had reviewed the element. The COT considered the draft EVM review of fluorine but agreed that it was more appropriate to wait until the EVM review and risk assessment was finalised in early 2002.

1.93 The COT asked that estimates of the intake of fluorine by children also be provided when the issue was reconsidered.

Food Standards Agency review of scientific committees

1.94 Members had received a letter from the Chairman of the Food Standards Agency asking for comments towards the Agency's review of scientific committees. The review is covering a range of issues including openness, conflicts of interest, views outside the mainstream etc. The Review Group had its first meeting in July 2001. This refined the issues and sought stakeholder views. The second meeting, held in September was attended by the Chairmen of the Advisory Committees and aimed specifically to cover issues raised by the Chairmen. Further consultation is planned, with a final report going to the Food Standards Agency Board in January 2002.

Joint meeting of COT/COM/COC

1.95 In response to the recommendations arising from Sir Robert May's "Review of Risk Procedures used by the Government's Advisory Committees dealing with Food Safety" [3] the COT and its sister committees the COC and COM identified a need for more interaction and co-operative working.

1.96 Toxicogenomics and proteomics had been highlighted as rapidly growing areas of toxicological science but their applicability in risk assessment had not been subjected to detailed consideration. The three committees agreed that these technologies should be considered in the context of assessing their current and future potential as tools in regulatory risk assessments. The meeting's objectives were identified as:

i) To provide advice to government departments and regulatory agencies on use of genomics and proteomics in toxicological risk assessment

ii) To facilitate closer working and greater collaboration between the COT, COC and COM.

1.97 The meeting, held on 8 October 2001 at the Department of Health in London, was attended by about 90 people including members of all three expert committees, members of other advisory committees, independent scientists and other interested stakeholders. In order to cater for those less familiar with the techniques, the programme commenced with a general overview of the methodology by experts in the field. This was complemented by three parallel Working Groups on:

i) Use of Genomics in Screening;

ii) Use of Proteomics in Screening;

iii) Use of Genomics/Proteomics in Risk Assessment.

Each working group was led by a facilitator and an invited expert. The final session included discussion of the outcomes from the Working Groups and agreed the overall conclusions

1.99 The meeting was well received. A statement is in preparation and a report of the meeting will be published in a scientific journal.

PAVA

1.100 The Home Office asked the COT to advise on the health effects of the use of Pelargonyl vanillylamide (PAVA or nonivamide), as a chemical incapacitant. PAVA was being used by Sussex Police as an alternative to CS spray, on which the COT had provided advice in 1999 (1999 Annual report, page 7). PAVA is the synthetic equivalent to capsaicin the active ingredient of pepper. It is also used as a food flavour and in human medicine for topical application as a rubifacient. The COT discussion on use of PAVA as an incapacitant will be completed after the COM consideration of the mutagenicity data.

[3] Office of Science and Technology, September 2001; http://www.dti.gov.uk/ost/whatsnew/index.htm

Statements of the COT

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Bisphenols in canned foods

Polycyclic aromatic hydrocarbons – Interim pragmatic guideline limits for use in emergencies

Reproductive effects of caffeine

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Tolerable Daily Intake for dioxins and dioxin-like polychlorinated biphenyls

STATEMENT ON AMNESIC SHELLFISH POISONING

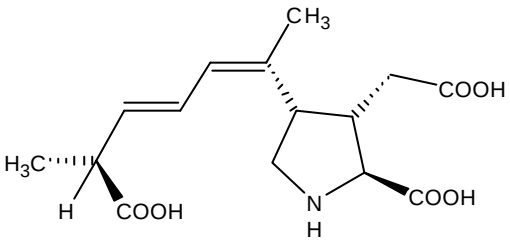
Introduction

1. We have been asked by the Food Standards Agency to review the issue of amnesic shellfish poisoning (ASP). The Committee was asked to consider whether the current EU action limit for bivalve shellfish (e.g. mussels, scallops, clams) of 20µg domoic acid/g tissue is adequate for the protection of public health. The Committee was also asked to comment on the public health implications of a proposed tired approach to scallop harvesting. This approach would allow harvesting of individual organs from whole scallops containing above 20µg domoic acid/g tissue. The individual organs could be marketed only if they contain levels less than or equal to 20µg domoic acid/g tissue.

Background

2. Amnesic shellfish poisoning was first recorded in Canada in 1987 following the consumption of contaminated mussels. Approximately 150 people became ill, and the outbreak resulted in the hospitalisation of 19 people and 4 deaths. The clinical effects were caused by domoic acid (figure 1), a water-soluble, amino acid which is produced by species of *Pseudonitzschia* phytoplankton, and accumulated by shellfish[1,2].

Figure 1: Chemical structure of domoic acid



3. Following the outbreak, the Canadian authorities imposed an action limit in mussels of 20 µg domoic acid/g tissue, above which harvesting of shellfish was suspended. The EU adopted this action limit for mussels and other bivalve shellfish including scallops and clams in 1997 (EC Directive 97/61/EC).

Toxicology

4. We reviewed the published toxicological data from animal studies and human case reports. There are limited data on the absorption, distribution, metabolism and excretion of domoic acid. However, these data indicate that domoic acid is not well absorbed in rodents and primates[3,4] and undergoes little metabolism prior to rapid excretion[5]. These data indicate species differences following oral exposure and suggest that primates have a relatively high sensitivity compared with rodents[4,6].

5. Domoic acid is a glutamate receptor agonist and binds with particularly high affinity to glutamate receptors in the central nervous system[7]. It is an excitotoxin and can produce a range of neuro-behavioural effects, which appear to be the most sensitive indicator of domoic acid toxicity.

6. Domoic acid is neurotoxic causing neuronal degeneration and apoptosis in specific regions of the hippocampus[8,9]. The lesions induced are consistent between rodent[11,12,13] and primate studies[6,14] and human[15,16] cases of ASP. Several mechanisms are thought to mediate the neurotoxicity. These involve perturbation of secondary messengers, including calcium and protein kinase C. However, it is thought that the critical toxic insult is the excessive accumulation of intracellular calcium[10].

7. The data indicate that rodent neonates are more susceptible to domoic acid toxicity than adults[17].

8. In rodent neonates, the spinal cord appears to be more sensitive than the brain to domoic acid toxicity[17]. However, a parallel comparison has not been carried out in adult rodents.

9. Domoic acid was not mutagenic *in vitro* in *V79* cells[18], but has not been tested in other systems. As there is potential for epoxide formation, it is suggested that further information on mutagenicity is required.

10. From review of the animal studies, we conclude that many used small group sizes and were inadequately reported. Additionally, many studies tested contaminated shellfish extract rather than purified domoic acid. This limited the utility of these studies, as the domoic acid content was not accurately quantified and the presence of other toxic components could not be excluded. Therefore, we consider the data were insufficient to identify a no-observed adverse effect level (NOAEL) or lowest-observed adverse effect level (LOAEL) which could support derivation of a Tolerable Daily Intake (TDI).

Human data

11. We reviewed the published case reports from two major outbreaks of ASP[1,2]. The most serious outbreak resulted in approximately 150 reported cases of ASP, the hospitalisation of 19 people and 4 deaths. The clinical symptoms ranged from gastrointestinal (GI) effects, to neurotoxic effects such as hallucinations, memory loss and coma. GI disturbances appeared within 24 hours and neurological effects within 48 hours of consumption of contaminated shellfish[1,16]. We note that the quantity of shellfish consumed was based on the recollections of a small number of patients. Additionally, the concentration of domoic acid was estimated from analyses of mussels collected from the affected areas after the outbreak had occurred. Therefore, we were unable to correlate the range and severity of the adverse effects with the dose of domoic acid consumed.

12. It has been suggested that the elderly are particularly vulnerable to ASP as the reported deaths occurred in individuals over 70 years of age. However, we are unconvinced that these limited data support such a premise as co-morbidity present in this group may have contributed to the deaths[1,2]. We note there are no data on the susceptibility of infants or children to ASP.

13. Although there have been no recorded outbreaks of ASP in the UK, we recognise that food poisoning incidents are under-reported. We note that the symptomology and rapid elimination of domoic acid from the body make ASP difficult to verify clinically. However, we suggest that urinary domoic acid may serve as a potential biomarker of exposure to this toxin but only if analysed soon after ingestion.

14. Due to the limited data, we were unable to ascertain if the GI disturbances were direct effects of domoic acid or a manifestation of excitotoxicity in the central nervous system. The latter is a plausible mechanism although it does not preclude the possibility of direct effects occurring in tandem. We regard neurotoxicity as the most significant effect of ASP in terms of public health.

Action Limit

15. We note that the current action limit is based on consumption estimates from the 1987 Canadian ASP outbreak indicating that mussels contaminated with ≥200µg/g domoic acid resulted in human illness. However, this was a retrospective estimate from a small number of affected individuals. A ten-fold uncertainty factor was incorporated to give an action limit of 20 µg/g[19,20]. The EU subsequently applied this action limit to other bivalve shellfish.

16. We regard the action limit as a pragmatic guideline rather than a toxicologically based safety limit. As noted in paragraph 10, the available data are not adequate to identify a NOAEL or LOAEL. In view of the severe and potentially irreversible neurotoxicity of domoic acid, we consider that an uncertainty factor of 10 is inadequate to allow for inter-individual variability in addition to the uncertainties in the estimation of the domoic acid content and quantity of mussels consumed. However, we consider that at present the toxicological and shellfish consumption data are too limited to support derivation of an alternative action limit. We suggest that further long-term toxicological studies are conducted, using appropriate models. Additional information on shellfish consumption is required to allow derivation of a TDI and a more robust action limit.

Tiered approach

17. Currently, EU directive 97/61/EC prescribes an action level for domoic acid of 20 µg domoic acid/g tissue for bivalve shellfish. If concentrations exceed this, harvesting of shellfish is stopped until levels drop below this. Detection of domoic acid in a range of shellfish, in particular King Scallops, has resulted in the frequent closure of harvesting grounds in Scotland.

18. The Food Standards Agency in Scotland is investigating a tiered approach to harvesting. This approach would allow harvesting of individual organs from whole scallops containing above 20 µg domoic acid/g tissue. The individual organs could be marketed only if they contain less than or equal to 20 µg domoic acid/g tissue.

19. The accumulation of domoic acid in shellfish is unpredictable, as very little is known about the environmental conditions that trigger phytoplankton blooms and the consequent production of domoic acid. There is also considerable inter-scallop and inter-organ variability in concentrations of domoic acid. Additionally, cross-contamination can occur during processing. We have paid particular attention to these factors in considering the public health implications of ASP.

20. In order to ensure adequate protection of public health we advise that shellfish and shellfish parts at point of sale should not exceed the current action limit. Therefore, we recommend that rigorous monitoring and enforcement at point of sale is incorporated into a tiered approach, if introduced. This is essential to account for the:

    i) unpredictable nature of domoic acid contamination of shellfish,

ii) considerable variability in the inter-scallop and inter-organ concentrations of domoic acid,

iii) possibility of cross-contamination during processing,

iv) pragmatic nature of the action limit,

v) risk of irreversible neurotoxicity.

Conclusions

21. We *consider* there are important and severe public health implications of ASP due to the irreversible neurotoxicity of domoic acid.

22. We have reviewed the toxicological data on domoic acid but *consider* these insufficient to establish a NOAEL that is appropriate for regulatory purposes. This is a reflection of the paucity of these data rather than the absence of harm. We *suggest* that if a TDI is to be established further toxicological studies using appropriate animal models are required.

23. In view of the small margin of safety between the current action limit of 20 µg domoic acid/g tissue and the concentration of domoic acid resulting in human illness we *consider* this limit as a pragmatic guideline and not a toxicologically based safety limit. We *advise* that shellfish at point of sale should not exceed the current action limit.

24. We *strongly recommend* that if a tiered approach is introduced it will require rigorous monitoring at point of sale and enforcement to ensure protection of public health.

25. We *consider* that more information on shellfish consumption is required.

26. We *note* that to date, there have been no reports of ASP in the UK and therefore, the current action limit may protect against major outbreaks. However, we *recognise* that in general, food poisoning incidents are under reported.

November 2001

COT statement 2001/08

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STATEMENT ON A SURVEY OF BISPHENOLS IN CANNED FOODS

Introduction

1. We have been asked to comment on the health implications of the results of a survey[1] conducted by the Food Standards Agency (FSA) to establish whether migration of bisphenol A (BPA) and bisphenol F (BPF) occurs from can coatings into retail samples of canned foods in the UK.

Background

2. BPA is a component of epoxy resins used in the manufacture of internal coatings for some food cans. BPA is also used in PVC coatings on the inside of some cans and in polycarbonate plastics used in consumer products. BPF is also used to make epoxy resins, but these resins are rarely used in food contact materials.

3. We note that the Government had previously commissioned a research project to develop robust methods of testing for migration of BPA from can coatings[2].

4. In view of the potential for migration from packaging, the EC's Scientific Committee on Food (SCF) recommended[3] a Tolerable Daily Intake (TDI) of 0.05 milligram/kilogram (mg/kg) body weight (bw) per day for BPA in the context of its uses in food contact plastics. The TDI for BPA was set in 1986 on the basis of long-term animal studies before the potential endocrine modulating activity of this compound was proposed. This TDI was converted into a specific migration limit of 3 mg/kg food in EC Directive 90/128/EEC (Plastic materials and articles intended to come into contact with foodstuffs). We note that the SCF is due to review the TDI for BPA in the near future.

Results of the Survey

5. Sixty-two samples of canned foods or drink (vegetables, beverages, fish in aqueous media, soup, desserts, fruit, pasta, meat products and non-reconstituted infant formulae) were obtained from retail outlets. The distribution of types of samples tested in the survey was similar across the UK. Collection of samples was approximately 80 per cent from supermarkets and included about 40 per cent 'own brand' foods to approximate consumer shopping habits. The cans were stored sealed at room temperature and then the contents were homogenised, solvent-extracted, filtered and analysed by GC-MS for BPA and BPF. The analytical methodology was of acceptable sensitivity and precision (limit of detection: 0.002 mg/kg; limit of quantification: 0.007 mg/kg).

6. No BPF was detected in any of the samples. In contrast, BPA was detected at up to 0.07 mg/kg in 37 samples and at 0.35 to 0.42 mg/kg in three cans of one sample. BPA was not detected in the four samples of infant formulae. We note that the BPA content of the contaminated samples was probably due to migration of residual monomer in the can coating, except for one sample with a higher level of contamination where BPA was used to cross-link the coating. We have been informed that the manufacturer has already replaced the coating system that resulted in the high level of contamination by a system that is more compliant with minimising potential BPA migration.

7. We were provided with estimates of BPA intake for adults and infants, which were based upon the mean contaminant level and high level consumption of the canned

foods in this survey (1.051 kg for adults and 0.375 kg for infants). Intake was estimated at 0.00036 to 0.00038 mg/kg body bw/day for adults (assuming lower and upper bound mean contaminant levels of 0.0207 mg/kg and 0.0217 mg/kg respectively for all foods except baby foods, and a body weight of 60 kg). Intake was estimated at 0.00083 to 0.00087 mg/kg bw/day for infants (assuming lower and upper bound mean contaminant levels of 0.0194 mg/kg and 0.0204 mg/kg respectively for all foods and a body weight of 8.8 kg).

Toxicological considerations

8. We are aware that the potential endocrine modulating activity of some man-made and naturally occurring chemicals, including BPA, continues to be investigated by many regulatory bodies and research groups world-wide. We have therefore paid particular attention to this activity in considering the health implications of intake of BPA from canned foods.

9. In 1997 this Committee considered[4] the health implications of a US study[5] on low-dose endocrine-modulating effects of BPA which had been widely quoted in the media. At that time, we did not consider that it would be justified to draw any conclusions about the health implications of human exposure to BPA based on these results. We also gave consideration to a compilation of toxicity studies, some unpublished, put together by the American Society of Plastics Industry which included reproductive toxicity (fertility), and developmental studies of BPA. We noted that effects were demonstrated on reproductive parameters in rats and mice, but only at very high doses. These were conventional toxicity studies demonstrating a no-effect level of 50 mg/kg bw/day, a value 25000 times higher than the low dose reported to increase prostate weight in the study of Nagel and co-workers mentioned above[5].

10. We note that an extensive review of BPA is in progress under the Existing Substances Regulations (ESR) (793/93/EEC). We were presented with a draft ESR report[6] which provided an up-to-date review of the large body of toxicological data on BPA, including several recent studies (some unpublished) designed to investigate the potential for endocrine modulating activity and other effects on the reproductive system. We considered the draft ESR report to be a valuable source of information in respect of our deliberations on BPA.

11. We note that BPA has been shown to have endocrine modulating activity in a number of *in vitro* and *in vivo* screening assays, with a potency generally ranging from 3 to 5 orders of magnitude less than that of the natural hormone oestradiol. The data indicate that there is a marked strain difference in the response to BPA in both rats and mice.

12. In developmental toxicity studies of orthodox regulatory design, using rats and mice, BPA did not induce structural malformations (including of the male and female reproductive organs), even at maternally toxic doses of greater than 500 mg/kg/day[7,8]. In a multigeneration study, reduced fertility was detected in rats exposed to 500 mg BPA/kg/day, but not at lower doses (0.001 to 50 mg /kg/day)[9]. Using a continuous breeding protocol, reduced fertility was also detected in mice exposed to 600 mg BPA/kg/day, and a decrease in epididymal weight in F1 males was observed at 300 mg/kg/day[10].

13. In contrast to the effects on rat and mouse fertility that were induced by exposure to BPA at 500mg/kg/day, effects on the development of reproductive organs have been

reported following BPA exposure at 100,000 to 1000 fold lower doses. Two research groups have reported increases in prostate weight and in anogenital distance, and a reduction in epididymal weight in the male offspring of mice exposed to 0.002 to 0.050 mg BPA/kg/day[5,11,12]. Other researchers, using larger numbers of animals, have attempted to replicate these studies, but have not observed any effects of BPA within this dose range[13,14]. It has been suggested that mouse strain differences might explain this discrepancy, but there are no data to support this assertion. Precocious vaginal opening has been observed in immature mice treated with 0.1 mg BPA/kg/day[15].

14. There is much current debate over the suggestion that some relationships between dose and toxic response may not follow a conventional upward trend, that is, there could be U-shaped response curves. The reported effects of BPA on reproductive organ development have not been shown to follow such a relationship, but have been termed "low-dose" because they occur well below the no-effect levels that have been observed in orthodox toxicity testing.

15. The Committee acknowledges the reports of apparent low-dose effects of BPA, and is aware that the numerical data from these studies have been independently validated[16]. The Committee considers that it is not appropriate, at this time, to base human health risk assessment on these reports, for several reasons. Firstly, it is not known whether the reported effects are transient or irreversible. Secondly, the functional significance of the reported effects is unknown, and this should be seen in the context of functional studies that observe effects only at very much higher doses of BPA. Thirdly, the observations of low-dose effects of BPA are not robust, in that they cannot be independently reproduced in other competent laboratories. Fourthly, other chemicals that can induce subtle effects on the developing reproductive organs inevitably cause more gross defects at higher doses, whereas BPA shows no such gross developmental toxicity.

Conclusions

16. We *note* that the FSA survey demonstrated that BPA migrates from can coatings into food at low levels. BPF was not found to migrate at detectable levels. From the results of this survey the intake of BPA by adults and infants was in the region of two orders of magnitude below the TDI established by the SCF in 1986, set before the potential endocrine modulating activity of this substance was proposed.

17. We *note* that BPA has shown endocrine-modulating activity in *in vitro* and *in vivo* screens. We *acknowledge* the reports of apparent low-dose effects of BPA. However in view of the uncertainty relating to the functional significance of the effects, as well as the lack of reproducibility, we considered that it is not appropriate, at this time, to base human health risk assessment on these reports.

18. The most recent multi-generation studies on BPA demonstrated effects on fertility in rats at high doses, and support earlier observations of a no observable adverse effect level (NOAEL) of 50 mg/kg bw/day. We *consider* that there is a substantial margin of safety (about 100,000 fold) between human exposure to BPA via canned food and doses that cause effects on fertility in animal studies.

19. We *acknowledge* the uncertainties that exist in the scientific understanding of potential endocrine effects of BPA. Nevertheless, on present evidence we *conclude* that the levels of BPA identified in canned foods analysed in the FSA survey are unlikely to be of concern to health, and that there is no reason for consumers to change their source

of foodstuffs as a result of these findings. It would be prudent to review this toxicological advice as further scientific evidence on possible low-dose effects of endocrine modulating substances unfolds.

April 2001

COT statement 2001/02

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STATEMENT ON A STUDY BY THE SMALL AREA HEALTH STATISTICS UNIT (SAHSU) ON HEALTH OUTCOMES IN POPULATIONS LIVING AROUND LANDFILL SITES

Introduction

1. In 1998, we were asked by the Department of Health to comment on the findings of an epidemiological study called the EUROHAZCON study [1]. This was a case-control study, which investigated the incidence of congenital anomalies (birth defects) around 21 landfill sites in Europe, ten of which were in the UK. The combined results from the 21 sites suggested that women who lived within 3 kilometres (km) of a landfill site were more likely to have a malformed foetus than women living further away from the site. We commented that the EUROHAZCON study was well conducted, but agreed with the authors that "there is a need for further investigation of whether the association of raised risk of congenital anomaly and residence near landfill sites is a causal one"[2]. At the time, we were informed that, in response to concerns raised about the findings of this study, Government Departments had commissioned a national epidemiological study of adverse health effects in relation to landfill sites in the UK from the Small Area Health Statistics Unit (SAHSU) at Imperial College and a preliminary protocol of that study was made available.

2. In November 2000 we addressed this topic again when we were asked to advise on a revised protocol for the SAHSU study. We were told that SAHSU had been provided with data on the location of landfill sites for use in this study which indicated that there was a total of 19,196 known, open or closed landfill sites in Great Britain and that most of the population lived within a few kilometres of a site. In order to accommodate this unexpectedly large number of sites, SAHSU had revised the study protocol so that health outcomes in the population living in postcodes within 2 km of a site would be compared with those in the rest of the population. A recent WHO workshop had suggested that any potential exposure from landfill sites was likely to be limited to 1 km from the site by the air pathway and 2 km by the water pathway[3]. The distance of 2 km proximity to a landfill site chosen by SAHSU to define the study population was therefore consistent with the recommendations of the WHO workshop. SAHSU estimated that 80% of the population of Great Britain lived within 2 km of a landfill site. Therefore, the study would be unusual in that the study population would be significantly larger than the reference population. We recommended that the study should continue but noted that care would be needed in the interpretation of the results.

3. The SAHSU study has now been completed and we have been asked to comment on the study and its results.

Study design and conduct

4. It is widely recognised that there are limitations in ecological studies of this kind. These types of study can only explore whether there is an association between the health outcomes analysed and the environmental hazard under investigation. They cannot say whether or not any reported association is causal. There is no assessment of whether the study population is actually subject to harmful exposures or if it is subject to different exposures than the reference population. It is assumed that those living near to the hazard are "exposed" and those living further away are "unexposed".

Ideally, a geographical study would incorporate a "dose-response" analysis by calculating relative risks in populations living within different distance bands from the hazard under investigation. It is unfortunate that this was not possible in the present study – SAHSU informed us that further subdivisions of distance less than 2 km from the site would not be meaningful in view of the uncertainties in the geographical data both on landfill sites and on postcode locations.

5. A further limitation of ecological studies is that they can only make adjustments for variables such as socio-economic deprivation on a group level and not on an individual level. In the SAHSU study, in common with other studies, socio-economic deprivation was adjusted for using a postcode-based index, the Carstairs index, which is formed by grouping several socio-economic variables derived from census data, which may differ at the individual level. Ecological studies cannot adjust explicitly for confounders such as family history of disease, lifestyle factors such as smoking, use of medicines and occupation, which might themselves be associated with the health outcomes being studied and which are unlikely to be completely accounted for by adjusting for deprivation alone. Therefore, there is a possibility of residual confounding. In addition, this particular study may have had problems of data quality. We are informed that the landfills data, although thoroughly checked for consistency by SAHSU, may be subject to inaccuracies or omissions eg regarding the exact location of sites or the types of waste deposited. There are also limitations in some of the health statistics data sets used in the study. For example, the congenital anomalies register for England and Wales is known to be incomplete, although we note that SAHSU also used hospital admissions data to provide an independent source of data for some anomalies.

6. Nevertheless, while recognising these limitations, we consider the SAHSU study to have been well conducted and we welcome it as a useful addition to the literature. It has the advantage of being much larger than previous studies and is population based, thereby ensuring that the study and reference populations were classified on the basis of proximity to all known landfill sites, rather than a few selected sites.

Study results

Birth outcomes

7. The study analysed data on several congenital anomalies and the prevalence of stillbirths and low and very low birthweights. The adjusted rates of these health outcomes in the study population, living within 2 km of a landfill site open during the study period, were compared with standard rates, obtained from model predictions of data from the reference population. We note that the area within 2 km of a landfill site tended to be more urban and more deprived than that beyond 2 km, with 34% versus 23% of the population in the most deprived tertile of the Carstairs score. We note also that there were differences between the two populations in terms of maternal age and ethnicity. All of these differences may influence the rates of outcomes reported, and, as noted above, adjustment for these may have been incomplete.

8. The study found that the risk ratio* for congenital anomalies overall was 1.01 (ie the adjusted rate was 1% higher in the study population than in the reference population).

* The risk ratio is the ratio of the rate in the study population, adjusted for deprivation and other factors, to the standard rate, obtained from model predictions of data from the reference population.

When individual congenital anomalies were considered, the adjusted risk ratio for neural tube defects was 1.05 but for cardiovascular defects, in contrast, it was 0.96 (ie the adjusted rate was 4% lower in the study population than in the reference population). The congenital anomalies register data indicated a risk ratio of 1.07 for hypospadias and epispadias, but this finding was not supported by hospital admissions data, for which the adjusted risk ratio was 0.96. For abdominal wall defects, the congenital anomalies register data indicated a risk ratio of 1.08 and the hospital admissions data yielded a similar estimate. The risk ratio for hospital admissions for surgical correction of gastroschisis and exomphalos was 1.19.

9. Adjusted rates for stillbirths were the same in the study and reference populations. For low birthweight and very low birthweight, risk ratios were 1.05 and 1.04 respectively.

10. We note that the risk ratios were all close to unity, and were much smaller than the estimated relative risks reported in the EUROHAZCON study. We note also that a "before and after" sub-analysis of sites which opened during the study period indicated that the risk ratio for congenital anomalies did not increase after the landfill sites opened and, indeed, that the ratios for abdominal wall defects appeared to decrease substantially. The sub-analysis was based on smaller numbers than the primary analysis but, coupled with the difficulties in interpretation of the study which we have outlined above, indicates that it is inappropriate to draw firm conclusions on the possible health effects of landfill sites from the results of this study.

11. The "before and after" analysis found higher risk ratios for low birth weight and very low birth weight after opening. We consider that there is a possibility that residual socio-economic confounding might account, at least in part, for the differences in these two parameters between the study and reference populations.

12. Another sub-analysis focussed on special waste landfill sites. We note that this analysis also was based on smaller numbers than the primary analysis and that a higher proportion of the population living around these sites was in the most deprived tertile of Carstairs score (36%) than in the main study population. We are informed that special waste sites are, in practice, co-disposal sites which take predominantly the same type of waste as most landfill sites, but are also licensed to take certain specified types of special waste. Without good information on emissions and controls at these and other landfill sites, it is not possible to assess whether special landfill sites may present a greater potential risk to health than other landfill sites. In this sub-analysis, the risk ratio for congenital anomalies overall was 1.07, and this may be compared to the risk ratio of 1.01 in the main analysis. The risk ratios for neural tube defects, and for low birth weight and very low birth weight, showed similar elevations to those found in the main analysis. The risk ratios for abdominal wall defects, and for hospital admissions for surgical correction of gastroschisis and exomphalos were lower than for the main analysis. For cardiovascular defects, the risk ratio was 1.11, whereas it was less than one in the main analysis. The congenital anomalies register data for hypospadias and epispadias indicated a risk ratio of 1.11 but, as in the main analysis, this finding was not supported by the hospital admissions data. The differences remain lower than those found in the EUROHAZCON study. The lack of consistency in the findings for all landfill sites and for special landfill sites further complicates any attempt to draw firm conclusions from this study about the possible health effects of landfill sites. Nevertheless, the finding of a risk ratio of 1.07 for congenital anomalies

overall for populations living around special waste landfill sites, whether or not it is related to the presence of the landfill sites, merits further investigation.

Cancer outcomes

13. The study analysed the incidence of childhood and adult leukaemias, hepatobiliary cancers, and cancers of bladder and brain. We understand that these endpoints were selected either to test hypotheses arising from the previous studies or on the basis of the known human carcinogenicity of certain chemicals known to be present in landfill sites. An additional uncertainty in this analysis was the use of short lag periods to allow for the latency period between initiation of cancer and the time of diagnosis/registration. SAHSU used a lag period of one year for childhood leukaemia and five years for the other cancer outcomes. We accept that this was a pragmatic approach, taken in order to increase the number of years of data available for analysis and to reduce the potential for dilution by migration. If, however, the latency period is longer, this index of potential exposure may be inappropriate, leading to dilution of any potential effect. However, taking this and other limitations of the study design into account, we consider that the finding of no excess risk for those living within 2 km of a landfill site for each of the cancer types studied provides a degree of reassurance.

Further work

14. We were informed that a programme of research and reviews is underway on congenital anomalies and landfill sites, and that this includes a project to measure emissions from landfill sites and to assess exposures of people living nearby. We welcome this as important information that was lacking from the present study and we have considered whether there is other work that could usefully be undertaken to address this issue. We consider that further exploration of the possibility of residual confounding in the SAHSU study would be a legitimate area of future research. Further, although this study found at most only small differences in adjusted rates of some birth outcomes between the study and reference populations, when considering landfill sites in general it remains possible that there are individual sites (or some subset of sites) which significantly affect the health of the local population. SAHSU has proposed that this could be investigated further by detailed mapping and statistical analysis of the existing data to provide an indication of any systematic variation in rates and to analyse any resulting variations in relation to possible explanatory variables (eg landfill characteristics, geology, other exposure sources, deprivation). We agree that this could be a useful way forward but note that the value of further analyses of the existing datasets may be limited by the known problems of some of these datasets.

15. An alternative approach to an ecological design would be to carry out case-control or cohort studies and to obtain estimates of exposure for individual study subjects, together with data on all relevant confounding factors and effect modifiers. Future studies of this type would greatly benefit from the development of accurate and specific marker(s) of exposure to chemicals released from landfill sites.

August 2001

COT statement 2001/04

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STATEMENT ON POLYCYCLIC AROMATIC HYDROCARBONS – INTERIM PRAGMATIC GUIDELINE LIMITS FOR USE IN EMERGENCIES

Introduction

1. The Committee was informed that the Food Standards Agency may be asked for advice on the risks to public health arising from the contamination of foodstuffs with Polycyclic Aromatic Hydrocarbons (PAHs). Our views were sought on the possibility of establishing pragmatic guideline limits for concentrations of PAHs in foodstuffs that could be used in emergency situations, where the available data are inadequate to support a full evaluation of health risks.

Background

2. The PAHs are a group of highly lipophilic chemicals that are present widely in the environment as pollutants. The principal sources of PAHs in the atmosphere are combusted fossil fuels, burnt refuse and coke ovens. Vehicle emissions are also a major source of PAHs. Humans are exposed to a mixture of PAHs from air, food and drinking water, as well as from tobacco smoke.

3. We have been informed that, although the PAHs are widespread environmental contaminants, excessive contamination of crops with PAHs can occur during incidents such as factory fires. In addition, shellfish can accumulate PAHs from oils spilt by grounded tankers or from waste oils which have been incorrectly disposed of. Furthermore, the improper processing of natural oils, or their adulteration with fossil fuels, can result in contamination with PAHs. The priority in such cases is to determine whether any food has been contaminated to an unacceptable level. Where localised contamination is thought to have occurred, analytical data for suspect food produce should be compared with data on similar produce gathered from comparable areas considered to be unaffected by the incident. In the absence of such data, the use of a pragmatic guideline limit would support the establishment of a risk management strategy, that would take into account additional information, including possible concentrations of PAHs in food as consumed and patterns of consumption.

4. Some members of this class of chemicals are carcinogens in experimental animals and it is generally accepted that occupational exposures to mixtures of PAHs are associated with human cancer. Given that these compounds exert their carcinogenic activity by a genotoxic mechanism, it is not possible to define a level of intake that is without possible risk, nor to set a Tolerable Daily Intake.

5. In 1996, our sister committees, the Committee on Carcinogenicity (COC) and the Committee on Mutagenicity (COM), were asked for advice by the Department of Environment and the Ministry of Agriculture, Fisheries and Food on evaluation of the individual PAH compounds, so that priority and representative compounds could be identified for monitoring and/or surveillance. The COC 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, namely: benz(a)anthracene (BaA), benzo(a)pyrene (BaP) and dibenz(a,h)anthracene (DBahA)[1].

Exposure to PAHs in the diet

6. PAHs are known to occur in foodstuffs as a result of environmental contamination and as a result of food processing and cooking (particularly smoke-curing and barbecuing). We have been provided with data on the concentrations of individual PAHs that have been detected in a number of foodstuffs[2].

7. A range of background concentrations of the individual PAHs of concern has been reported in uncooked meat, dairy products, fish, vegetables, fruits, cereals, and animal and vegetable oils, and these are usually less than 5 µg/kg. Background concentrations vary considerably, depending upon the location from which they are obtained. Data from the United Kingdom on the concentrations of the PAHs of concern in various unsmoked, uncooked foodstuffs are provided in Table 1.

8. In comparison, higher concentrations may occur in grilled and barbecued meats: reported levels of BaA, BaP and DBahA range from undetectable to 144, 212 and 8.8 µg/kg, respectively[2].

Table 1: Ranges of concentrations of PAH compounds of concern in United Kingdom uncooked foodstuffs (µg/kg fresh weight) *

Foodstuff	PAH compound	Reported range
Meat and meat products	Benz(a)anthracene	0.02 – 0.13
	Benzo(a)pyrene	0.01 – 0.26
	Dibenz(a,h)anthracene	0.01
Fish and fish products	Benz(a)anthracene	Trace – 0.14
	Benzo(a)pyrene	Trace – 0.35
	Dibenz(a,h)anthracene	0.03
Vegetables and cereals	Benz(a)anthracene	0.05 – 3.17
	Benzo(a)pyrene	ND – 1.42
	Dibenz(a,h)anthracene	0.01 – 0.05

* From data collated in reference 2

ND : Not detected

Derivation of pragmatic guideline limit

9. We *consider* that it would be reasonable to use guideline limits that reflect overall background contamination of uncooked staple foodstuffs with PAHs. Allowing for the variation and uncertainty in the reported levels, we recommend guideline limit values of 15 µg/kg for any one of the three PAHs of concern. This concentration is above the expected range of up to 5 µg/kg as found in most uncooked foods, and takes into account the range found in grilled or barbecued foods.

Conclusions

10. We *recognise* that some individuals may have increased exposure to PAHs arising from consumption of foods cooked in a manner (e.g. grilled or barbecued) that results in the food containing increased concentrations of these compounds.

11. We *consider* that, in emergency situations, and in the absence of data on background levels for comparison, it may be appropriate for risk management strategies to include use of pragmatic guideline limits. Such pragmatic guideline limits could assist in identifying produce containing PAHs at concentrations that might constitute a small but unquantifiable added risk to health.

12. We *consider* that overall background contamination by PAHs of uncooked staple foods that constitute a significant proportion of the diet provides a reasonable basis for proposing pragmatic guideline limits. For such limits we recommend values above the normal range of background contamination, but below the upper range that may occur in grilled or barbecued foods. Therefore we *recommend* a pragmatic guideline limit of 15 µg/kg in foodstuff for any one of the three individual PAH compounds of greatest concern, i.e. benzo(a)pyrene, benz(a)anthracene or dibenz(a,h)anthracene.

13. We recommend that where adequate background data exist which demonstrate that the levels in foodstuffs do not result from the emergency under consideration, the pragmatic guideline limits should not be used. .

14. We *stress* that these pragmatic guideline limits should not be applied other than in emergencies and that efforts should be made to reduce the background contamination of foodstuffs by PAHs.

15. Finally, we *stress* that this is interim advice based upon sparse data, which are insufficient to provide the sound basis normally used for COT decisions. There is a need for further information on dietary intakes of PAHs resulting from specific contamination incidents, which may help to support risk assessment. Therefore the limits described in this statement are considered to provide an "**interim pragmatic guideline** " only.

March 2001

COT statement 2001/01

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STATEMENT ON THE REPRODUCTIVE EFFECTS OF CAFFEINE

Introduction

1. A number of human epidemiological studies, reporting increased risks of adverse reproductive effects with caffeine consumption during pregnancy, have been published since this Committee last considered the safety of caffeine in 1984. Several important studies have also been published since the most recent consideration by the European Scientific Committee on Food (SCF) in 1999. In the light of this increasing body of human data, the Committee was asked to review the evidence for possible reproductive effects of caffeine. We have considered a large number of both animal and human studies, including recent epidemiological data.

Background

Intake

2. Caffeine is present in a wide range of foods and beverages including coffee, tea, cocoa, chocolate, colas and energy drinks. It is also present in a number of prescription and over-the-counter medicines including cold and flu remedies, headache treatments, diet pills, diuretics and stimulants. While over-the-counter medications are labelled that they should not be taken during pregnancy without consulting a GP, most foods and beverages contain no such labelling (with the exception of some energy drinks). However, most pregnant women are likely to consume caffeine from one or more of these sources.

3. Table 1 indicates typical caffeine contents of a range of foods and beverages. Values for energy drinks, chocolate, drinking chocolate and cola are taken from a 1998 survey. Typical values for coffee and tea are quoted from the literature, as the caffeine contents of these beverages vary greatly, depending on brewing method, brand and preferred strength. The caffeine intake will also depend on the volume consumed at a single serving and the values cited in Table 1 assume a standard cup size of 190ml.

Table 1: Caffeine contents of some commonly consumed beverages and food

Source	**Typical caffeine content (mg) per serving**
Instant coffee	Approximately 75 mg per 190 ml cup[1]
Brewed coffee (filter or percolated)	Approximately 100-115 mg per 190 ml cup[1]
Decaffeinated coffee (brewed or instant)	Approximately 4 mg per 190 ml cup[1]
Tea	Approximately 50 mg per 190 ml cup[1]
Drinking chocolate	1.1 – 8.2 mg when made up as per manufacturers instructions in 200 ml water[2]
Energy drinks (containing either added caffeine or guarana)	28 – 87 mg per 250 ml serving[2]
Cola (regular and diet)	11 – 70 mg per 330 ml serving[2]
Chocolate	5.5 – 35.5 mg per 50 g bar[2]

4. The most recent data on caffeine consumption in the UK are provided by a 1988 survey of consumption of coffee, tea and colas. The mean caffeine intake from these sources was estimated to be 3.98 mg/kg body weight per day (i.e. 239 mg/day for a 60 kg person) for the general population and 3.43 mg/kg body weight per day (i.e. 206 mg/day for a 60 kg person) for pregnant women[3]. If an average content of 75 mg per 190 ml cup of instant coffee is assumed, these intakes correspond to approximately 3 cups per day.

Previous considerations

5. The Committee last considered the safety of caffeine (present as an additive and naturally occurring) in 1980-1984[4]. At that time, we noted that renal excretion of intact caffeine was very slow, so the rate of elimination of caffeine was dependent on the rate at which it is metabolised. The rate of elimination of caffeine was markedly decreased during pregnancy, the half-life increasing from 4-6 hours in the non-pregnant adult to 18 hours in late pregnancy. As a result, serum caffeine levels may be much higher in pregnant women than in non-pregnant adults for an equal intake. We also noted that caffeine freely crosses the placenta and that plasma caffeine concentrations in the neonate are similar to maternal plasma concentrations. For these reasons particular attention was paid to the potential effects of caffeine in pregnancy and on the neonate.

6. In our previous discussions, we considered a number of animal studies on caffeine and effects on reproduction and several epidemiological studies. However, at that time human data on caffeine intake were limited and were considered inadequate to draw any clear conclusions. The studies had failed to take into account potential confounding factors such as alcohol intake and smoking, which are known to affect pregnancy outcome, and data on caffeine intake were often lacking. We were satisfied that the available human data did not suggest any increased risk of congenital malformations as a result of caffeine intake during pregnancy, but there remained some doubt about possible effects of coffee on human birth weight.

7. The SCF considered the safety of caffeine as part of its review on ingredients in so-called “energy drinks” in January 1999[5]. Regarding risk to pregnancy, it noted that contradictory results have been reported in human studies. It concluded that maternal caffeine consumption during pregnancy did not appear to have any measurable adverse consequences for the human fetus at intakes up to 300 mg/day, but that the question of possible effects on pregnancy and the offspring at regular intakes above this level remained open.

Consideration of possible adverse effects on reproduction

Toxicokinetics

8. We note that smoking approximately doubles the rate at which caffeine is metabolised[6]. We also note that there is evidence of a trend between cigarette smoking and caffeine consumption, with smokers consuming more caffeine on average than non-smokers. There is evidence to suggest that while it is assumed that enzyme activities in those who give up smoking quickly return to the levels of non-smokers, it takes up to 6 months for there to be a significant reduction in caffeine intake. As a result, serum caffeine levels may be increased by up to 2½ times previous levels for

several months[6]. We also note that caffeine elimination is very slow in infants up to 3 months of age, due to lower rates of metabolism.

9. We note that the rate of caffeine elimination varies between individuals. Some of this variation may be due to polymorphisms in genes encoding enzymes involved in the metabolism of caffeine, in particular the cytochrome P450 enzymes, CYP1A1 and CYP1A2, that are involved in caffeine demethylation. However, although different rates of expression of these enzymes have been observed, the contribution of genetic polymorphisms is unclear. Lifestyle factors, such as smoking and regular taking of drugs that induce or inhibit the enzymes involved in caffeine metabolism may be more significant than genetic factors to inter-individual variability in caffeine elimination.

Animal studies

10. The potential reproductive effects of caffeine have been studied in a wide range of species and strains of animal. Studies in rats and mice have shown increased rates of fetal malformations, such as cleft palate and ectrodactyly (absence of digits) when single parenteral caffeine doses of 50 mg/kg body weight or higher have been given between the 9th and 14th days of gestation. No increased rates of malformation have been observed at single doses below 50 mg/kg body weight in either rats or mice.

11. Repeat doses of 12.5 mg/kg body weight caffeine per day and higher, given by oral gavage to rats throughout pregnancy, have been associated with significantly decreased birth weights. 7 As effects have been observed at all dose levels studied it has not been possible to determine a No Observed Adverse Effect Level (NOAEL). It is not possible to determine whether these effects are secondary to decreased maternal body weight gain, which has also been seen in some studies.

12. Studies in the Cynomolgus monkey, Macaca fascicularis, have shown a high rate of stillbirths and miscarriage with maternal caffeine intakes of 10-15 mg/kg body weight per day given via the drinking water 8. This is equivalent to a 60kg woman consuming 8 to 12 average cups of coffee per day (assuming 75 mg caffeine/cup). We note that the main serum metabolite of caffeine in monkeys is theophylline, whereas in humans it is paraxanthine and that information on the comparative toxicities of these metabolites is not available.

Human data

13. We have considered a number of epidemiological studies of maternal caffeine consumption and risk of spontaneous abortion or low birth weight. These cohort and case-control studies assessed caffeine intake at various stages of pregnancy by the use of dietary questionnaires. Caffeine intakes were assessed by multiplying the number of servings of a beverage or food by an estimated mean caffeine concentration. Different studies assume different caffeine contents of beverages and this accounts for some of the variation in the levels of caffeine intake associated with adverse effects on reproduction in different studies. One study used serum paraxanthine levels as a marker for caffeine intake[9] after previously showing that serum paraxanthine levels were closely correlated to caffeine intakes reported by dietary questionnaire[10].

14. A number of studies have reported significantly increased risks of spontaneous abortion with caffeine intakes greater than 300 mg/day11-16 and some studies have shown increased risks at lower intakes, ranging from about 150 mg/day and above[14,16]. In these studies, caffeine intakes were commonly estimated from assessments of

coffee, tea and cola consumption. One study used coffee consumption only[16]. Other sources of caffeine, including chocolate, drinking chocolate, chocolate syrup, chocolate brownies and caffeine-containing medications were additionally used in other studies[11,13]. Several studies considered more than two levels of intake and demonstrated dose-response relationships, i.e. the greater the reported caffeine intake during pregnancy, the greater the odds ratio for spontaneous abortion[11,15,16]. The studies used different populations and different categories of caffeine intake and the dose-response curves do not superimpose. It is therefore not possible to identify a threshold above which the increased risk becomes significant. Not all studies of caffeine intakes up to about 300 mg/day have shown an association[17,18]. Where an association was found it is not possible to establish whether the association was causal or whether it could be due to an unknown factor or because confounding for social factors was only partially removed by adjustment.

15. It has been suggested that caffeine intake decreases more during pregnancy in women who have symptoms of nausea than in women who do not. There is evidence that women who have symptoms of nausea are less likely to miscarry than women who do not. If women without nausea were to consume more caffeine, then absence of nausea could be related to the underlying cause of their apparent risk, without the higher caffeine intake being necessarily causal. Several studies have attempted to take account of this theory and adjust the data accordingly, either by questioning study participants on their nausea status at various time-points throughout pregnancy or by assessing caffeine intake in the first month of pregnancy, before the onset of nausea[11,13,19,20]. A case-control study, investigating the relationship between maternal caffeine intake, nausea and spontaneous abortion, showed no association between caffeine consumption and risk of spontaneous abortion in women who did not report nausea. However, the study did show a significant association between caffeine consumption of 300 mg/day and above and spontaneous abortion in women who did report nausea (odds ratio = 5.4, 95% confidence interval 2.0-14.6) [13]. This would suggest that the associations between maternal caffeine intake and spontaneous abortion cannot be entirely accounted for by the relationship between nausea and the viability of the pregnancy, although an association was only shown at caffeine intakes of >300 mg/day.

16. Several studies have shown an association between maternal caffeine intakes greater than 300 mg/day during pregnancy and low birth weight [21-24]. Two of these studies also showed an association at lower intakes, ranging from estimated intakes of about 70 to 150 mg/day and above[23,24]. Assessments of coffee, tea and cola consumption were used to estimate caffeine intake[21-24]; one study also took caffeine-containing drugs into account[23]. Again, dose-response relationships were demonstrated[21,22], although it is not possible to establish a level of intake above which the increase becomes significant as the levels of intake associated with low birth weight differ between studies. Not all studies have shown an association between caffeine intake and low birth weight at caffeine intakes above 300 mg/day[26-28]. Again, it is not possible to establish whether the associations shown in a number of the studies are causal or whether they could be due to other risk factors or because confounding for social factors was only partially removed.

17. We also considered a meta-analysis of studies of maternal caffeine intake during pregnancy and risk of spontaneous abortion or low birth weight, which compared maternal caffeine intakes during pregnancy of more than 150 mg/day with less than 150 mg/day[25]. Calculated odds ratios were significantly increased for spontaneous

abortion (odds ratio = 1.36; 95% confidence interval, 1.29-1.45) and low birth weight (odds ratio = 1.51; 95% confidence interval, 1.39-1.63), defined as a birth weight of less than 2,500 g. However, although the odds ratios in the individual studies were adjusted for confounding factors, the meta-analysis used unadjusted data only. It was noted that adjustment of individual studies for confounding factors significantly decreased the odds ratios for spontaneous abortion and low birth weight calculated by these studies.

18. Coffee was the main source of caffeine in most studies and we cannot exclude the possibility that the effects seen in these studies are due to a component of coffee other than caffeine. Several studies have compared the results for various individual sources of caffeine or have re-analysed the data excluding coffee drinkers. These have not shown a significant difference between different sources of caffeine. However, these analyses had low statistical power, as acknowledged by the authors[11,14,20].

19. Several studies have investigated maternal caffeine consumption during pregnancy and risk of pre-term birth and have not shown an association[29,30]. However, one study showed an association between consumption of more than 3 cups of coffee per day and pre-term birth as a result of premature membrane rupture[31].

20. We considered a number of studies that investigated potential effects on fertility. Studies in animals have indicated no adverse effects on male fertility at levels in excess of normal or likely human intakes. The evidence for effects on female fertility is contradictory, but human studies have not shown any effect on time to conception at caffeine intakes less than 300 mg/day[32,33].

21. Maternal caffeine intake appears to have pharmacological effects on the fetus. High maternal caffeine intakes (greater than 500 mg/day) have been shown to increase the time the fetus spends in a state of arousal during the third trimester[34]. Cardiac arrhythmias have been shown in newborn infants born to mothers with high caffeine intakes (>500 mg/day) during pregnancy[35]. These resolved within one week. An association between heart rate in newborn infants and maternal caffeine intake during pregnancy has been shown in one study[36].

22. We considered two studies investigating incidence of sudden infant death syndrome (SIDS) and maternal caffeine intake during pregnancy. One of the studies showed an association, but did not adequately measure cigarette consumption. Smoking is a known risk factor for SIDS[37]. The other study did not show an association[38]. Overall we concluded that there was no reliable evidence of an association between maternal caffeine intake during pregnancy and SIDS.

Conclusions

23. We *note* that the risk of low birth weight and spontaneous abortion increases with increasing maternal caffeine intake during pregnancy. However, a threshold level of caffeine intake, above which maternal caffeine intake presents a risk to pregnancy, cannot be determined. Different studies assume different caffeine contents of beverages and this leads to some variation in the levels of caffeine intake associated with adverse effects on reproduction in different studies. We *consider* it prudent to assume that caffeine intakes above 300 mg/day show a plausible association with low birth weight and spontaneous abortion, given the available evidence from studies in experimental animals and epidemiological studies. However, on the basis of the available evidence, it is not possible to define this association as causal. We *note* that

300 mg/day caffeine is equivalent to four cups of instant coffee or about six cups of tea, assuming average caffeine contents.

24. We *note* that for caffeine intakes of 150 to 300 mg/day there is less evidence for an association, with greater inconsistency in the results of epidemiological studies than for intakes above 300 mg/day.

25. We *note* that data on maternal caffeine consumption during pregnancy and associations with adverse effects on reproduction other than low birth weight and spontaneous abortion, such as pre-term birth and adverse effects on the fetus are inconclusive. We do not consider there to be reliable evidence for associations with these parameters at moderate consumption levels (below 300 mg/day).

26. There do not appear to be effects of caffeine consumption on male fertility. Evidence for adverse effects on female fertility is inconclusive.

27. We *note* that the studies used to establish this association focused on caffeine intake from coffee, and that a possible influence of other constituents of coffee cannot be excluded. We also recognise that coffee and tea are just two sources of caffeine and do not necessarily represent the main sources of caffeine intake for all people.

28. Further studies are required to establish whether the observed association is causal. These might include the use of biomarkers of caffeine intake.

October 2001

COT statement 2001/06

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STATEMENT ON THE TOLERABLE DAILY INTAKE FOR DIOXINS AND DIOXIN-LIKE POLYCHLORINATED BIPHENYLS

Introduction

Dioxins and polychlorinated biphenyls (PCBs)

1. Dioxins are persistent organochlorine compounds that are widely dispersed environmental contaminants and which accumulate in fatty foods. The term "dioxins" is commonly used to refer to a group of 75 polychlorinated dibenzo-*p*-dioxin (PCDD) and 135 polychlorinated dibenzofuran (PCDF) congeners, of which less than 20 are considered to be biologically active. Dioxins are produced in a number of thermal reactions, including incineration of municipal waste, domestic fires and bonfires, forest fires and internal combustion in automobile engines. They are also generated as trace contaminants during the synthesis of many organochlorine compounds (e.g. chlorophenoxy herbicides such as hexachlorophene, chlorodiphenyl ether herbicides) and during some industrial processes (e.g. bleaching of pulp and paper with chlorine gas).

2. Polychlorinated biphenyls (PCBs) are environmentally stable, lipophilic chemicals that were widely manufactured for a range of industrial applications between the 1930s and 1970s. Use of PCBs for industrial purposes has been discontinued but these substances may still be released to the environment during disposal of materials and obsolete equipment. There are 209 theoretically possible PCB congeners, of which 12 non-*ortho* or mono-*ortho* compounds exhibit similar biological activity to PCDDs and PCDFs, and are therefore referred to as "dioxin-like PCBs".

3. There is continuing public concern about the health hazards of dioxins and related compounds. These compounds are persistent in the environment and tend to accumulate in biological systems. One of the most extensively studied PCDD congeners, 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), exhibits a broad range of toxic effects in laboratory animals, some at very low doses.

4. Exposure of the general population to dioxins and dioxin-like PCBs is primarily from food. The estimated exposures from the UK Total Diet Study samples for all age groups have declined substantially. In 1982, average intakes for dioxins and dioxin-like PCBs were 7.2 and 18 pg TEQ/kg bw/day for adults and toddlers, respectively. In 1997 the averages had fallen to 1.8 and 4.6 pg TEQ/kg bw/day for adults and toddlers, respectively[1]. Over the same period, the intake estimates for high level (97.5^{th} percentile) consumers have fallen from 13 and 28 pg TEQ/kg bw/day to 3.1 and 7.2 TEQ/kg bw/day, for adults and toddlers, respectively. Dioxins and PCBs are detectable in almost all types of food. Highest concentrations are found in meat, fish, eggs and dairy products. However, cereals, fats and oils contribute significant proportions of the total dioxin and PCB intake because they are major components of the diet. The decline has been attributed in part to controls on emissions to the environment and the discontinuation of production and use of dioxin-like PCBs. It is anticipated that exposures will continue to decline with present and planned environmental controls.

5. The highest dioxin exposures in humans have generally been associated with occupational exposure or accidental contamination of the environment or edible oils. Occupational exposure studies have been undertaken at plants in the USA, Germany, the Netherlands and the UK that manufacture chlorophenols and/or chlorophenoxy

herbicides. Application of chlorophenoxy herbicides has been associated with much lower levels of exposure. Exposures to more highly chlorinated PCDDs have been estimated for workers exposed to pentachlorophenol and/or other chlorophenates at saw mills or manufacturing plants. In addition, an explosion in a chemical plant at Seveso in 1976, resulted in widespread release of TCDD to the environment and exposure of the local population. The ingestion of edible oils contaminated with high levels of polychlorinated compounds including PCBs and PCDFs was associated with toxicity in food poisoning incidents in Yusho and Yu-Cheng, which we reviewed in 1997[2].

Previous COT evaluations

6. The COT and our sister Committees on Carcinogenicity (COC) and Mutagenicity (COM) have considered dioxins and dioxin-like PCBs on several occasions in the past[1-8]. In 1989 we made a comprehensive statement about the health hazards of PCDDs and PCDFs[3]. We made a second statement in 1991 when UK exposure data on these compounds from food became available[4]. On that occasion, we endorsed the Tolerable Daily Intake (TDI) of 10 pg/kg bw/day 2,3,7,8-TCDD recommended by an expert group convened by the WHO Regional Office for Europe[9] and we recommended that, when considering mixtures of PCDDs and PCDFs, the TDI can be regarded as 10 pg/kg bw/day 2,3,7,8-TCDD Equivalents (TEQ). We further stated that, in view of the estimated long elimination half-lives of this class of compounds, it would be more appropriate to regard the TDI as a time-weighted average tolerable intake. We reviewed PCDD and PCDFs again in 1995, when we concluded that the new information available at that time did not necessitate the alteration of the previously agreed TDI[6].

7. The Toxic Equivalency Factor (TEF) approach was initially used to facilitate risk assessment of PCDDs and PCDFs (i.e. dioxins). In 1997, we tentatively accepted that the TEF approach could be extended to include the dioxin-like PCB congeners[2] and in 1998 we endorsed the revised WHO-TEFs for dioxins and dioxin-like PCBs[7].

Recent International evaluations

World Health Organisation.

8. In 1998 the WHO European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS) conducted a re-evaluation of the TDI for dioxins and dioxin-like PCBs. The Executive Summary of this report was published in a special issue of Food Additives and Contaminants [10], devoted to the 1998 WHO-ECEH/IPCS Consultation on Dioxins, allowing an evaluation of the basis on which the WHO consultation reached its conclusions.

9. The WHO consultation recommended a TDI for dioxins and dioxin-like PCBs of 1-4 pg WHO-TEQ/kg based on the NOAEL/LOAELs of those effects considered to be the most sensitive in experimental animals, namely endometriosis, developmental neurobehavioural effects, developmental reproductive effects and immunotoxicity.

Scientific Committee on Food

10. The Scientific Committee on Food (SCF) undertook a reassessment of the TDI for dioxins and dioxin-like PCBs for the European Union, adopting a temporary opinion in November 2000. This was revised in June 2001, in the light of newly published data allowing calculation of the total amount of dioxin in the fetus (the fetal body burden) associated with maternal exposure at steady state[11]. The SCF concluded that, because TCDD and related compounds have very long half-lives in the human body, the tolerable intake should be expressed on a weekly rather than a daily basis. Based on the LOAEL from a study showing developmental effects in male rat offspring following repeated subcutaneous administration of TCDD[12], the SCF established a tolerable weekly intake (TWI) of 14 pg WHO-TEQ/kg bw[13].

Joint FAO/WHO Expert Committee on Food Additives

11. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) also considered dioxins and dioxin-like PCBs in June 2001. The JECFA used a similar body burden approach to that used by the SCF and also took into account exposure from background contamination considered to be present in feed provided to laboratory animals. It proposed a provisional tolerable monthly intake (PTMI) of 70 pg WHO-TEQ/kg bw[14], based upon the lowest LOAEL and a NOAEL for developmental effects in male rat offspring[12, 15].

Environmental Protection Agency

12. The U.S. Environmental Protection Agency (EPA) commenced a reassessment of dioxin exposure and human health effects entitled, "Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds" in April 1991. In 1994, it released its initial external review draft describing health effects and exposures. In our 1995 statement [6], we welcomed the US EPA initiative to investigate further the health hazards of 2,3,7,8-TCDD and related compounds. The document provided a thorough review of the literature on the effects of these compounds on various biological systems. However, we did not consider it provided any new information or analysis that necessitated the alteration of the previously agreed TDI of 10pg TEQ/kg bw/day or of our previous advice.

13. Following public comment and advice from its Science Advisory Board, the EPA undertook an extensive revision of its review, particularly two key sections on Dose-Response Modelling and Integrated Summary and Risk Characterization[16]. These chapters were subject to public comment and further Science Advisory Board review during late 2000 and Spring 2001. The EPA's draft assessment considered that cancer was the most appropriate end-point for risk assessment and undertook an evaluation based on their risk assessment guidelines for carcinogens. The results of this evaluation are not yet available.

Evidence considered in the current evaluation.

14. In 2000, we were asked to review the risk assessments of dioxins carried out by the WHO, the SCF, and the US-EPA. We concluded that it was appropriate to conduct our own evaluation of the data, informed by these international assessments and other

relevant information, before reconsidering the TDI for dioxins and dioxin-like PCBs. As part of this evaluation it was essential that the evidence concerning human cancer risks for dioxins be evaluated to determine whether or not it is appropriate to assume the existence of a threshold and hence whether a TDI could be established. We are grateful for the assistance provided by the COC and we have taken account of its conclusions [17] in completing our evaluation.

15. In undertaking our evaluation we have had access to the published assessments of all three international evaluations. The EPA documents provided the most detailed and comprehensive review of the published literature. We have supplemented this by evaluation of the original publications identifying critical end-points and recently published data.

Mechanism(s) of action of TCDD

16. Most of the actions of TCDD and related compounds can be ascribed to the consequences of an initial binding to what has become known as the Ah or aryl hydrocarbon receptor (AHR), although this binding protein is now more properly termed a ligand activated transcription factor. This binding results in multiple changes in gene transcription leading to increases in biotransformation enzymes, modulation of cell cycling proteins and other responses. Inappropriate gene expression resulting from the high affinity binding and long term occupancy of the receptor may be the basis of the toxicity of TCDD. However, although the mechanisms of early molecular changes are well understood, the relationship between changes in gene regulation and observed toxicity is still unresolved.

17. It has become apparent that the sequence of events from TCDD binding to gene transcription involves other transcription factors, chaperones (such as HSP90) and regulatory proteins. The net result is the association of TCDD-AHR with another factor, the Ah receptor nuclear translocator (ARNT), in the nucleus followed by binding of the complex to 'dioxin' responsive regulatory elements (DREs) in enhancer regions upstream of particular genes. Downstream activation of promoter regions then occurs with production of mRNA from the genes. Most of the molecular events for transcription of the CYP1A1 gene have been elucidated. For other genes the sequence of events is far less clear but probably occurs in a similar manner [18] and the number of known AHR-regulated genes is still increasing.

18. Mechanistic studies on the role of the AHR in the toxicity of TCDD have shown that proteins similar to the AHR have been found in many organisms suggesting that this receptor has an essential biological function [19]. Sequencing studies on the AHR have shown that it is a member of a family of gene regulating proteins known as PAS (PER-ARNT-SIM) [20]. In mammals, these proteins (which include hypoxia inducible factor 1-α [HIF1α] and ARNT) regulate the transcription of specific genes. Heterodimerisation of the AHR with ARNT is apparently essential for the TCDD activated AHR to induce specific DNA binding and transactivation *in vitro*. However, heterodimerisation of ARNT can also occur with HIF1α and with a newly discovered factor AHR repressor (AHRR) [21]. TCDD/AHR might act by competing against these, or even competing against the binding of a hypothetical normal endogenous ligand of the AHR that has yet to be found. Other studies have shown that levels of the AHR, AHRR, ARNT and HIF1α may be regulated by cell type and activation and by stages of growth and differentiation[22].

19. A number of lines of evidence *in vivo* support the role of AHR in TCDD toxicity. For instance, polymorphism of the AHR, with varying affinities for TCDD, in mice correlates with variable susceptibility to toxic effects[23]. Different strains of mice that do not possess the functioning gene for the AHR (referred to as *Ahr* null or 'AHR knockout' mice) have been shown to be extremely resistant to very high doses of TCDD for a variety of toxic endpoints. Binding capabilities of dioxins and dioxin-like PCBs to the AHR, as shown by structure activity relationships, generally show similar ranked order to their elicitation of biochemical responses.

20. However, in seeking to understand the mechanisms of action in order to inform risk assessment, it might be inappropriate to place exclusive emphasis on the AHR. At very high doses of TCDD (in the *Ahr* null mouse) the chemical may have toxic actions which are not mediated by the receptor. Similarly, in some *in vitro* experiments, various effects of TCDD have been interpreted as non-AHR dependent.

21. In terms of binding to the AHR, some ligands may be competitors of TCDD-induced gene regulation. Conditional disruption of the *Arnt* gene has recently shown that ARNT is required for AHR-stimulated gene activation by TCDD in liver, but this association has yet to be extended to toxicity[24]. Other, as yet unidentified, AHR ligands may be present, or TCDD-AHR complex may participate in cell dysfunction by unknown routes not involving the regulation of gene expression via DREs and ARNT. Although no endogenous ligand of AHR has been proven, a number of naturally derived chemicals are ligands.

22. Some data suggest that the binding affinity, and the effect of binding, of TCDD to the AHR, are much lower in humans than in rodents, even the resistant DBA/2 mouse[25]. This could contribute an extra safety margin but the difference in response may vary with endpoint. Some polymorphisms of the human AHR gene have been reported but the functional significance of these polymorphisms is still under investigation[26]. We note that, in view of this uncertainty, it is not possible to exclude the possibility that the most sensitive humans are as responsive as the most sensitive rodents. Overall, we agree that the evidence that toxicity is mediated via the AHR, and the limited evidence that dioxin/receptor interaction does not inevitably lead to a toxic response, are sufficient to consider a threshold approach to the risk assessment.

Toxicokinetics of dioxins

Absorption

23. The extent of gastrointestinal absorption of dioxins is reported to vary with the medium or vehicle of administration, and the lipophilicity of the individual congeners. The percentage absorption of TCDD is approximately 60% in rodents[27]. Similar absorption has been reported for other chlorinated dibenzodioxins and dibenzofurans, although the absorption of octachlorodibenzodioxin (OCDD) is less than 20%. PCDDs and PCDFs are incompletely absorbed because they are not in solution within the gut lumen, and absorption is dependent on the digestion and emulsification of the food matrix.

Distribution

24. Once absorbed, probably via chylomicrons, TCDD rapidly leaves the blood compartment with a distribution half-life of approximately 30 minutes in rats, after which time it is primarily found in adipose tissue, the liver, skin, muscle and other tissues. TCDD present within the blood is largely associated with lipoproteins. Studies in rats have shown that after initial rapid distribution there is a slower redistribution from muscle and other organs, primarily to the liver and adipose tissue, skin and thyroid gland; the concentrations in these organs show a slow increase over a period of about 4 days following a single intraperitoneal dose[28]. This pattern of distribution is probably representative of distribution in humans and there is a high correlation between adipose tissue concentrations and the levels in serum.

25. The duration of the distribution phase is very short compared with the elimination phase. After tissue distribution, which takes about 4 days, *in vivo* elimination is adequately represented by a single mono-exponential decrease and half-life. The distribution phase is important in the interpretation of effects produced *in utero* in rats after a single oral dose given late in pregnancy, which are the basis for determining the tolerable intake.

Metabolism and elimination

26. Although early studies suggested that TCDD is not metabolised, it is now recognised that it is slowly converted to polar metabolites that are eliminated as glucuronides. The main metabolites of TCDD formed with rat hepatocytes *in vitro* are 1-hydroxy-2,3,7,8-TCDD and 8-hydroxy-2,3,7-TCDD. Other metabolites have been identified in dog, including tri- and dichloro-hydroxy and dihydroxy- compounds. Oxidative metabolism does not appear to give rise to significant bioactivation or formation of DNA adducts and the limited available data indicate that the metabolites are less toxic than the parent compound[29]. A major route of elimination of the hydroxy- metabolites is as glucuronic acid conjugates in the bile. Unmetabolised PCDDs and PCDFs are not detected in bile but are excreted in the faeces and faecal fat by direct intestinal elimination.

27. The half-life of TCDD has been reported to be about 20 days in the rat, 12 days in mice, 90 days in the guinea pig and between 6 and 11 years in humans[28,30-32]. The elimination half-life in humans correlates positively with the percentage body fat, indicating slower elimination in individuals with higher fat composition. Consistent with this is evidence suggesting an age-related decrease in half-life in the elderly, as the fat stores are mobilised during redistribution from the subcutaneous to abdominal areas[33]. Mobilisation of fat stores during lactation contributes to the presence of dioxins in breast milk[34], and this is associated with a decrease in the maternal body burden during breast-feeding.

Human data

Introduction

28. The human effects observed in one or more studies are summarised in Table 1. In assessing the effects of dioxins and dioxin-like PCBs in humans, we have selected those studies that provide the most information on the relationship between outcomes and exposure to TCDD-contaminated materials. Case reports were not reviewed and

only cohort studies with calculated standardised mortality ratios (SMRs) or equivalent, were discussed in assessing mortality. Many of the studies reviewed are cross-sectional in design and there are inherent limitations of this type of study. We noted that the lack of adequate exposure data was a frequent limitation of the available epidemiology. Exposure is measured in different media and expressed in different units across the studies, which makes comparison difficult. Some studies were only able to use indirect estimates of exposure, which cannot be directly related to dioxin levels. Development of a tolerable intake requires studies with quantitative assessment of exposure.

29. We focused our evaluation on studies in which exposure was assessed by measurement of dioxin concentrations in serum or body fat, which could be correlated with body burden and intake. The body burdens in human studies have been estimated in two ways. Firstly, the body burden may be calculated from the concentrations of dioxins in lipid and the percentage body composition as fat. This does not allow adequately for sequestration of dioxins within the liver, but this should produce only a minor error in the calculation of body burden. The second method is calculation of the body burden based on estimates of intake and half-life. In humans the intakes of dioxins will have varied historically and there is uncertainty about past exposures. In addition, little is known about the half-life of dioxins and dioxin-like PCBs at different life stages. Calculation of body burden based on daily intake has to allow for the bioavailability from the food matrix and the half-life or clearance from the body. A limitation to this method for considering mixtures of dioxins and dioxin-like PCBs is that reliable estimates of the half-life of TCDD and also its congeners are necessary.

30. We also focused on the relationship between exposure and response, particularly (although not exclusively, if other important health end points were investigated) for the health end points that are relevant/comparable to the results of animal studies. We noted that the EPA report identified six studies or series of studies in humans, which measured serum levels of TCDD and compared them with possible health effects[35-43]. We were also informed of an additional study in Dutch chemical workers, which provided exposure data [44] and a series of Dutch studies on cognitive development[45-53].

Table 1. Effects associated with human exposure to dioxins.

Effect	**Epidemiological evidence**
Chloracne	Proven association No clear dose relationship
Gastrointestinal effects and liver enzymes	Transient increases in some liver enzymes
Cardiovascular diseases	Positive association in occupational studies, but not in airforce veterans exposed to herbicides in Vietnam (Operation Ranch Hand). Dose-response in some studies
Changes in lipid levels	Results not consistent
Diabetes	Overall results not consistent Increased risks of morbidity in Seveso and Ranch Hand study
Reproductive hormones	Inconsistent results

Reproductive outcomes	Change in sex ratio of offspring with highly exposed fathers in Seveso No data yet on possible effects such as endometriosis and fertility in women – Seveso endometriosis study on-going
Thyroid function	Results not entirely consistent. Some small differences reported in thyroid hormone uptake levels.
Neurological / psychological effects	Inconsistent findings. Some effects reported in Ranch Hand study and Seveso (polyneuropathies, abnormal co-ordination) No association with depression
Respiratory system	Inconsistent evidence Irritative effects and reduced lung function in some studies
Urinary system	No major renal or bladder dysfunctions observed.
Immunological effects	Inconsistent findings.
Neurobehavioural developmental effects	Some observed differences in on-going Dutch studies
Cancer	Regarded as a probable human carcinogen (based on human, animal and mechanistic data)

31. With the exception of the series of Dutch studies on cognitive development, the studies reported the effects of high level occupational exposure or the results of accidental release. Occupational or accidental exposure would be associated with higher peak body burdens, followed by gradual elimination and were therefore difficult to compare with steady state conditions associated with background human exposure via the diet or with repeated exposure in animal studies. Also, the occupational studies have not addressed the reproductive effects that represent the most sensitive endpoints in the animal studies.

Studies of cognitive development

32. A series of Dutch studies involved cohorts in Rotterdam and Groningen, representing a highly industrialised region and a less industrialised, more rural area, respectively. The cohorts were sub-divided between breast-feeding for a minimum of six weeks and formula fed using a single batch of one commercial formula. Plasma from maternal and cord blood samples and milk samples were analysed for dioxins and PCBs, including some PCB congeners considered not to have dioxin-like properties. The infants were monitored at ages from 3 to 42 months, with assessments of motor and cognitive development, as well as indicators of thyroid function[46-52]. Similar studies were conducted on a smaller cohort in Amsterdam[53]. We invited additional expertise to ensure that these studies were reviewed adequately, particularly the relevance of the methodology, and we gratefully acknowledge the assistance provided.

33. We noted that the measures used were standard for the age of children assessed, and the best available in the absence of an *a priori* hypothesis of specific effects. However, we were informed that in very young children it is only possible to perform crude tests which do not provide a clear distinction between motor and cognitive development. Such tests therefore serve more as screening tests than definitive measures and thus the interpretation of any observed change may be hard to assess. Tests of motor function had been conducted from shortly after birth until about 30

months of age. Tests of cognitive function were conducted from 3 months to about 42 months. Different patterns had been observed in the studies conducted at different ages and very little change was observed in the middle of the age range. We noted that Prechtl's neurological examination (as conducted on the Rotterdam and Groningen cohort [47,48]) was considered the most stringent, but with the disadvantage of generating a large number of false positives. For infants, the Bayley Scales, based on a very large sample and well standardised, is probably the best instrument. This scale is divided into a mental development index (MDI) that measures how motor tasks (e.g. control of hands) are applied and a psychomotor index (PSI) that measures gross movements (e.g. walking). However, the Bayley Scales provide a measure of timing of appearance of certain skills and not the quality with which they are carried out. We noted that the study used a 1969 version of the test whereas a more advanced version was published in 1993.

34. The paper of Patandin *et al.* [52] reported changes at 42 months using the Kaufman Assessment Battery. This was considered to be critical to our assessment, since cognitive function is stabilising at this age and becomes predictive of function in later life. In contrast, Bayley scales are used for younger age groups (3 months to 3 years) and have low predictivity. The Kaufman scores at 42 months suggested an effect of pre-natal dioxin exposure leading to an effect on cognitive development.

35. It was difficult to determine whether the effects were due to dioxin exposure or to confounding factors. Complex correlations were found between dioxin and PCB levels and confounding factors, such as breast-feeding, smoking and maternal education. Linear regression analysis had been used to assess the influence of confounding factors. It was not clear whether this was appropriate, and the data were insufficient to determine whether the statistical approach might result in over- or under-correction. Overall, if the effects were real, they were most likely to be due to pre-natal exposure. Breast-feeding ameliorated the effects. However concerns over the known and potential confounders made it impossible to reach firm conclusions.

36. We noted that distinction between pre-natal and post-natal exposure to dioxins and PCBs was an issue of concern relating to the Dutch studies. Prenatal exposure was based on analyses at 8 months gestation, and it was not clear whether these were fully representative of exposure throughout pregnancy. However, when considering effects on thyroid function it should be noted that these effects may be confounded by changes in maternal thyroid hormone production prior to thyroid development in the fetus. None of the populations examined in the Dutch studies were considered to have exposures greater than the normal background range, differences were found between industrial and rural locations and there was a very large natural variation. In addition breast-feeding appeared to be a major confounder, with the highest proportion of breast fed infants having the highest dioxin concentration. Similarly level of maternal education appeared to correlate best with high exposure as did smoking. The paucity of information on the mathematical models used in the study made it impossible to determine whether effects were "real" or due to confounders.

37. We concluded that it was not possible to determine whether any cognitive changes represented temporarily delayed milestones of development or a persistent decrement and that follow-up studies were needed. These should be carried out two to three years after the original study or during the teenage years, as increasingly sensitive measures can be used in older children. Decreased variability in older children also tends to make the tests more sensitive. In the absence of such studies we do not

consider it possible to come to clearer conclusions about the outcome of dioxin exposure.

Sex ratio.

38. A recent study has reported on the sex distribution of children born between 1 April 1977 and 31 December 1996, with one or both parents exposed to TCDD in the Seveso incident, for whom TCDD serum concentrations were available relating to the time of the incident [54]. The exposed individuals were between 3 and 45 years old in 1976. Compared with an unexposed population, there was a dose-related decrease in the proportion of male children born to TCDD-exposed fathers. We note that this difference was statistically significant at paternal serum TCDD concentrations of 118 pg/kg or more. This could be estimated to correspond to a body burden of 24 ng/kg bw, which is equivalent to a daily intake of 12 pg/kg bw/day. However, the high exposure resulting from the Seveso incident is not comparable to steady state exposure, and the body burden derived from the peak serum concentrations in 1976 may not be the most appropriate dose surrogate for reproductive effects occurring in subsequent years.

Endometriosis.

39. Eskenazi and co-workers published initial details of the Seveso Women's Health Study [55]. The primary objectives of this study are to investigate whether there is a relationship between TCDD exposure and the following end-points; endometriosis, menstrual cycle characteristics, age at menarche, birth outcomes of pregnancies conceived after 1976, time to conception, clinical infertility and age at menopause. Insufficient results are currently published to assess the effects of TCDD exposure in the Seveso incident on endometriosis and other reproductive end-points in women. We considered that further consideration of female reproductive outcomes should be deferred until further papers on the Seveso Women's Health Study become available.

Immunotoxicity.

40. We noted that, compared with studies in experimental animals, there is much less information regarding immunotoxicity in humans. Nevertheless, there are suggestions that human immune function may be less susceptible to TCDD and dioxin-like PCBs that of rodents.

41. Evidence for immunotoxicity in humans resulting from occupational or accidental exposure to TCDD or related PCBs is inconsistent. However, a common feature of some investigations has been a modest exposure-related reduction in the frequency of peripheral CD4 T lymphocytes. The extent to which these effects represent an early indication of immunosuppression is unclear.

42. A recent paper has examined infectious and atopic diseases and immunological parameters in children with background levels of exposure to PCBs[56]. These are the cohorts from Rotterdam discussed in paragraphs 32 to 37, above. A large number of analyses are reported, many of which are simply correlation coefficients and some of the statistically significant results are likely to have occurred by chance. The authors concluded that exposure to PCBs and dioxins might be associated with a greater susceptibility to infectious diseases and a lower prevalence of allergic diseases. However, we noted a number of contradictions in the reported results, in addition to

the uncertainty over control for confounders as noted in paragraph 35. We concluded that the study did not provide convincing evidence of a causal relationship between pre-natal exposure or total body burden to PCBs and increased susceptibility to infectious diseases or decreased incidence of allergic disease.

Cardiovascular disease

43. Some studies have reported a positive association between exposure to TCDD, or to PCDDs and PCDFs, and the incidence of ischaemic heart disease[39,40,42,44]. These studies have indicated that a significant increase in ischaemic heart disease is associated with a body burden at or above 25 ng TCDD/kg bw, or 55 ng TEQ/kg bw for PCDDs and PCDFs. However, they did not adequately allow for confounding by other risk factors, such as smoking and diet. No studies included measurement of dioxin-like PCBs exposure or its contribution to the body burden.

Cancer

44. The Committee on Mutagenicity (COM) and the Committee on Carcinogenicity (COC) considered 2,3,7,8-TCDD in 1988/9 and concluded that this compound was carcinogenic in rodents but that this was unlikely to be due to a mutagenic mechanism. The COC gave further consideration to the carcinogenicity of 2,3,7,8-TCDD in 1993, when more epidemiological data were available. The Committee concluded that the new data strengthened the possibility of an epidemiological link between occupational exposure to 2,3,7,8-TCDD and an increase in total cancers in humans, although there was no consistent association with cancer at any specific anatomical site(s). It was considered that there was insufficient evidence for a clear causal link but it would be prudent at present to regard 2,3,7,8-TCDD as a possible human carcinogen [5].

45. The COC reviewed TCDD in 1998, following the publication of the IARC monograph which concluded that TCDD should be considered as a definite human carcinogen [57]. The COC agreed that TCDD is a potent carcinogen in laboratory animals, but that the information from the most heavily occupationally exposed cohorts suggested that there was, at most, only a weak carcinogenic effect in these individuals. It therefore concluded that there were insufficient epidemiological and toxicological data on TCDD to conclude a causal link with cancer in humans, but it would be prudent to consider TCDD as a "probable weak human carcinogen" [58].

46. The COC has reconsidered its 1998 statement in the light of recently published data on cancer epidemiology, including the twenty-year follow-up of the Seveso incident[59], and mechanisms of carcinogenicity. It agreed that TCDD should be regarded as a "probable human carcinogen" on the basis of all the available data. The COC agreed that although a precise mechanism for carcinogenesis in laboratory animals or humans could not be elucidated from the available information, the data (i.e. negative genotoxicity in standard assays, and evidence from studies of mechanisms) suggested that a threshold approach to risk assessment was likely to be appropriate. The COC did not consider it possible to quantify the margin-of-safety risk assessment in view of the difficulties in selecting the appropriate metric of exposures. However, it noted that the excess cancer mortality reported in the heavily exposed industrial cohorts was small and commented that any increased risk of cancer at background levels of exposure is likely to be extremely small and not detectable by current epidemiological methods[17].

Animal data

47. There are few regulatory rodent toxicity studies and no regulatory non-rodent studies on the dioxins, and most of the available data relate to TCDD. Most of the regulatory toxicity studies were performed at least 20 years ago and cannot be considered adequate for the determination of NOAELs. The recent studies were conducted to non-standard protocols and many of the studies examining the most sensitive end-points also failed to identify NOAELs. We have reviewed the experimental toxicology of TCDD, with particular consideration to those showing effects at the lowest doses.

Immunotoxicity.

48. We noted that the available data presented a complicated picture, with diverse protocols, including the use of different species and strains; various routes and durations of exposure and a wide range of doses. Nevertheless, some general points could be made:

49. In rodent studies the most consistent effect is a reduction in antibody responses to sheep red blood cells (SRBC). The SRBC assay is primarily a measure of the integrity of humoral immunity. However, as initiation and maintenance of antibody responses to SRBC requires not only B lymphocytes, but also functional T lymphocytes and antigen processing/presenting cells, this assay provides something of an overall view of adaptive immunity.

50. The most sensitive adverse effect level resulting from exposure to TCDD in which an immune alteration has been implicated was reported by Burleson *et al* [60]. An increased mortality of mice following challenge with influenza A virus was found following a single exposure to 0.1, 0.05 or 0.01 µg/kg TCDD. However, there is no evidence that the observed increase in susceptibility to virus challenge was necessarily attributable to impaired immune function and mortality was not associated with increased titres of virus in the lungs of mice exposed to TCDD. Therefore it could not be concluded that the lowest dose in this study represents the LOAEL for TCDD-induced immunotoxicity in mice.

51. We concur with the conclusion of the WHO, EPA and SCF reviews in considering the studies of Gehrs and colleagues to be important in assessing the immune effects of dioxins[61, 62]. Pregnant rats (Fischer 344 strain) received a single oral dose (on gestational day 14) of 0.1, 0.3, 1.0 or 3.0 µg/kg TCDD. Exposure at all doses was associated with a persistent (up to 14 months) reduction in males of delayed-type hypersensitivity (DTH) responses to bovine serum albumin. Maternal doses of 0.3 µg/kg TCDD and above were required for persistent suppression of DTH reactions in female offspring. On the basis of these investigations it is likely that 0.1 µg/kg TCDD should be regarded as the LOAEL for immune effects in young rats.

52. A second conclusion drawn from these studies was that maximal inhibition of immune function required both lactational and *in utero* exposure. This was more effective than lactational exposure alone, which was in turn more effective that *in utero* exposure only. It was noted that these differences in potency related to rats and might differ in humans.

Developmental and reproductive toxicity

53. The studies on developmental and reproductive effects in experimental animals mainly involved administration of TCDD alone, but there were comparative data for other congeners on teratogenicity and ovarian function. TCDD was able to elicit a number of different developmental effects although the sensitivity differed. The most sensitive and robust end-point was the effect on epididymal sperm count.

54. The EPA provided an excellent comprehensive review of the literature on developmental and reproductive toxicity, and although some new studies had emerged since it was written these did not have a major impact. The human sensitivity (based on *in vitro* data on embryonic AHR concentrations in different species) appeared to be in the middle of the range shown by experimental animals. Whilst the AHR was clearly implicated in the teratogenicity of TCDD, its role in other developmental effects was less clearly established. The reproductive effects were correlated with body burden at the critical stage of sexual differentiation (GD 15-16, as noted by SCF and JECFA[13,14]) and it appeared that equivalent fetal body burdens on day 16 of gestation were achieved by administration of different bolus doses on day 8 and day 15 of gestation.

55. We noted that the most sensitive end-points were observed following bolus administration and paid careful consideration to the relevance in deriving a tolerable intake. These studies are considered in detail in paragraphs 64-70. We noted that there was evidence to support an extrapolation from a bolus dose to a chronic exposure, as considered in paragraphs 71-74. The only multigeneration study was old[63] and was subjected to detailed evaluation in previous considerations by the Committee[4]. We considered that the results from this multigeneration study supported the body burden estimates but that there were questions about the statistics which required further evaluation.

56. We were informed that data from animal developmental studies did not show differences in the sex ratio of offspring, as had been reported for humans in the Seveso region. However, we accepted the animal studies were not designed specifically to address this issue.

Endometriosis.

57. In our 1995 statement we noted a study reporting an increased incidence of endometriosis in rhesus monkeys 10 years after completion of a study in which TCDD was administered in the diet for a period of about 4 years[64]. A recently published paper follows up the same group of monkeys 13 years after completion of the dietary study, reporting that the incidence of endometriosis correlated with serum levels of certain PCB congeners, but not TCDD[65]. Monkeys involved in a study in which lead was administered were also found to show an association between serum PCB levels and endometriosis. The authors could not account for the source of PCB exposure to these animals.

58. We noted that a number of aspects of this observational study undermined confidence in the results and in the earlier findings and concluded that it was not possible to draw reliable conclusions.

Acute, subchronic and chronic toxicity

59. TCDD causes a wide range of toxic responses after short and long term exposure with large differences in sensitivity between species/strains of animals to particular responses. Most of the reported toxic responses could be produced in every species provided an appropriate dose was given. The wide variability in sensitivity and the particular toxic response produced within and between species, makes it difficult to identify an appropriate endpoint for risk assessment. Lethality (as determined by LD50) varies with species from the highly sensitive guinea pig to the relatively insensitive hamster. There is also considerable variation within species. The value of these studies for risk assessment is doubtful given the age of the various studies, and the use of different strains, dosing regimens, routes of administration and observation period. No single site of toxicity has been identified as the cause of lethality; each species has a different spectrum of organ toxicity with a wasting syndrome and hepatotoxicity as the most common features. The wasting effect occurs in several species, but no single explanation for this effect has been described. Hepatotoxicity includes a wide range of liver effects in many species with rats and mice at the sensitive end and guinea pigs and hamsters as the least sensitive species. There is considerable variation in response within different strains of rat. The chronic dietary administration studies of Kociba *et al.* [66] reported that the lowest dose of 0.001 µg/kg bw/day was a NOAEL for hepatocellular nodules, although low body weights were recorded at various times during the study and only animals surviving to the end of the study were necropsied. In this study, the tumour incidences were significantly increased at a number of sites at the 0.1 µg/kg bw/day dose level.

60. We noted that there was no adequate basis for decisions on acceptable risk levels in humans based on the standard toxicity studies. Two of the most sensitive endpoints across the species seemed to be induction of CYP 1A1 and oxidative stress. Although CYP 1A1 induction is not considered to be a toxic response, it could underlie toxicity resulting from disruption of various endogenous processes. However, we noted that induction of CYP isozymes does not always show a good correlation with responsiveness in different mouse strains, indicating that it cannot be directly linked to toxicity. Oxidative stress had been detected in mouse brain[67], although it was not clear whether this was related to CYP induction.

Overall assessment

Use of body burden as a dose surrogate.

61. We considered that the most appropriate measure of exposure for assessing the sensitive endpoints of TCDD toxicity were the associated tissue concentrations, rather than the administered dose. Ideally the concentration in the target tissue would be the most appropriate measure of dose for comparing effects in different species, but this is impracticable for humans. The tissue concentration is directly related to the body burden at steady-state so that calculated body burdens are a valid surrogate. We therefore consider that the exposure/dose-response relationship for TCDD and related compounds should be based on body burden not external dose. The body burden approach allows for the massive interspecies differences in the half-life, and the potential for accumulation. An additional advantage of using body burdens, compared with previous dose-response assessments based on external dose, is that the body

burdens can be estimated for occupational and accidental exposures, and body burden-response relationships assessed. We concur with the recent evaluations that, despite some limitations, the body burden provides the appropriate dose metric, and that there is sufficient scientific evidence to support the use of body burden.

Human daily intakes and body burden

62. Following dietary exposure to dioxins and dioxin-like PCBs, the body burden will be accumulated over a period of 15-30 years in humans, during which time the environmental concentrations of these substances have decreased. In consequence, the body is not truly at steady-state, and hence there will be errors in the daily intake when calculated from current concentrations in body lipids. A pharmacokinetic model that allows for decreasing environmental concentrations with time indicates that the simple steady-state assumption over-estimates daily intake by approximately 20%. Some equations relating daily intake to body burden (based on adipose levels) do not include a specific term for bioavailability, and this would need to be considered for each route/protocol for exposure. This analysis is particularly important in relation to interpretation of human epidemiology studies where the body burden and daily intake is based on analysis of adipose tissue concentrations.

63. Overall, the data indicate that dioxins and dioxin-like PCBs may be associated with a number of effects, including cancer and cardiovascular disease, but generally at body burdens at least 10-fold higher than those occurring in the general population. Most of the studies involve groups that have been exposed to very high levels of dioxins resulting from occupational or accidental exposure and the pattern of exposure does not reflect long-term dietary exposure.

Evaluation

Key studies

64. We conducted a detailed review of the human data linking health effects to dioxin exposure, and a summary of these data is available on the COT website (http://www.foodstandards.gov.uk/committees/cot/summary.htm). We concluded that the available human data did not provide a sufficiently rigorous basis for establishment of a tolerable intake. This was because:

    i) the epidemiological studies do not reflect the most sensitive population identified by animal studies,

    ii) there are considerable uncertainties in the exposure assessments and inadequate allowance for confounding factors;

    iii) the patterns of exposure did not reflect exposures experienced in the general UK population, which are mainly from diet.

    We therefore found it necessary to base our evaluation on the data from studies conducted in experimental animals.

65. In accordance with the advice of the COC[17], we considered it appropriate to take a threshold approach to establishing a tolerable intake. This is based upon the negative genotoxicity in standard assays and evidence from studies of mechanisms.

66. Because a threshold-based approach was considered appropriate, we examined all of the toxicological effects, in addition to cancer, in order to identify the most sensitive end-points. We concluded that the most sensitive indicators of TCDD toxicity were the effects on the developing reproductive systems of male rat fetuses exposed *in utero*. These data were used despite inconsistencies in the findings reported, and the fact that none of the recent observations were made following sub-chronic or chronic dietary administration that would give constant (steady-state) maternal body burdens. We note that tolerable intakes were also derived from these endpoints in the recent SCF and JECFA evaluations. The key studies used different strains of rats and tended to give contradictory findings. A change in urogenital distance was found after single oral doses given on day 15 of gestation (GD15) of 50ng/kg bw[15], 200ng/kg bw [68] and 1000ng/kg bw[69]. We considered that the data on ano-genital distance were not robust because of lack of correction for body weight or other means of normalisation, and should be regarded as an intermediate marker with no functional significance. Decreases in sperm numbers, production, reserve or morphology were found after single oral doses of 50ng/kg bw and above (GD15) [68-70] and subcutaneous dosage to give a body burden of 25ng/kg bw[12], but not, in one study, at 800ng/kg bw (oral dose on GD15) [15]. Changes in the weight of the urogenital complex, including the ventral prostate were reported after an oral dose of 200ng/kg bw on GD15[15] but not at 300ng/kg bw subcutaneously [12].

67. Despite some inconsistencies, we considered that the effects on sperm production and morphology represented the most sensitive effects. These were indicative of the functional adverse reproductive effects in the rat that were produced by long-term administration in the multigeneration study of Murray *et al* at doses resulting in a 10-fold higher body burden than those in the studies of sperm production [63]. We also note that the sperm reserve in the human male is much less than that in the rat, and therefore these changes are considered relevant. No NOAEL was available for these effects, but the study of Faqi[12] provided the lowest LOAEL. We noted limitations in this study but considered that the results could not be discounted and therefore, that this should be used as the basis for deriving the tolerable intake.

68. We considered that a tolerable intake based on these effects would also protect against any risk of carcinogenicity from dioxins and dioxin-like PCBs. This conclusion is based on the mode of action of dioxins and difference between the body burdens at background levels of exposure and those associated with increased cancer risk as observed by the COC [17].

69. Three of the studies [15,68,70] reported adverse effects in male rat offspring following a single oral dose of TCDD given on GD15, and one[12] following repeated weekly subcutaneous injections. In all cases the effects were observed postnatally and the pattern of both *in utero* and post-natal exposure would be different. Because of the long half-life of TCDD (21 days in rats), and its presence in milk, the male offspring would be exposed to decreasing concentrations until the time of measurements. The recent SCF and JECFA evaluations[13,14] used recently published toxicokinetic studies[11,27] that allow the fetal body burdens to be calculated on GD16, on the assumption that this is the appropriate site of action, and period of sensitivity.

70. We have adopted a similar approach to the SCF and the JECFA. However, in view of the numerous assumptions in this approach (described below), we have used a simplified calculation of fetal and maternal body burdens associated with these different dosage regimens and their conversion to the steady-state dietary intakes that

would result in the same fetal body burdens. Calculation of a tolerable intake for humans is complex and requires a number of steps: calculation of the fetal body burden of rats under the experimental conditions; correction of the corresponding maternal body burden in rats to represent chronic daily intake *via* the diet; the use of uncertainty factors to give an equivalent tolerable human maternal body burden; and finally, derivation of a daily intake by humans that would result in the tolerable human maternal body burden.

Calculation of body burden

71. On the assumption that the critical period of exposure is GD16, the adverse effects following a single oral dose on GD15 would have been initiated at a time when the dose was undergoing tissue distribution. At this time, more of the maternal body burden would have been associated with well-perfused tissues, such as the liver, and the reproductive system and less with adipose tissue. It is possible to estimate the fetal exposure on GD16 by allowing for differences in the maternal dosage protocol using the toxicokinetic data of Hurst *et al*, following a single oral bolus dose on GD15 [27] and following dietary administration of 1, 10 and 30ng/kg bw per day for 5 days per week from 13 weeks before mating [11].

72. A problem with the interpretation of the Hurst *et al.* papers[11,27], which measured radioactivity after dosage with radioactive TCDD, is that the ratios of maternal to fetal body burdens on GD16 were not independent of dose, as would be predicted for such low doses. This non-linearity is difficult to explain on biological grounds and may have arisen as an artefact of the low levels of radioactivity measured. The SCF evaluation used regression analysis with a power model forced through the origin to correct maternal dosage and derive a correction factor of 2.6 for the higher fetal body burdens when dosed on GD15 compared with daily treatment[13]. These regressions used the ratios of maternal:fetal body burdens in ng/kg bw after single doses of 50 and 200ng/kg bw on GD15 [27] (30:5.3 and 97.4:13.2, respectively) and after daily oral doses equivalent to 0.71, 7.1 and 21.3ng/kg bw/day [11] (20:1.4, 120:7.5 and 300:15.2 respectively). The JECFA evaluation confirmed the results of the power model but also used a linear model that gave a correction factor of 1.7, and the JECFA concluded that both models fitted equally well to the available data[14]. Although the power and linear models fitted equally well, they gave different correction factors, especially at very low body burdens. This resulted in a discrepancy (see JECFA, 2001) [14] when applied to the correction of the 5ng/kg bw subcutaneous maintenance dose used in the study of Faqi *et al* [12] (see below).

73. Because the correct mathematical model cannot be determined based on goodness of fit, and because the regressions are determined largely by body burdens higher than those relevant for derivation of a tolerable intake, we decided to adopt a simpler method of correction using the ratios calculated directly from the lowest doses in each of the studies by Hurst *et al*[11,27]. After a single oral dose of 50ng/kg bw on GD15, the fetal body burden on GD16 was 5.8-fold lower than the maternal body burden (5.3ng/kg bw compared with 30.6ng/kg bw) [27]. After sub-chronic oral treatment with 1ng/kg bw/day for 5 days a week, which gave a maternal body burden of 19ng/kg bw, the fetal body burden on GD16 was 14.6-fold lower than the maternal body burden (1.3ng/kg bw compared with 19ng/kg bw) [11]. Thus a bolus dose given on GD15 results in 2.5-fold higher fetal body burdens (14.6/5.8) on GD16, than would occur if the same maternal body burden had arisen as a result of sub-chronic treatment.

Derivation of the TDI

74. In order to derive a tolerable intake for humans, it was necessary to convert the subcutaneous dosage regimen used in the Faqi study [12] into a steady-state maternal body burden on GD16. The study involved a bolus dose of 25ng/kg bw, 14 days before mating, and subsequent weekly maintenance doses of 5ng/kg bw. Assuming that the first day of mating corresponds to GD0, these weekly maintenance doses would have been given on GD-7, GD0, GD7, etc. By GD16, the doses given up to GD7 would have distributed to all tissues, representing steady-state distribution and resulting in a maternal body burden of 18.3ng/kg bw. This value is comprised of 9.3 + 2.3 + 3.0 + 3.7ng/kg bw remaining in the body from the doses given on GD-14, GD-7, GD0 and GD7, respectively, assuming a half-life of 21 days. The maternal body burden from the 5ng/kg bw maintenance dose given on GD14 would give a "non-equilibrium" maternal body burden of 4.5ng/kg bw on GD16. Using the correction factor described in paragraph 73, it can be estimated that a steady state maternal body burden of 2.5-fold higher (i.e. 11.3ng/kg bw) would be needed to produce the same fetal body burden as this “non-equilibrium” dose. Therefore the calculated total steady-state maternal body burden on GD16 arising from the subcutaneous dosing protocol at the LOAEL is approximately 30ng/kg bw, which would be about 33ng/kg bw after allowing for the TCDD intake from food.

75. Conversion of the calculated equivalent steady-state maternal body burdens from these studies in rats into an equivalent human body burden requires the use of uncertainty factors to allow for the use of a LOAEL and to allow for species differences and human variability. Both the SCF and the JECFA evaluations used a default factor of 3 to allow for the use of LOAEL, and an overall factor of 3.2 ($10^{0.5}$) to allow for species differences and inter-individual variability[71,72]. The latter factor is lower than the default of 100 normally used because it incorporates the following chemical-specific adjustment factors:

- inter-species differences in toxicokinetics: uncertainty factor of 1.0 because the body burden approach allows for toxicokinetic differences;
- inter-species differences and human variability in toxicodynamics: uncertainty factor of 1 to cover both of these aspects based on the assumption that in general, rats are more sensitive than humans, but the most susceptible humans might be as sensitive to TCDD as rats;
- human variability in toxicokinetics: uncertainty factor of 3.2 to allow for potential increased accumulation, and hence body burden, of dioxins in the most susceptible individuals. This is only relevant for congeners with shorter half-lives than TCDD, because an individual with a 3.2-fold longer TCDD half-life would not reach steady-state body burden.

Applying the uncertainty factor of 9.6 (3 x 3.2) to the calculated maternal steady-state body burden from the study of Faqi *et al* (LOAEL=33ng/kg bw) gives a tolerable human equivalent maternal body burden of 3.4ng/kg bw.

76. Estimation of the daily intake of TCDD that would result in this body burden has to take into account the fraction absorbed (bioavailability) from the diet by humans (both the SCF and JECFA evaluations concluded that the bioavailability of TCDD in humans is 50%), and the very long half-life in humans (which the JECFA concluded

was an average of 7.6 years, while the SCF used a figure of 7.5 years). The human equivalent body burdens can be converted into daily intakes by the equation:-

$$\text{daily intake (pg/kg/day)} = \frac{\text{body burden (pg/kg bw) x ln 2}}{\text{bioavailability x half-life in days}}$$

77. Using a bioavailability of 0.5 and a half-life of 2740 days (7.5 years), the tolerable human equivalent steady-state body burden from the study of Faqi *et al* [12] would be produced in humans by a daily intake of 1.7pg/kg bw/day. Given the imprecision and assumptions inherent in these calculations we concluded that the tolerable daily intake for dioxins and dioxin-like PCBs should be based on this value rounded to a single figure, i.e. 2pg WHO TEQ/kg bw per day. We note that SCF and JECFA have used longer averaging periods, but because intakes are usually expressed on a daily basis, we considered that establishment of a tolerable daily intake was more appropriate and transparent. This value is consistent with tolerable intakes derived recently using similar data (WHO: 1-4pg WHO TEQ /kg bw/day[10]; SCF: 14pg WHO TEQ /kg bw/week[13]; JECFA: 70pg WHO TEQ /kg bw/month[14]).

78. We note that the body burden is the most appropriate dose metric for establishment of a tolerable intake and, because of its long half-life, the body burden of TCDD at steady state is about 2000 fold higher the average daily intake. For example, an intake of 10 times the TDI on a single day would result in a 0.5% increase in the body burden. Therefore short term variation in intake does not significantly alter the body burden, and occasional exceedance of the TDI would not be expected to result in harmful effects, provided that intake averaged over a prolonged period is within the TDI.

Conclusions

79. We *conclude* that dioxins and dioxin-like PCBs have the potential to cause a wide range of adverse health effects. The health effects most likely to be associated with low levels of exposures relate to the developing embryo/fetus.

80. We *recommend* that a tolerable daily intake of 2 pg WHO-TEQ/kg bw per day is established, based upon effects on the developing male reproductive system mediated via the maternal body burden.

81. We *consider* that this TDI is adequate to protect against other possible effects, such as cancer and cardiovascular effects.

82. We *note* that the most recent intake estimates for the UK population are 1.8 pg/kg bw/day for the average consumer and 3.1 pg/kg bw/day for the 97.5 percentile consumer and that dietary intakes are decreasing.

83. There are no short-term measures that can be used to decrease the body burden of dioxins and dioxin-like PCBs in humans because of their long half-lives and widespread presence at low levels in food.

84. Similarly, because of the long half-life, short-term exceedances of the tolerable intake are not expected to result in adverse effects. Nevertheless, it is not possible to identify a duration and degree of exceedance at which adverse effects might occur.

85. Finally, we *confirm* our previous advice that, although intakes of dioxins and dioxin-like PCBs by breast-fed babies are higher than is desirable, encouragement of breast-

feeding should continue on the basis of convincing evidence of the benefits of human milk to the overall health and development of the infant.

October 2001

COT statement 2001/07

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2001 Membership of the Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

Professor H F Woods CBE BSc (Hons) BM BCh DPhil FFPM FRCP (Lon & Edin)

Sir George Franklin Professor of Medicine

Division of Molecular and Genetic Medicine

University of Sheffield

MEMBERS

Professor P Aggett OBE MB ChB FRCP MSc DCh FRCPCH

Vice Chairman.

Head of Lancashire Postgraduate School of Medicine and Health

Dr S Ariyanayagam MBBS MRCGP MRCOG MFFP DCh Lond (From 1 October 2001)

Public Interest Representative

Professor N A Brown BSc PhD (up to 30 September 2001)

Professor of Developmental Biology, Department of Anatomy and Developmental Biology, St George's Hospital Medical School, University of London

Dr P Carthew BSc(Hons)MSc PhD FRCPath

Senior pathologist, SEAC Toxicology Unit, Unilever.

Professor J K Chipman BSc(Hons) PhD CBiol FIBiol MRCP

Professor of Cell Toxicology, University of Birmingham

Dr P Jackson BA(Oxon) MA(Oxon) MB ChB MRCP PhD FRCP (From 1 October 2001)
Reader in clinical pharmacology and theraputics, University of Sheffield

Dr M Joffe MD MSc(Econ) FRCP FFPHM

Reader in epidemiology and public health, Imperial College School of Medicine, London

Professor I Kimber BSc(Hons) MSc PhD FIBMS CBiol MIBiol

Head of Research, Syngenta Health Assessment and Environmental Safety, Syngenta Central Toxicology Laboratory,Macclesfield

Professor J Lunec BSc(Hon) PhD FRC Path (From 1 October 2001)

Director of Clinical Pathology, University of Leicester

Dr A Piersma MSc PhD (From 1 October 2001)

Senior staff scientist, National Institute of Public Health and the Environment, Holland

Professor A G Renwick OBE BSc PhD DSc (up to 30 September 2001)

Professor of Clinical Pharmacology, University of Wales College of Medicine

Professor I R Rowland BSc(Hons) PhD

Professor of Human Nutrition and Director of Northern Ireland Centre for Diet and Health (NICHE), University of Ulster

Dr L Rushton BA(Hons) MSc PhD CStat

Head of Epidemiology, Medical Research Councl, Institute for Environment and Health, University of Leicester

Ms J Salfield BSc(Hons) MSc MIFST CertED RPHN

Public Interest Representative

Dr A G Smith BSc(Hons) PhD CChem FRSC

Head of Molecular Toxicology, Medical Research Council Toxicology Unit, University of Leicester

Dr L Stanley BA PhD (From 1 October 2001)

Principal lecturer in biomedical sciences, School of Pharmacy and Pharmaceutical Sciences, De Montfort University, Leicester

Professor S Strobel MD PhD FRCP FRCPCH

Consultant paediatric immunologist, Institute of Child Health

Dr A Thomas MB CHB PhD FRCP (up to 30 September 2001)

Consultant Physician in General (internal) Medicine and Geriatric Medicine, and Director of Medical Education, Plymough Hospitals NHS Trust

Professor J A Timbrell BSc(Hons) PhD DSc MRCPath FRFC FIBiol

Professor of Biochemical Toxicology, King's College, London

Dr M Tucker BSc(Hons) PhD FRCPath

Independent pathologist

SECRETARIAT

Dr D Benford BSc(Hons) PhD	Scientific Secretary
Mr J M Battershill Bsc MSc	Scientific Secretary - Department of Health
Mr K Butler	Administrative Secretary
Dr D Gott BSc(Hons) PhD	
Ms C A Mulholland BSc(Hons)	
Dr C Tahourdin BSc(Hons) PhD	
Dr J Shavila BSc(Hons) MSc PhD	
Dr N Thatcher BSc(Hons) PhD	
Mr B Maycock BSc(Hons) 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)	HBOS (formerly 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 the Harry Bottom Charitable Trust.
Professor P J Aggett (Deputy Chairman)	Nestec Wyeth Borax	Ad hoc Consultancy Ad Hoc Consultancy Lecturing and Chairing Meetings	Nestec FDF Abbot Unilever Meat and Livestock Commission	Departmental commissioned research and student placements
Dr Sati Ariyanaygam (from 01.10.2001)	NONE	NONE	NONE	NONE
Professor N A Brown (up to 30.09.2001)	Nestec, Wyeth, Borax	Ad hoc Consultancy Lecture and Chairing Meetings	Nestic, FDF Abbot Unilever Meat and Livestock Commission	Departmental commissioned research and student placements
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

Member	Personal Interest		Non-Personal Interest	
	Company	**Interest**	**Company**	**Interest**
Dr P Jackson (from 01.10.2001)	Bristol Myers Squibb	Lecture fees	Novartis	Research grant
			Merck Sharp Dohme	Research grant
	Merck Sharp Dohme	Lecture fees		
			Servier	Research grant
	British Heart Foundation	Lecture fees	Bristol Myers Squibb	Research grant
			Pfzier	Research grant
			Astrazeneca	Research grant
			Medtronic AVE	Research grant
			Department of Health	Research grant
Dr M Joffe	Ilzro	Research grant	NONE	NONE
Dr I Kimber	British Airways	Share Holder	Unilever plc	Grant for Research
	British Petroleum-Amoco	Share Holder		
	ICI	Share Holder		
	Halifax	Share Holder		
	AstraZeneca	Share Holder		
	AstraZeneca	Employee		
Professor J Lunec (from 01.10.2001)	NONE	NONE	Scilucent LLC. USA	Funding research group to investigate toxicology of soya-bean oil implants
Dr A Piersma (from 01.10.2001)	NONE	NONE	NONE	NONE
Professor A G Renwick (up to 30.09.2001)	International Sweeteners Association	Consultant	Hoffman-La Roche Unilever SmithKline Beecham Pfizer Flavor and Extract Manufacturers Association (FEMA)	Research Support Research Support Research Support Research Support
Professor I R Rowland	Colloids Naturels International (CNI) Rouen, France	Consultancy	Various	Departmental teaching & research funded by various Food companies
	Danisco	Consultancy		

Member	Personal Interest		Non-Personal Interest	
	Company	**Interest**	**Company**	**Interest**
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
Dr L Stanley (from 01.10.2001)	NONE	NONE	NONE	NONE
Professor S Strobel	NONE	NONE	NONE	NONE
Dr A Thomas (up to 30.09.2001)	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 THE MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Preface

This is my first year as Chairman of the Committee and I would like to start by recording my thanks and appreciation to Professor James Parry who stood down as Chair of COM during the year. Jim provided outstanding leadership and expertise to the Committee setting a very hard standard for me to follow.

The Committee on Mutagenicity (COM) provides advice on 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 expertise of independent committee members is required to provide recommendations on potential hazards and risks and frequently suggestions for further studies.

During 2001, the Committee undertook a number of very complex reviews including in particular one of the insecticide Dichlorvos. The statement for this review was not finalised during 2001 but was published early in 2002 and can be found on the COM web site (http://www.doh.gov.uk/com/com.htm). The report of the COM's consideration given in this Annual report illustrates the complexity of the task undertaken by the COM and the dedication shown by members, which has included attendance at extraordinary meetings at very short notice. The COM also finalised advice on phosphine (used as a pesticidal fumigant), 1,3-dichloropropan-2-ol and 2,3-dichloropropan-1-ol which are contaminants that may be present in drinking water, and terephthalic acid which is used as a starting chemical for production of food packaging materials.

The Committee also finalised its review of risk assessment of *in-vivo* mutagens (genotoxic carcinogens) which has been published on the Internet. This is an important document setting out the principles used by the Committee in the risk assessment of mutagenic chemicals.

Finally I would like to thank all members for the support given to me during my initial year as Chair and I look forward to the forthcoming year's business.

Professor P B Farmer Chair

MA DPhil CChem FRSC

1,3-Dichloropropan-2-ol and 2,3-dichloropropan-1-ol

2.1 1,3-Dichloropropan-2-ol (1,3-DCP) and 2,3-dichloropropan-1-ol (2,3-DCP) are members of a group of chemicals called chloropropanols. This group includes 3-monochloropropane 1,2-diol (3-MCPD), which has recently been considered by both the COM (1) and the COC (2). Like 3-MCPD, 1,3-DCP and 2,3-DCP can be present as contaminants in epichlorohyrin/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 therefore 1,3-DCP and 2,3-DCP may potentially be present in drinking water from their use. 1,3-DCP, like 3-MCPD, can also be present as process contaminant of food where acid-hydrolysed vegetable protein has been used as an ingredient, such as soy and similar oriental sauces.

2.2 Members agreed that the metabolism of 1,3-DCP was likely to produce a reactive epoxide intermediate that could damage DNA. Members were aware that there were very few data on the absorption, distribution, and excretion of 2,3-DCP. Theoretically, 2,3-DCP could be metabolised to produce epichlorohydrin (and subsequently glycidol) and therefore there were structural alerts for genotoxicity and carcinogenicity. The committee agreed that both 1,3-DCP and 2,3-DCP were mutagenic *in-vitro* and it would be prudent to regard 1,3-DCP and 2,3-DCP as potentially genotoxic *in-vivo* and agreed that both compounds should be tested for genotoxicity *in-vivo* using the approach set out in the COM guidelines.

2.3 The Food Standards Agency has subsequently commissioned appropriate research. The results will be considered by COM when available. A finalised COM statement can be found at the end of this report.

Dichlorvos

Background to COM review

2.4 Dichlorvos (O-(2,2-dichlorovinyl)-O,O-dimethylphosphate, DDVP) was first introduced into the UK as an agricultural pesticide in 1962. Non-agricultural uses were first assessed under the voluntary Pesticides Safety Precaution Scheme in 1975-78. A review by the ACP of approvals issued under the Control of Pesticides Regulatory (COPR 1986) was undertaken in 1994. At the time of the COM review dichlorvos was used by amateur and professional users as a public hygiene insecticide (e.g. use of hand held aerosols for surface/space spray and slow release products e.g. strips, cassettes). A relatively small number of products were approved for use in animal husbandry and in agriculture and horticulture on edible crops (e.g. cucumbers) and on non-edible crops (e.g. chrysanthemums). Dichlorvos has also been used in a veterinary medicinal product for control of fleas in cats and dogs.

2.5 The COM considered generic aspects arising from the paper by Sasaki YF *et al* (1) on the performance of the *in-vivo* COMET assay with respect to the newly published strategy in the COM guidance at its 8 February 2001 meeting. With respect to dichlorvos, members noted that this chemical contained a structural alert for mutagenicity and was a direct acting *in-vitro* mutagen. Members noted that negative

results had been documented in a number of *in vivo* assays with dichlorvos. These were reported to be adequate but on the data available it was not possible to assess tissue exposure. The Committee considered that a full review of the mutagenicity data was needed in order to assess the significance of the results obtained in the new Comet assay.

COM review (26 April 2001)

2.6 The toxicokinetics, mutagenicity and carcinogenicity data sections from the draft evaluation prepared by HSE for the Advisory Committee on Pesticides meeting on 5 April 2001 were made available to the COM. The Committee evaluated the data from all of the mutagenicity studies cited in the HSE review. The COM also considered additional information and mutagenicity data submitted by industry to HSE prior to the COM meeting on 26 April 2001.

2.7 Members agreed that dichlorvos is a weak methylating agent (compared to methyl methanesulphonate; MMS). The Committee agreed that dichlorvos was mutagenic *in-vitro*, both in the presence and absence of exogenous metabolic activation. The Committee reached an initial conclusion that a pattern of mutagenic effects had been documented in the *in-vivo* studies which suggested that dichlorvos induced mutagenic effects in the skin following topical application (i.e. at the site of contact) and in systemic tissues (liver and bone-marrow) under conditions where repeated doses were administered. The Committee agreed provisionally that dichlorvos should be regarded as an *in-vivo* mutagen and that there was a potential risk of mutagenicity at site of contact tissues and systemically following repeated exposure.

2.8 A draft statement was forwarded to the data holders (referred to below as "industry") for comment (in accordance with the procedures for openness; see Annex 3) and to Advisory Committee on Pesticides.

2.9 The data holders expressed a number of significant reservations regarding the approach used by the COM to assess the evidence and the interpretation of the positive results in the *in-vivo* mutation assays. These comments were forwarded to the Chairman who considered that industry should have an opportunity to present their arguments and the new data cited in their submissions. The Secretariat was therefore asked to arrange an extraordinary meeting of the COM to further consider all the available information on dichlorvos.

Extraordinary meeting of 23 July 2001

2.10 Members considered a number of new papers forwarded by industry for this meeting. The Chairman asked the Committee to identify the key areas for questioning. These were identified as:

i. relative rates of hydrolysis compared to methylation of DNA by dichlorvos.

ii. the concept of a threshold for dichlorvos induced DNA methylation.

iii. any data relating to oxidative damage in hepatocytes induced by dichlorvos and relevance of these data to the response seen in the in-vivo mutation study in transgenic animals

iv. the use of the intraperitoneal route for the assessment of direct acting mutagenic effects in the liver.

v. The weight of evidence approach to the assessment of mutagenicity.

Industry presentation

2.11 The industry team comprised representatives of the data holders and experts in mutagenicity assessment acting as consultants. The team distributed a new set of overheads which differed in detail from those submitted to the secretariat. The presentation lasted approximately 30 minutes. Members raised a number of questions with the industry team. The team withdrew from the room whilst Members discussed the comments raised by industry.

COM discussion of industry presentation

2.12 Industry accepted that dichlorvos was a direct acting *in-vitro* mutagen but argued that since the rate of dichlorvos hydrolysis was many orders of magnitude faster than DNA alkylation there was an effective threshold for alkylation *in-vivo.* This was consistent with many in-vivo negative studies covering a range of endpoints. Consideration was then given to the four studies reported by the COM in the 1st draft statement as giving positive results and which underpinned the conclusion reached by COM.

2.13 The mouse hair follicle assay nuclear anomaly assay was considered by industry to be not an appropriate assay to identify genotoxicity and could not be used to detect mutagens. This suggestion was agreed by the Committee but it was felt that the results did indicate that the skin was a target organ for dichlorvos.

2.14 Industry's concerns with the mouse skin micronucleus assay were that this was not a validated assay, and results were available from only one laboratory. Other laboratories had difficulty with this test and no data were available on sensitivity, specificity or reproducibility. The Committee accepted that this was not a standard assay but noted that for supplementary in-vivo assays, especially to investigate local site effects, novel methods often provided useful data. Furthermore there was no convincing explanation to discount this positive result.

2.15 Industry believed that the methodology used in the repeated dose rat bone-marrow chromosome aberration study was inadequate to draw any conclusions from the reported increase in numerical aberrations due to inadequate methodology. It was also noted, by industry, that this study gave unique results when compared to the other available repeat dose studies. The Committee agreed that the method was limited but the results were indicative of effects on numerical aberrations. Members agreed it would be more appropriate to refer to numerical aberrations rather than aneuploidy.

2.16 Finally, industry expressed concerns regarding the inappropriate study design of the Muta™Mouse study involving high doses with the liver as the target. Industry suggested that the route of administration (namely intraperitoneal) was fundamentally flawed for direct acting compounds. There were also concerns about the excessively high dose level and the lack of details to address the specification of the test material used. The Committee felt that the methods used in this study had limitations but the data were indicative of a mutagenic effect of dichlorvos at the site-of-contact.

2.17 A major concern of industry was the lack of appropriate consideration of weight of evidence with regard to the in-vivo mutagenicity studies. However the Committee agreed that the positive data showed a pattern for in-vivo site of contact mutagenicity and the systemic studies were irrelevant for the evaluation of site-of-contact effects.

2.18 Members noted that no additional information was provided by industry with regard to the potential for oxidative effects of dichlorvos in the liver.

2.19 Further consideration was given to the negative results in the single dose UDS assay in the forestomach of mice using gavage dosing. The Committee concluded that it was not possible to exclude genotoxic effects from these data given the relative insensitivity of the method used as indicated by the response with the positive control chemical, and that repeat dosing would most likely be required to identify any mutagenic effects of dichlorvos in this assay. In addition the Committee noted the views of the Chairman and a member of the COC on the carcinogenicity bioassays in the mouse, and in particular on the oesophageal and stomach tumours.

2.20 The Committee concluded that dichlorvos should be regarded as an in-vivo mutagen at the site-of-contact. High doses of dichlorvos induced mutagenic effects in the skin following topical application and in the liver following intraperitoneal dosing. The Committee agreed that dichlorvos induced tumours of the forestomach in mice after gavage dosing; also that the oesophageal tumours seen after dietary administration were probably related to dichlorvos treatment. The Committee felt that it would be prudent to assume a genotoxic mechanism on the basis of the available data. The Committee agreed that in the absence of appropriate mechanistic data a precautionary approach should be adopted and no threshold could be assumed for the mutagenic and carcinogenic effects of dichlorvos.

Actions following meeting of 23 July 2001

2.21 A revised draft statement was forwarded to the data holders for comment. The finalised statement was not published until early December 2001. The data holder sought a judicial review regarding the regulatory review of dichlorvos pesticide products. The judicial review took place during 5-9 November 2001. Mr Justice Crane handed down his judgement on the case held in the High Court between AMVAC Chemicals UK Ltd (Claimants) and Secretary of State for Environment, Food and Rural Affairs (DEFRA) and Secretary of State for Transport, Local Government and Regions (DTLR) (Defendants) on 3rd December 2001. Essentially the Claimants (AMVAC Chemical UK Ltd) won on one of the three issues they had raised namely that the decision to suspend pesticide approvals was flawed in relation to the amount of notice given to the claimant and the decision was therefore quashed. Mr Justice Crane saw no reason why those advising ministers should not review their advice, taking into account all the Claimants submits, without significant delay.

2.22 This meant that the COM was asked to consider the relevant material submitted to the court by AMVAC to ascertain whether this warranted any revision of the COM statement on dichlorvos. This information essentially comprised exhibits from a number of independent scientific experts and a number of papers on the generic issues of evaluation of various types of mutagenicity assays. In addition other pesticide approval holders were asked to forward any further data by 4 January 2002.

2.23 A further extraordinary meeting of COM was arranged for the 9 January 2002 to consider this data. The outcome of this additional meeting will be reported on the COM internet site (www.doh.gov.uk/com/com.htm).

Phosphine

2.24 Phosphine is used as a pesticide to fumigate grain and as a rodenticide. The COM considered the mutagenicity of phosphine in 1997. Although in-vitro data indicated it had mutagenic potential, *in-vivo* data in rodents (bone marrow, liver and germ cell) were negative. However a clastogenicity study by Garry *et al* (Science, 246, 251-255, 1989) in fumigant workers in Minnesota did give rise to concern, although it was recognised that this study had limitations. The COM recommended that consideration be given to a feasibility study in UK pesticide applicators using modern methods for assessing genotoxic effects. This recommendation was passed by PSD to industry. After detailed consideration of this request the industry view was that such a study would be impractical due to the small numbers of workers involved and the fact that nearly all were also potentially exposed to another mutagen, methyl bromide. Industry has suggested a study of those workers involved in the manufacture of phosphine, outside the 'closed' area where full PPE is worn. However the numbers are relatively small (about 20) and the exposures low. In addition a new inhalation carcinogenicity bioassay in the rat which had been conducted to acceptable regulatory standards and which was negative had now become available. The Committee was asked to review its conclusions reached in 1997 in the light of this new information. Members were also asked to note the most recent estimates of exposure to phosphine during the manufacture of phosphides, which had been tabled for information.

2.25 Members discussed the available mutagenicity data and reaffirmed that phosphine was highly reactive and agreed that the mutagenicity data provided some evidence for positive results *in-vitro* but the available *in-vivo* assays were negative. The Committee agreed that the negative rat inhalation carcinogenicity bioassay provided additional reassurance with regard to possible site of contact mutagenicity. It was noted that there was no evidence that phosphine had a direct interaction with DNA, and that any mutagenic effect *in-vitro* might be related to the production of reactive oxides which could react with cellular components. It came to the notice of the Committee that phosphine has been reported to induce oxidative DNA damage *in-vitro* and *in-vivo.*

2.26 Members recalled that exposures to phosphine in the studies reported by Garry *et al* indicated concentration of phosphine up to 4.1 ppm. The Committee noted a recent communication received by the secretariat from the Lawrence Livermore Laboratory USA in respect of a study which had been published as an abstract only in 1996. This reported that the negative results of cytogenetic screening study in workers potentially exposed to phosphine during fumigation work, was probably due to the efficiency of exposure reduction provided by Personal Protective Equipment worn by the operators. The Committee agreed that occupational exposure to phosphine was significantly reduced in fumigators and other workers exposed to phosphine by the use of Personal Protective Equipment. It was therefore unlikely that any occupational groups could be currently identified within the UK where an appropriate biomonitoring study could be undertaken. Members were aware that the use of phosphine as a pesticide in the UK was expected to increase in the future as a result of restrictions to be enforced with respect to the use of other fumigants (methyl bromide). The Committee was made

aware of proposals to measure exposure in workers undertaking fumigation operations and those re-entering grain stores. The Committee agreed that it would like to see a review of any results that became available. Members commented that exposure of workers to phosphine during the manufacture of electronics might represent a separate occupational group which could be considered in any further studies of exposure to phosphine.

2.27 The Committee concluded that additional new data, (namely negative rat inhalation carcinogenicity study), together with the negative *in-vivo* mutagenicity data in bone marrow and liver in animals provided sufficient reassurance regarding the *in-vivo* mutagenicity of phosphine, taken together with the information relating to very low potential exposure arising from pesticides use. Thus the COM's original suggestion for consideration of a biomonitoring study in UK pesticide applicators was no longer necessary. The Committee noted that use of phosphine was likely to increase in the future and asked to be informed of any results of exposure measurements made available to the UK regulatory authorities. A finalised COM statement can be found at the end of this report.

Terephthalic acid

2.28 The COT had considered terephthalic acid in 2000 and requested advice from the COM on the potential *in-vivo* genotoxicity of this compound, which produced bladder tumours in a carcinogenicity bioassay in the rat. It was important to exclude a genotoxic mechanism.

2.29 Members noted that TPA contained no structural alerts for mutagenicity and was practically insoluble in water. In discussion, the Committee agreed that the non-genotoxic mechanism (formation of insoluble crystals in the bladder and subsequent chronic tissue damage) suggested that the induction of bladder tumours with this compound was plausible. The Committee commented on the poor quality of the *in-vitro* mutagenicity test data but accepted that the evidence from the studies using bacteria suggested that TPA was not mutagenic in a limited number of *Salmonella typhimurium* strains. The cytogenetics test in lung fibroblasts had not been conducted to internationally accepted standards, i.e. no short duration exposure had been used and no tests using exogenous metabolic activation had been undertaken. Members were aware that TPA could be used in a potentially large number of food contact applications and thus intakes could be higher than quoted in the COT statement (2000/08). The Committee agreed that an adequately conducted *in-vitro* cytogenetics test in mammalian cells was required to bring the *in-vitro* mutagenicity data on this compound up to an acceptable level.

2.30 Members reviewed the *in-vivo* micronucleus assay conducted with TPA in ICR mice. The test material had been given as a suspension in corn oil by the intraperitoneal route and there was no direct measurement of exposure to the bone marrow and no toxicokinetics data were available to assist. However, signs of toxicity reported suggested that the test material had been absorbed into the blood circulation and thus dose selection had been adequate. Members noted that the low spontaneous background incidence of micronuclei in ICR mice complicated the interpretation of the data. The Committee agreed that this study had given negative results but a

decision on further *in-vivo* mutagenicity testing should await the outcome of the *in-vitro* cytogenetics testing requested by the Committee.

2.31 The Committee noted that TPA is used widely in food contact applications and agreed that the available *in-vitro* mutagenicity data in bacterial test systems suggested that TPA is not mutagenic in a limited number of *Salmonella typhimurium* strains. It was also agreed that the available *in-vivo* micronucleus assay conducted with TPA had given negative results. The Committee recommended that an adequately conducted *in-vitro* cytogenetics test in mammalian cells is needed before any definite conclusions were reached that this compound did not have any significant mutagenic potential, and thus that the bladder tumours in the rat carcinogenicity bioassay arose from a non-genotoxic mechanism.

2.32 A draft statement was forwarded to the data holders who have agreed to undertake the study requested by the COM. A finalised COM statement can be found at the end of this report.

Review of Committee Procedures

Reviews of risk procedures used by Government Advisory Committees dealing with food.

2.33 The Committee considered an expert report written by Sir Robert May (Chief Scientist), Sir Liam Donaldson (Chief Medical Officer) and Sir John Krebs (Chairman of the Food Standards Agency). The Committee had provided details of the risk assessment procedures used during the drafting of the report. A meeting had also been organised for Chairmen to discuss how their Committees approached risk assessment and to gather ideas on ways in which risk is currently assessed, managed and communicated.

2.34 The COM considered the final report and commented that due to the complex nature of some of the work undertaken by the Committee, flexibility was required rather than a formalised approach to risk assessment. It was agreed that the COM guidance published in December 2000 was suitable as a format for Committee discussions. The Committee agreed that improved communication between the COM, COC and COT would be helpful. It was suggested that these committees could receive each other's minutes. It was also suggested that joint meetings and cross-membership could be usefully pursued. Members agreed that the secretariat should identify potential ideas for a joint scientific meeting of all three committees. (A joint meeting on use of genomics proteomics in toxicology was subsequently arranged and held on 8 October 2001, see section 2.46 below).

Code of Practice for Scientific Advisory Committee: 2nd Round of Consultation

2.35 Members recalled that they had previously commented on the first draft of a Code of Practice for Scientific Advisory Committees. The second version contained a number of suggested amendments and included discussion of some topics, which arose from

the findings of the Inquiry into BSE by Lord Phillips. Members were asked for their comments on the new draft, which would be fed back to the Office of Science and Technology.

2.36 Members noted that the minutes of COM discussion were often highly technical, and that there was a need to make the information as comprehensible as possible to the public, and that further consideration should be given to this challenging issue. The Committee agreed that the format used by the COT for stakeholder meetings and open scientific meetings would be appropriate for the COC and COM. Members asked for more flexibility in assessing the need for extra members of COM and to co-opt additional expertise when required. The Committee accepted the practice that a consensus view was reported in statements, but considered that it should be clearly stated where one or several members disagreed with a conclusion. Members felt that on appointment more information could be provided on the length of service possible and of training opportunities. Members felt that the Committee should be able to suggest topics where research was required, although it was acknowledged that any proposal would have to be included in the overall research priorities review process of the sponsoring Government Department.

2.37 The finalised report was subsequently published on 19 December 2001.

Test Strategies and Evaluation

Risk assessment of *in-vivo* mutagens (and genotoxic carcinogens)

2.38 The Committee completed a generic review of the approach to be used to the risk assessment of *in-vivo* mutagens and genotoxic carcinogens. This work had been undertaken to complement the revised COM guidance on a strategy for testing of chemicals for mutagenicity which had been completed in December 2000. The COM reaffirmed that for *in-vivo* mutagens, it is prudent to assume that there is no threshold for mutagenicity. Where a potential threshold related mechanism can be identified, adequate data should be generated on a chemical-by-chemical basis to support this mechanism. In many situations this will involve *in-vitro* studies of mechanism. An appropriate strategy should be devised for each chemical under consideration and this may, in some instances, include *in-vivo* studies. The regulatory approach to such chemicals can then be based on the identification of a critical NOAEL and use of uncertainty factors. A finalised COM statement can be found at the end of this report.

Tobacco induced lung carcinogenesis: The importance of p53 mutations

2.39 It has been noted that p53 mutations in lung cancer are different from those in other cancers and that an excess of G→T transversions is characteristic for these tumours. G→T transversions have been likened to a 'molecular signature' of tobacco smoke mutagens in smoking-associated lung cancers. The Committee discussed a recent publication (*Rodin SN & Rodin AS (2000). Human lung cancer and p53: The interplay between mutagenesis and selection PNAS vol.97. (No 22) p12244*) where the authors had argued that p53 mutations in lung cancer may not be due to exposure to

PAHs but represent selection of pre-existing or endogenous mutations by physiological stress aggravated by smoking. This argument could be used to invoke a 'threshold' approach to the risk assessment of tobacco smoke induced lung carcinogenesis, ie a departure from the approach adopted by COM/COC for genotoxic carcinogenesis.

2.40 Members agreed that there were important reservations regarding the rationale used by Rodin and Rodin and agreed that it was important to provide a detailed publication in the scientific literature to outline the COM views. The Committee agreed that it was important to record that there were over 3,000 chemicals in tobacco smoke and that these included known genotoxic carcinogens such as the tobacco specific nitrosamines which were also likely to be important in the aetiology of tobacco-smoke induced lung carcinogenesis. Members agreed with Rodin and Rodin that many of the chemicals in tobacco-smoke accentuated the formation of tumours and acted as promoters. Overall it was agreed that a clear conclusion on the role of mutations in p53 tobacco smoke induced lung cancer should be published.

2.41 The Committee concluded that tobacco smoke contained many genotoxic carcinogens, some of which are implicated in the aetiology of lung cancer induced by smoking. The evidence from the study of mutational patterns and hotspots in the p53 gene from lung tumours of smokers and non-smokers suggests that carcinogens in tobacco smoke induce mutations in tumours. This supports the view that tobacco smoke is demonstrably a genotoxic carcinogen and any exposure is likely to be associated with some increased risk of cancer (ie effects do not have a threshold).

International Workshop on the Categorisation of Mutagens

2.42 The Committee was asked to provide comments on the recently agreed Globally Harmonised Scheme (GHS) for classification and the agreed criteria for mutagens. The system was similar to that of the EU except that it only contains 2 classes (1 and 2) category 1 being divided into known mutagens (1a) and those that should be regarded as if they induce heritable mutations in germ cells of humans (1b). Category 2 mutagens are those which cause concern for man owing to the possibility that they may induce heritable mutations in the germ cells of humans. Thus category 1b on the GHS was equivalent to category 2 on the EU system, and category 2 on the GHS was equivalent to category 3 on the EU system.

2.43 The "detailed" criteria for the GHS classification were similar to those in the EU systems with 2 exceptions. For category 1b mutagens, data from positive *in-vivo* heritable germ cell tests were needed or data from positive *in-vivo* somatic cell assays plus evidence that the substance had the potential to cause mutations in germ cells eg from mutagenicity/genotoxicity tests in germ cells *in-vivo,* or by demonstrating the ability of the substance to interact with the genetic material of germ cells (this was in line with earlier COM recommendations whereas the EU approach is in terms of reaching germ cell DNA rather than interaction with DNA). For category 2 mutagens in the GHS, activity from somatic cell in *in-vivo* assays for mutagenicity was needed, or from genotoxicity assays *in-vivo* supported by positive *in-vitro* data.

2.44 It was also noted that chemicals which were positive *in-vitro* in mammalian mutagenicity assays and which show chemical structure activity relationships to

known germ cell mutagens, should be considered for classification as category 2 mutagens. Members agreed that this proposal went further than earlier COM advice.

Ongoing Work

Chemical evaluation

2.45 The committee is carrying out a mutagenic evaluation of the following chemicals:

i) Dimetridazole;

ii) Flunixin;

iii) Flunixin-meglumine and

iv) Meglumine.

Joint Meeting of the COT, COC and COM

2.46 In response to the recommendations arising from Sir Robert May's "Review of Risk Procedures used by the Government's Advisory Committees dealing with Food Safety," the COT, COC and the COM, identified a need for more discussion and joint working between these three committees. Toxicogenomics and proteomics had been highlighted as rapidly growing research areas of toxicological science. The Committees decided to hold a joint meeting to discuss their applicability in risk assessment. A brief summary of the meeting is presented in the COT section of this Annual Report. A detailed publication is currently being drafted and a statement will be published on the COT/COC/COM websites.

Statements of the COM 2001

1,3-Dichloropropan-2-ol and 2,3-dichloropropan-1-ol

Update on phosphine (post 1997)

Terephthalic acid

Risk assessment of *in-vivo* mutagens (and genotoxic carcinogens).

MUTAGENICITY OF 1,3-DICHLOROPROPAN-2-OL (1,3-DCP) AND 2,3-DICHLOROPROPAN-1-OL (2,3-DCP)

Introduction

1. 1,3-Dichloropropan-2-ol (1,3-DCP) and 2,3-dichloropropan-1-ol (2,3-DCP) are members of a group of chemicals called chloropropanols. This group includes 3-monochloro propane 1,2-diol (3-MCPD), which has recently been considered by both the COM[1] and the COC[2]. Like 3-MCPD, 1,3-DCP and 2,3-DCP can be present as contaminants in epichlorohyrin/amine coplymers used as flocculants or coaglulant aids in water treatment. These polyamine flocculants have been available for many years as approved products for use in water treatment and therefore 1,3-DCP and 2,3-DCP may potentially be present in drinking water from their use. 1,3-DCP, like 3-MCPD, can also be present as process contaminant of food where acid-hydrolysed vegetable protein (acid-HVP) has been used as an ingredient, such as soy and similar oriental sauces.

2. 1,3-DCP has not previously been considered by COM but has been considered by COC, originally in 1991 and more recently in 2001. The COC noted that in a carcinogenicity study undertaken by Hercules Inc[3]. 1,3-DCP was administered in the drinking water of 80 male and 80 female Wistar rats for 104 weeks. Statistically significant positive trends were observed for benign and malignant tumours (intermediate and high dose level) in the liver, kidney, tongue/oral cavity and thyroid. The COC's conclusions for 1,3 DCP have been published. [4] 2,3-DCP has not been previously considered by COM, but COC conclusions on this substance are contained in the COC statement[4].

3. In 1993 the FAO/WHO Expert Committee on Food Additives (JECFA) concluded that, because of its carcinogenicity, 1,3-DCP is an undesirable contaminant in food and that levels should be reduced to as low as technologically achievable[5]. 2,3-DCP has not been considered by JECFA. The Drinking Water Inspectorate (DWI) and the Food Standards Agency (FSA) asked the COM for advice on these two chemicals in view of the recently published statements by the COM and COC on 3-MCPD.

COM evaluation

4. The Committee was aware that 1,3-DCP and 2,3-DCP are closely related compounds and structurally similar to 3-MCPD which had been considered at its previous meeting. Members were informed that, at present, there are no mandatory contaminant levels for these chloropropanols, either in food or drinking water.

1,3-DCP

5. Members agreed that the metabolism of 1,3-DCP was likely to produce the reactive epoxide intermediate that could damage DNA. Members were aware that 1,3-DCP had been found to be mutagenic to *Salmonella typhimurium* in strains TA 1535 and/or TA 100. [6-13] Studies with mammalian cells have produced increased frequencies of sister chromatid exchanges and chromosome aberrations. [14,15] A positive result has been obtained in the mouse lymphoma assay. [16,17] 1,3-DCP was negative in the wing spot test in *Drosophila melagonaster* (a somatic mutation and recombination test). [18] No *in-vivo* mammalian studies have been carried out. [6]

6. Members were aware that there was very little data on the absorption, distribution, and excretion of 2,3-DCP. Theoretically, 2,3-DCP could be metabolised to produce epichlorohydrin (and subsequently glycidol) and therefore there were structural alerts for genotoxicity and carcinogenicity.

7. The Committee noted 2,3-DCP was mutagenic in *Salmonella typhimurium* strains TA 100 and TA 1535 in a study with and without metabolic activation[10], and mutagenic in another Ames test. [9] Positive results were also obtained for sister chromatid exchange with Chinese Hamster V79 cells both with and without metabolic activation. [14] Members were aware that in the limited studies conducted, 2,3-DCP was genotoxic *in-vitro* with and without metabolic activation in bacterial and mammalian cells. No *in-vivo* studies in mammals have been carried out.

COM Conclusion

8. The committee concluded that it would be prudent to regard 1,3-DCP and 2,3-DCP as potentially genotoxic *in-vivo* and agreed that both compounds should be tested for genotoxicity *in-vivo* using the approach set out in the COM guidelines.

May 2001

COM/01/S2

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STATEMENT ON PHOSPHINE USE AS A PESTICIDE IN FUMIGATION OF GRAIN AND AS A RODENTICIDE: UPDATE OF COM CONCLUSIONS REACHED IN 1997

Introduction

1. Aluminium and magnesium phosphides are used in pesticides to fumigate stored products (principally grain). In addition aluminium and zinc phosphide are used in rodenticide products. The active compound in both cases is phosphine gas which is liberated when the phosphides come into contact with moisture. These compounds have been used for this purpose in the UK for over 25 years. In view of their high acute toxicity, their use is limited to trained operators.

2. The COM provided advice on the mutagenicity of phosphine to the Advisory Committee on Pesticides (ACP) in 1997. The text of the COM advice to the ACP is reproduced below as background information. The COM concluded that there was some limited evidence (from human monitoring studies) to suggest that phosphine was an *in-vivo* mutagen, and suggested that further investigation in pesticide workers would aid in reaching a definite conclusion.

Background: COM conclusions reached in 1997

3. The Committee reached the following conclusions with respect to the mutagenicity of phosphine and metal phosphide:

   i. Aluminium and magnesium phosphide are used in pesticides to fumigate stored food products (principally grain). In addition, aluminium and zinc phosphide are used in rodenticide products. The active compound in both cases is phosphine gas which is liberated when the phosphide comes into contact with moisture. These compounds have been used for this purpose in the UK for over 25 years. The Advisory Committee on Pesticides (ACP) requested the advice of the COM on the mutagenicity of these pesticides. The Committee reviewed all the available published and unpublished data forwarded by the Pesticides Safety Directorate and placed a particular emphasis on the studies in humans. The Committee agreed the following conclusions which are applicable to phosphine and all the above mentioned metallic phosphides.

In-vitro studies

   ii. Phosphine (or zinc phosphide) has consistently given negative results in assays for gene mutation in bacteria (*Salmonella typhimurium*). There was some concern that the high toxicity of phosphine could mask potential mutagenic effects. Phosphine has however been shown to have clastogenic potential in cytogenetic studies in mammalian cells and zinc phosphide has given positive results in the mouse lymphoma assay. In both cases activity was seen in the presence and absence of an exogenous metabolic activation system. Phosphine and metallic phosphides therefore have mutagenic potential *in-vitro*.

In-vivo studies in animals

iii. Phosphine has been extensively studied *in-vivo* using bone marrow/peripheral blood assays for clastogenicity. Although some inconsistent data were obtained, overall it can be concluded that negative results were obtained. Negative results were also obtained in a liver UDS assay and in a dominant lethal assay in mice, although this latter assay was of limited quality. Overall, the Committee concluded that there was no convincing evidence that phosphine and metallic phosphides had shown mutagenic activity *in-vivo* in rodents.

Studies in humans

iv. The Committee considered three published reports in detail.

v. Garry VF *et al* (Science, 246, 251-255, 1989) studied small groups of grain workers in Minnesota. They reported an increase in total chromosome aberrations (excluding gaps) and of deletions, breaks and complex aberrations in a group of 9 individuals with reported exposure to phosphine alone compared to a control group of 24 individuals with no exposure to pesticides and a control group of 15 grain workers employed in the inspection and processing of grain. Limited personal sampling data were available to show exposures up to 4.1 ppm phosphine in enclosed space applications and up to 0.64 ppm for open-air applications. The Committee noted the small number of phosphine workers examined and the limited details available regarding matching of control and exposed groups for smoking habits. The Committee observed the considerable overlap in the range of results for pesticide applicators exposed to phosphine and grain worker controls. Overall it was concluded that pesticide applicators exposed to phosphine alone had elevated levels of chromosome damage in this study.

vi. In a subsequent study the same group of authors (Garry VF *et al* Cancer Epidemiology, Biomarkers and Prevention, 1, 287-291, 1992) reported similar findings in a group of 6 pesticide applicators exposed to phosphine compared to a control group of 26 individuals. The Committee agreed that the results, ie increased levels of chromosome damage were compatible with the earlier study by these authors.

vii. In a separate Australian study, Barbosa A and Bonin AM (Occupational and Environmental Medicine, 51, 700-705, 1994) analysed micronuclei in peripheral blood lymphocytes taken from 31 fumigators working with phosphine and 21 control subjects. Limited exposure data were provided for 3 individuals employed as fumigators and reported exposure concentrations of 0.1-0.8 ppm for a period of 1 hour. The authors report that the groups were matched for sex age and smoking habit but few details were provided. No evidence of an increase in micronuclei in phosphine exposed fumigators was documented. No increase in urinary mutagenicity (Salmonella assay) was seen in fumigators.

Conclusions human studies

viii. The Committee concluded that it would be prudent to assume that phosphine was a human genotoxin on the basis of the results reported in studies of Garry *et al*. The Committee agreed that the negative data reported by Barbosa and Bonin may be due to the fact that they studied workers exposed to lower levels of phosphine

and used a methodology of lower sensitivity than Garry *et al*. However, there were limitations in the studies published by Gary et al, for example in subject and control selection and matching, and phosphine exposure estimations. The Committee felt that further data were needed to ascertain whether these results were reproducible (by other groups) before any definitive conclusions could be drawn. Consideration should be given to the feasibility of a study in UK pesticide applicators using modern methods for assessing genotoxic effects. The COM would be willing to advise on the design of such a study.

Referral from ACP secretariat February 2001

4. The ACP secretariat asked for further advice from the COM in view of additional data which had been submitted by industry (namely a negative inhalation carcinogenicity bioassay in the rat) and proposals outlining a strategy for assessing genotoxic effects in workers occupationally exposed to phosphine.

COM consideration of new data

5. Members reaffirmed that phosphine was highly reactive and agreed that the mutagenicity data provided some evidence for positive results *in-vitro* but the available *in-vivo* assays were negative. The Committee agreed that the negative rat inhalation carcinogenicity bioassay provided additional reassurance with regard to site of contact mutagenicity[1]. It was noted that there was no evidence that phosphine had a direct interaction with DNA, and that any mutagenic effect *in-vitro* might be related to the production of reactive oxides which could react with cellular components. It has come to the notice of the Committee that phosphine has recently been reported to induce oxidative DNA damage in-vitro and *in-vivo*[2,3]. The Committee was aware that occupational exposure to phosphine was significantly reduced in fumigators and other workers exposed to phosphine by the use of Personal Protective Equipment[4]. It was therefore unlikely that any occupational groups could be currently identified within the UK where an appropriate biomonitoring study could be undertaken. Members were aware that the use of phosphine as a pesticide in the UK was expected to increase in the future as a result of restrictions to be enforced with respect to the use of other fumigants (methyl bromide). The Committee was aware of proposals by the ACP to require monitoring of exposure in workers undertaking fumigation operations and those re-entering grain stores. The Committee noted that they would like to see a review of any results when available.

COM Conclusion

6. The Committee concluded that additional new data, (namely negative rat inhalation carcinogenicity study), together with the negative *in-vivo* mutagenicity data in bone marrow and liver in animals provided sufficient reassurance regarding the *in-vivo* mutagenicity of phosphine, taken together with the information relating to very low potential exposure arising from pesticides use. Thus the COM's original suggestion for consideration of a monitoring study in UK pesticide applicators was no longer necessary. The Committee noted that use of phosphine was likely to increase in the future and asked to be informed of any results of exposure measurements made available to the UK regulatory authorities.

February 2001
COM/01/S1

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STATEMENT ON THE MUTAGENICITY OF TEREPHTHALIC ACID

1. Terephthalic acid (TPA) is used as a starting material in the manufacture of polyethylene terephthalate (PET). PET has been used to coat the internal surface of food cans.

2. The European Commission's Scientific Committee for Food (SCF) had reviewed the toxicology of TPA in the 80's and set a temporary Tolerable Daly Intake of 0.125 mg/kg bw/day, which was based on 3-month and 2-year oral studies in rats[1]. The TDI was temporary pending the submission of full reports of these studies. The major finding in the long-term study with TPA was the occurrence of malignant and benign tumours of the urinary tract at high doses. It was proposed that these tumours were likely to arise from urothial hyperplasia associated with bladder stone formation rather than a genotoxic mechanism.

3. In October 2000, the Committee on Toxicity (COT) considered the health effects of TPA in the context of a survey on the migration of this compound from can coatings into food[2]. The COT concluded that the concentrations of TPA that had been determined in foods analysed in the survey were not of concern for public health on the basis of the then available information. However, the COT noted that the toxicity studies on TPA were not carried out to current standards. The COT requested that, in the light of the urinary tumours occurring in rats fed the highest dietary concentration of TPA in long-term studies, the view of the COM be sought on the potential *in vivo* genotoxicity of this compound.

In Vitro mutagenicity data

4. Members noted that TPA contained no structural alerts for mutagenicity and was practically insoluble in water. The Committee agreed that the formation of oxalate based insoluble crystals in the bladder and subsequent chronic tissue damage was a plausible non-genotoxic mechanism for the induction of bladder tumours with this compound.

5. The Committee noted the availability of a number of reports of investigations of the *in vitro* mutagenicity of TPA in bacteria using *Salmonella typhimurium* strains[3-5]. These studies were poorly reported and some used inadequate protocols or non-standard methods. However, TPA was consistently negative in these investigations. In one report[5], a range of TPA concentrations of up to 10.0 mg/plate was used in the presence and absence of an activation system with no evidence of toxicity in the bacteria. Although TPA was found to be negative, it was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 only. In other studies in *Salmonella typhimurium* strains which tested the activity of TPA at single[4] or multiple dose levels[3], insufficient details were provided to draw any conclusions. Overall, the Committee accepted that the evidence from the studies using bacteria suggests that TPA is not mutagenic in a limited number of *Salmonella typhimurium* strains.

6. The availability of an *in vitro* cytogenetics test in lung fibroblasts was also noted by the Committee[6]. Although TPA was found to be negative when tested at a concentration of 2 mg/l using an exposure period of 48 hours, the study did not

address the influence of an exogenous metabolic activation system. In addition, the effect of shorter exposure periods were not investigated.

7. The Committee was aware that TPA may be used in a large number of food contact applications and agreed that an adequately conducted *in-vitro* cytogenetics test in mammalian cells was required to bring the *in-vitro* mutagenicity data on this compound up to the recommended level for a compound with widespread exposure.

In Vivo mutagenicity

8. Members reviewed a recent *in-vivo* micronucleus assay conducted with TPA in ICR mice[7]. The test was conducted to current standards but was not supported by any toxicokinetic data and gave no direct measurement of exposure to the bone marrow. Signs of toxicity were reported which suggested that the test material had been absorbed into the systemic circulation and thus dose selection had been adequate.

9. The Committee agreed that this study had given negative results but this did not provide adequate reassurance regarding the mutagenic potential of TPA in the absence of an adequate in-vitro package of data.

Conclusions

10. The Committee noted that TPA is used widely in food contact applications and agreed the following conclusions:

11. The available *in vitro* mutagenicity data in bacterial test systems suggested that TPA is not mutagenic in a limited number of *Salmonella typhimurium* strains.

12. It was also agreed that the available *in-vivo* micronucleus assay conducted with TPA had given negative results.

13. The Committee recommended that an adequately conducted *in-vitro* cytogenetics test in mammalian cells is needed before any definite conclusions were reached that this compound did not have any significant mutagenic potential, and thus that the bladder tumours in the rat carcinogenicity bioassay arose from a non-genotoxic mechanism.

November 2001

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STATEMENT ON RISK ASSESSMENT OF *IN-VIVO* MUTAGENS (AND GENOTOXIC CARCINOGENS)

Introduction

1. The general advice of the COM when considering the risk assessment of chemicals which are mutagenic *in-vivo* has been that it is prudent to assume a linear, non threshold dose response. Thus it is assumed that any exposure to an *in-vivo* mutagen is associated with some damage to DNA and consequently an increased risk of mutation leading to an increased risk of adverse health effects albeit that this may be small. In such instances the Committee has recommended that exposures be reduced to a low as is reasonably practicable. The COC has adopted a prudent approach to the assessment of chemical carcinogens which assumes that genotoxic carcinogens have the potential to damage DNA at any level of exposure and that such damage may lead to tumour development. Thus for genotoxic carcinogens it is assumed that there is no discernible threshold and that any level of exposure carries a risk[1,2].

2. The COM agreed to review its general approach to the risk assessment of *in-vivo* mutagens following the publication of the COM guidance on a strategy for testing chemicals for mutagenicity[3]. The Committee has previously considered specific chemicals, on a case-by-case basis, with regard to deviations from its general approach to *in-vivo* mutagens. This statement summarises the conclusions reached at the February meeting 2001.

Evidence for existence of in-vivo thresholds for mutagenic effects

3. The Committee recalled that there were two mechanisms for which sufficient evidence is available for the COM to conclude that a threshold for mutagenicity exists namely (i) aneugenicity induction by tubulin inhibitors (specifically methyl benzimadazole carbamates (MBCs), benomyl, carbendazim and thiophanate-methyl) and, (ii) the rapid detoxication of hydroquinone and phenol via the oral route. The Committee has undertaken detailed reviews of the mutagenicity data on these chemicals and full statements have been published on the COM Website (*www.doh.gov.uk/com.htm*) and in the Committee's Annual Reports[1]. A brief overview is given below with the objective of providing background information on the approach used to provide the critical data used by the COM in its evaluation.

Methyl benzimadazole carbamates (MBC) induced aneugenicity

4. 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 in particular as antihelmintics in both food producing and companion animals. These chemicals act by interfering with microtubule formation during mitosis. The COM has provided advice to the U.K 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[4-6].

5. 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[4]. 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 methodologies for identifying thresholds in 1993, namely appropriate in-vitro experiments in human lymphocytes using the detection and quantification of non-disjunction and chromosome using FISH (Fluorescent in-situ hybridisation) analysis of selected chromosomes for centromeric DNA. The Committee considered that it was not possible to determine thresholds for aneugenicity using the currently available in-vivo assays. 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) could be estimated for these two chemicals[7-10]. 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[11].

Hydroquinone and phenol

6. In 1994, the COM agreed that both hydroquinone and phenol should be regarded as somatic cell *in-vivo* mutagens[12-19]. 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 from appropriate toxicokinetic studies 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[20]. 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. 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]. The new toxicokinetic study in which rats were given a single intratracheal dose of 14C-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.

7. The data on MBCs and hydroquinone and phenol show that if there were specific data on a chemical to invoke such mechanisms then the possibility of threshold could be considered. Members agreed that data on threshold-related mechanisms would in most instances come from *in-vitro* studies. Any observed NOEL from such *in-vitro* mechanistic studies could be used to inform a risk assessment but it was unlikely that the data could be used in a quantitative way. In those cases where a potential threshold

mechanism is based on chemical detoxication, then appropriate *in-vivo* data will be required.

Possible mechanisms for thresholded mutagenicity

8. The Committee reviewed a number of papers which reported proceedings of an international symposium held in Salzberg in September 1998[23]. Two broad categories of potentially threshold mechanisms were interaction with non-DNA targets and rapid detoxication which were consistent with the COM's experience regarding MBCs and hydroquinone and phenol. In addition further papers presented to the symposium noted that threshold dose responses could involve exposure to redundant or multiple cellular targets which inactivation or modification before a toxic response is produced[24]. Lists of potential cellular targets for threshold-related genotoxicity were presented at the Symposium which essentially identify protein targets such as microtubules, DNA synthetases, topoisomerases[25]. The Committee agreed that appropriate supporting evidence would be required in order to invoke any of these mechanisms on a chemical-by-chemical basis.

Conclusions

8. The COM reaffirmed that for *in-vivo* mutagens, it is prudent to assume that there is no threshold for mutagenicity. Where a potential threshold related mechanism can be identified, appropriate data should be generated on a chemical-by-chemical basis. In many situations this will involve *in-vitro* studies of mechanism. An appropriate strategy should be devised for each chemical under consideration and this may, in some instances, include *in-vivo* studies. The regulatory approach to such chemicals can then be based on the identification of a critical NOAEL and use of uncertainty factors.

June 2001

COM/01/S3

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2001 Membership of the Committee on the Mutagenicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

Professor J M Parry BSc PhD DSc (up to 31 October 2001)

Professor of Genetics. School of Biological Sciences, University of Wales, Swansea

MEMBERS

Professor J Ashby BSc PhD CChem FRCS

Senior Research Associate, Mutagenesis, carcinogenesis and endocrine disruption, Syngenta Central Toxicology Laboratory

Dr Julie Clements BSc PhD

Head of Genetic and Molecular Toxicology, Covance

Professor C Cooper BSc PhD DSc

Head of Molecular Carcinogenesis Section, Institute of Cancer Research, Haddow Laboratories

Professor P B Farmer MA DPhil CChem FRSC (Chairman from 1 November 2001)

Professor of Chemistry, MRC Toxicology Unit, University of Leicester

Dr N J Gooderham PhD CChem FRSC
Reader and Head of Molecular Toxicology, Division of Biomedical Sciences, Imperial College of Science, Technology and Medicine

Ms M Langley BA

Lay Member

Dr I Mitchell BA PhD

Consultant in Genetic and Molecular Toxicology, Kelvin Associates

Professor D H Phillips BA PhD DSc FRCPath

Professor of Environmental Carcinogenesis, Institute of Cancer Research

Professor D J Tweats BSc PhD CBiol FIBiol FRCPath

Director of Preclinical Safety Sciences, Glaxo Wellcome Research & Development Ltd

SECRETARIAT

R J Fielder BSc PhD Dip RCPath (Scientific)

Diane Benford BSc PhD (Scientific – Food Standards Agency)

K N Mistry (Administrative)

J M Battershill BSc MSc

Declaration of interests during the period of this report

Member	Personal Interest		Non-Personal Interest	
	Company	*Interest*	*Company*	*Interest*
Prof J M Parry (Chairman)	Compass Catering JIB Insurance National Power Smith Kline 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	Celltech Abbey National plc Woolwich Alliance & Leicester Bradford & Bingley Friends Provident Torotrak SBS Group	Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder Share Holder	Scotia Zeneca	Research Studentship & 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	GlaxoSmithKline	BBSRC Collaborative Studentship

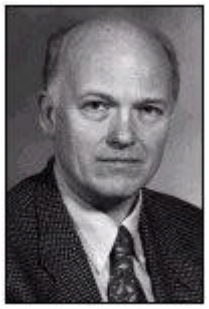
Member	Personal Interest		Non-Personal Interest	
	Company	*Interest*	*Company*	*Interest*
Ms M Langley	Business Consolidating Services	Director	NONE	NONE
	CREE Research	Share Holder		
	Cyber Care	Share Holder		
	Eshelon	Share Holder		
	Quelcom	Share Holder		
	Ciebel	Share Holder		
	Wibex	Share Holder		
	Halifax	Share Holder		
	BT	Share Holder		
	MMO2	Share Holder		
Prof D Phillips	Abbey National plc	Share Holder	NONE	NONE
	BG Group	Share Holder		
	Bradford & Bingley	Share Holder		
	Centrica	Share Holder		
	CGNU	Share Holder		
	Lattice Group	Share Holder		
	National Grid	Share Holder		
Dr I Mitchell	Kelvin Associates	Associate/Consultant Director/Creditor	NONE	NONE
	IM Enterprises	Pensioner, Option and		
	Glaxo Smithkline	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		

Member	Personal Interest		Non-Personal Interest	
	Company	*Interest*	*Company*	*Interest*
Prof D J Tweats	Glaxo Wellcome	Salary Employee	NONE	NONE
		Share Option Holder		
		Share Holder		
	Halifax	Share Holder		
	Co-operative Bank plc	PEP Holder		

* Appointed as Chair wef 1 November 2001

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

Preface



The Committee on Carcinogenicity (COC) evaluates chemicals for their human carcinogenic potential at the request of the Department of Health and Food Standards Agency and other Government Departments including the Regulatory Authorities. All details concerning membership, agendas, minutes and statements are published on the Internet.

During the year 2001 the Committee has provided advice on a wide range of chemicals. Three statements were published; 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which is principally a food contaminant, 1,3-dichlorpropanol and 2,3-dichloropropanol which may be present as contaminants in drinking water, and interim conclusions on the evidence which has been used to support claims that there is an increase in the incidence of intrahepatic cholangiocarcinoma. With regard to this latter statement, the Committee made a number of recommendations for further work to assist in the evaluation of the reported increase in this tumour. The Committee has initiated a revision of its guidelines with particular emphasis being placed on risk assessment of chemical carcinogens. In this respect the Committee has undertaken some discussion of the ranking of chemical carcinogens according to carcinogenic potency.

The Committee has reviewed the Government's response to the Phillips' report on the BSE enquiry and agreed that most of its procedures were appropriate but initiated a number of changes, for example publishing lay summaries and glossaries of statements.

Finally I would like to record my thanks and appreciation to Professor James Parry who stood down as COM chair during this year. Jim had a wealth of expertise and experience and provided sound advice to COC. He will be missed by members.

Professor P.G. Blain (Chairman) CBE

BMedSci MB PhD FRCP (Lond) FRCP (Edin) FFOM CBiol FIBiol

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

3.1 2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD or TCDD) is a member of a class of chemicals known as dioxins. The term dioxins refers to a group of chlorinated hydrocarbons comprising the polychlorinated dibenzo-*p*-dioxins (PCDDs) and the polychlorinated dibenzofurans (PCDFs). In September 2000, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) commenced a review of the risk assessments of dioxins carried out by the World Health Organisation (WHO) (1), the Scientific Committee on Food (SCF; http://europe.eu.int/comm/food/fs/sc/scf/out78_en.pdf), and the United States Environmental Protection Agency (US-EPA; (www.epa.gov/ncea/pdfs/dioxin/). As part of this evaluation the COT asked for COC advice on the evidence concerning human cancer risks. The COT also asked COC for a view on the approach suggested by the EPA as outlined in its draft risk assessment of dioxins.

3.2 The Committee had reviewed the available information on TCDD in 1993 and again in 1998 following the publication of the World Health Organisation's International Agency for Research on Cancer monograph on TCDD in 1997. In 1998 the Committee had concluded that that there were insufficient epidemiological and toxicological data on TCDD to conclude a causal link with cancer in humans, but that it would be prudent to consider TCDD as a "probable weak human carcinogen".

3.3 Epidemiology studies on the association between exposure to TCDD and other dioxins and cancer published since 1998 are predominantly updates of cohort investigations previously considered by the COC. The Committee agreed that, in general, these publications presented analysis of more data and had improved statistical power compared to previous studies reviewed in 1998. Although the studies were all of cohorts with exposures to mixtures of chemicals, the authors had attempted to model past exposure to TCDD and to investigate dose-response. An attempt had been made in some studies to make allowance for confounding factors (eg smoking) by making internal comparisons but in no case were individual smoking data available. It was noted that the approach taken in most of the studies to assess dose-response was back-extrapolation of TCDD or TCDD-equivalent (TEQ) levels in blood to estimate the body-burdens at the time of occupational exposure. This was generally considered to be the most appropriate approach that could be taken, particularly if the half-life of TCDD used in such calculations had been adjusted for body fat and age.

3.4 There was considerably more information available on the mechanisms by which TCDD could induce cancer and this was of particular help to the Committee in deriving conclusions.

3.5 Overall the Committee agreed that TCDD should be regarded as a probable human carcinogen on the basis of all the available data. The Committee agreed that, although a precise mechanism for carcinogenesis in laboratory animals or humans could not be elucidated from the available information, the data (ie negative genotoxicity in standard assays, and evidence from studies of mechanisms) suggested that a threshold approach to risk assessment was likely to be appropriate. In this respect Members commented that any increased risk of cancer at background levels of exposure is

likely to be extremely small and not detectable by current epidemiological methods.

3.6 The Committee considered that the approach taken by the EPA in its draft risk assessment was consistent with the general approach outlined by the agency in its proposed guidelines for carcinogen risk assessment. In conclusion, the Committee agreed that the review of cancer epidemiology studies and risk characterisation of cancer undertaken by EPA as part of its review of TCDD and related compounds was a detailed and valuable scientific assessment but the derivation of quantitative estimates used by the EPA (ED_{01} and the slope factor and risk at background exposure levels) were not considered appropriate by COC for risk assessment.

3.7 A finalised COC statement can be found at the end of this Annual Report.

1,3-Dichloropropan-2-ol and 2,3-dichloropropan-1-ol

3.8 1,3-Dichloropropan-2-ol (1,3-DCP) and 2,3-dichloropropan-1-ol (2,3-DCP) are members of a group of chemicals called chloropropanols. This group includes 3-monochloropropane-1,2-diol (3-MCPD), which has recently been considered by both the COM and the COC (see 2000 Annual Report). Like 3-MCPD, 1,3-DCP and 2,3-DCP can be present as contaminants in epichlorohydrin/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 therefore 1,3-DCP and 2,3-DCP may potentially be present as contaminants in drinking water arising from this use. 1,3-DCP, like 3-MCPD, can also be present as a process contaminant of food where acid-hydrolysed vegetable protein has been used as an ingredient, such as soy and similar oriental sauces.

3.9 The COM had advised that both 1,3-DCP and 2,3-DCP were mutagenic in-vitro and it would be prudent to regard 1,3-DCP and 2,3-DCP as potentially genotoxic in-vivo and agreed that both compounds should be tested for genotoxicity in-vivo using the approach set out in the COM guidelines.

3.10 The Committee assessed a long term carcinogenicity bioassay undertaken with 1,3-DCP in Wistar rats, which had been previously considered by COC in summary form in 1991. At that time COC had concluded that 1,3-DCP was genotoxic and carcinogenic, although a formal committee statement was not issued. Additional information on the study was now available to the Committee, together with additional mutagenicity and metabolism data, which have been reported since 1991.

3.11 In the carcinogenicity bioassay, all tissues were examined microscopically for neoplastic lesions in all rats of the control and high dose groups at 104 weeks and in those animals in the low and mid dose groups which had died spontaneously or which were killed in extremis. In addition the following tissues were examined microscopically in all animals in low and mid-dose groups; adrenal glands, oesophagus, kidneys, lungs, thyroid gland and tongue. The Committee agreed that 1,3-DCP was clearly carcinogenic in this study and concluded that it would be prudent to assume that 1,3-DCP is a genotoxic carcinogen and that exposures to 1,3-DCP should be reduced to as low a level as technologically feasible.

3.12 There were very few toxicological data for 2,3-DCP available to the committee and carcinogenicity studies have not been carried out. Theoretically, 2,3- DCP could be metabolised to produce mutagenic chemicals, i.e. epichlorohydrin (and subsequently glycidol) and therefore has structural alerts for genotoxicity and carcinogenicity. The Committee agreed it would be prudent to assume that 2,3-DCP may possess genotoxic activity in-vivo. Although no carcinogenicity data are available, it would however be prudent to reduce exposures to 2,3-DCP to as low a level as technologically feasible.

3.13 Additionally, in view of the possible human exposure through drinking water and certain foods, the Committee recommended that relevant regulatory authorities should review the likely exposures of these compounds with the intention of achieving the above recommendations.

3.14 A finalised COC statement can be found at the end of this Annual Report

Increase in mortality rates from intrahepatic cholangiocarcinoma in England and Wales 1968-1998.

3.15 The Committee first considered this subject in 2000, when it reviewed a pre-publication paper by Professor Howard Thomas and colleagues (of the Department of Medicine at Imperial College of Science Technology and Medicine), which reported an increase in the mortality from intrahepatic cholangiocarcinoma over the period 1968-1998. The Committee at that time advised that changes in diagnostic standards over time could have accounted for the reported increase but concluded that it was important to keep this topic under review. During 2001, the Committee saw a revised version of the paper from Professor Thomas and colleagues, subsequently published (*Taylor-Robinson SD, Toledano MB, Arora S, Keegan TJ, Hargreaves S, Beck A, Khan SA, Elliott P and Thomas HC (2001). Increase in mortality rates from intrahepatic cholangiocarcinoma in England and Wales 1968-1998. Gut, **48**, 816-820*), and other new information on this issue.

3.16 The Committee noted that a substantial increase in the reported rate of mortality from intrahepatic cholangiocarcinoma had been documented in both England and Wales and in the US over the last 30 years or so and concluded that changes in diagnostic standards over time could contribute to this increase. It was therefore important to undertake additional investigations before a definite conclusion could be reached as to whether there had been a real increase in this tumour. The Committee recommended that:

i.) an integrated pathological and clinical review of cases was needed to ascertain the accuracy of diagnosis of intrahepatic cholangiocarcinoma and, in particular, the potential for diagnostic transfer from secondary adenocarcinoma in the liver, for example, from carcinoma of the pancreas to intrahepatic cholangiocarcinoma.

ii.) further evaluation of time-trends for intrahepatic cholangiocarcinoma in other countries, particularly with reference to the introduction of improvements in diagnostic imaging, would be helpful.

iii.) consideration should be given to examination of information on the diagnosis of intrahepatic cholangiocarcinoma in a number of specialist liver units before and after the introduction of better diagnostic imaging techniques.

iv.) whether or not the recorded increase in incidence of intrahepatic

v.) cholangiocarcinoma was artefactual, it was clearly higher than was previously believed to be the case. Given the poor prognosis from this cancer, it was important to improve our understanding of its aetiology

vi.) the topic should be kept under review.

3.17 A full COC statement can be found at the end of this Annual Report

Review of Committee Procedures

Committee procedures in the light of Phillips enquiry

3.18 The report of the BSE Enquiry, published in October 2000, included a number of findings relating to the role of scientific advisory committees and the Government's assessment and use of scientific advice. The Government's interim response to the Enquiry's report was put out for public consultation (9 February to 30 April 2001).The Committee now considered the Government's final response which incorporated views expressed during the consultation exercise.

3.19 The Committee commented on a number of areas highlighted by the report as being important. Members agreed that the Committee already fulfils the requirement for horizon scanning with respect to generic issues relating to cancer risk assessment. However, identifying future cancer 'scares' was far more problematic. In respect of the recommendation to seek wider consultation and scientific views outside the mainstream, members considered that they would consult on important generic documents, but this would need to be considered on a case by case basis.

3.20 The Committee agreed that openness could be increased by making more background papers available on the internet (after discussion and approval by members), to support the detailed statements which the Committee already publishes. Joint meetings on general areas of interest to the COC/COT/COM, which would be open to the public, could be held once every year or 18 months, such as the recent symposium on genomics and proteomics. It was considered that COC statements were detailed and acknowledged areas of difficulty and uncertainty.

3.21 Members considered that mechanism are already available which enable quick *ad hoc* advice to be sought when required. This might involve obtaining advice from the chairman and/or members by post/e-mail, or the setting up of working groups and additional extraordinary meetings if necessary. The Committee agreed that contingency planning was not applicable to the COC, as it is not involved in risk management.

3.22 The COC considered that it would be difficult to improve communication of its advice to the public because of its specialist and very technical nature. Members were informed that, to help improve communication, a 'what's new' section had been placed on the COC website. It was considered that glossaries and lay summaries could help with public understanding. Members agreed that it would be useful to have an item each year where members suggested areas or topics that needed further consideration in the light of new evidence.

Test strategies and Evaluation

Ranking of carcinogens; Comparison of methods using some air pollutants

3.23 The Committee had previously considered, in 1995, the utility of ranking chemical carcinogens by use of the potency estimates based on the results of animal tests. The DH Toxicology Unit at Imperial College of Science Technology and Medicine, in collaboration with the secretariat, had now produced a paper for the Committee which attempted to rank a number of air pollutants using a range of methods derived from both animal carcinogenicity data and epidemiology studies.

3.24 The first objective was to compare available methods for ranking the carcinogenic potency of air pollutants and determine whether the methods allow derivation of broad potency categories for these chemicals. The second objective was to consider the performance of the T25 as a method for ranking carcinogenic potency, further to the discussion in 1995. The T25 is the daily dose (expressed as mg/kg bw/day) resulting in a tumour incidence of 25% at a specific tissue site, after correction for spontaneous incidence, within the standard study period for that species. Part of the consideration of the latter objective would involve comparing the performance of T25 with the TD50. The TD50 is defined as the daily dose rate required to halve the probability of an experimental animal of remaining tumourless at the end of its standard life-span, which requires more complex mathematical analysis of the dose response curve. The COC acknowledged that there was a need for a pragmatic method for ranking the potency of carcinogens based on animal data for use in the identification of carcinogens present in chemicals (as impurities) and in mixtures at low levels as part of the classification and labelling procedure. The COC has previously advised that the TD50 should only be used for genotoxic carcinogens. The paper presented an analysis of the rank orders of potency for seven carcinogens; arsenic, benzene, 1,3-butadiene, benzo[a]pyrene (taken as representative of polycyclic aromatic hydrocarbons), cadmium, nickel and ozone. A range of measures was used for each carcinogen: ED10 (dose associated with a 10% extra risk of tumours), inhalation unit risk estimate, NEHEL (No Expected Human Effect Level), TD50 and T25. Members agreed that the exercise had provided some useful information on the derivation of different indices of potency but it would be necessary to undertake a further evaluation of a greater number of compounds in order to draw any conclusions regarding a comparison of these methods. It was not possible to draw any conclusions with regard to the air pollutants examined, as relevant potency estimates were not available for all of the chemicals under consideration. The information presented in the paper highlighted the need to

evaluate the usefulness of proposed potency estimates based on animal data by comparing them with estimates based on appropriate human data.

3.25 The Committee asked that a further paper looking at a wider range of chemicals should be provided for consideration at a future meeting.

ECETOC* Workshop on use of T_{25} in chemical carcinogen regulation

3.26 The Committee previously discussed the T25 in 1995, when it concluded that the T25 should be used with caution, and that the TD50 was still the most practical quantitative estimate of carcinogenic potency available for the ranking of genotoxic carcinogens.

3.27 ECETOC (*European Centre for Ecotoxicology and Toxicology of Chemicals) held a workshop on 10 November 2000 to carry out a scientific analysis of the proposed uses of the T25 in chemical carcinogen regulation. An overview of this workshop was available and the Committee was asked whether it wished to revise its view of the T25 in the light of the workshop and other developments.

3.28 The COC considered that the use of the T25 in potency ranking of genotoxic carcinogens was an acceptable pragmatic approach but that the parameter should not be over interpreted. Ranking of genotoxic carcinogens was of value in prioritisation of chemicals for risk re-evaluation. It was noted that there was no basis for the use of the T25 to rank non-genotoxic carcinogens, for which tolerable exposure levels could be derived using an approach based on knowledge of mechanism, identification of no adverse effect level, and use of uncertainty factors. The Committee considered that the use of the T25 was acceptable for ranking potency of genotoxic carcinogens and to aid in the classification of carcinogens for packaging and labelling purposes. The Committee deferred a discussion of the use of the T25 in Quantitative Risk Assessment in view of the forthcoming review of the COC guidelines.

Ongoing reviews

Presentation on initial preliminary results of meta-analysis of alcohol and breast cancer

3.29 The Committee heard a presentation from the Department of Epidemiology and Public Health at Imperial College on the initial results of a meta-analysis study of the association between drinking alcohol and breast cancer. Members asked for a number of additional analyses to be undertaken. The full report will be considered when available.

Genotype and environment interaction on susceptibility to cancer

3.30 The Committee reviewed three papers prepared by the Department of Health's Toxicology Unit. There was detailed discussion of these papers and follow up papers prepared by the secretariat at all three committee meetings during 2001. A draft statement is due to be considered at the first meeting in 2002.

Minimum duration of carcinogenicity studies.

3.31 The Committee reviewed two published papers, which had expressed opposing views on the need for carcinogenicity studies to entail dosing of rats for up to at least 2 years. This is the current requirement in all internationally accepted guidelines for testing (such as the OECD guidelines). A statement is due to be considered at the first meeting in 2002.

Review of COC guidelines

3.32 The Committee agreed to update its guidelines (published in 1991) with particular emphasis on producing advice on the risk assessment of chemical carcinogens.

Use of transgenic animal models in short term tests for carcinogenicity

3.33 The International Life Sciences Institute and the Health and Environmental Sciences Institute (ILSI/HESI) in the USA recently published research on the use of a variety of animal models in carcinogenicity testing (in *Toxicologic Pathology*, volume 29, supplement pp1-351). These models all use shorter exposure periods than the classical lifetime rodent carcinogenicity bioassays. The Committee has reviewed this research and forwarded a statement to ILSI/HESI.

Joint meeting of the COT, COC and COM

3.34 In response to the recommendations arising from Sir Robert May's "Review of Risk Procedures used by the Government's Advisory Committees dealing with Food Safety," the COT, COC and the COM, identified a need for more discussion and joint working between these three committees. Toxicogenomics and proteomics had been highlighted as rapidly growing research areas of toxicological science. The Committees decided to hold a joint meeting to discuss their applicability in risk assessment.

A brief summary of the meeting is presented in the COT section of this Annual Report. A detailed publication is currently being drafted and a statement will be published on the COT/COC/COM websites.

Statements of the COC

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

1,3-Dichloropropan-2-ol and 2,3-dichloropropan-1-ol

Increase in mortality rates from intrahepatic cholangiocarcinoma in England and Wales 1968-1998.

CARCINOGENICITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

Introduction

1. 2,3,7,8- tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD or TCDD) is a member of a class of chemicals known as dioxins. The term dioxins refers to a group of chlorinated hydrocarbons comprising the polychlorinated dibenzo-p-dioxins (PCDDs) and the polychlorinated dibenzofurans (PCDFs). In September 2000, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) commenced a review of the risk assessments of dioxins carried out by the World Health Organisation (WHO)[1], the Scientific Committee on Food (SCF; http://europe.eu.int/comm/food/fs/sc/scf/out78_en.pdf), and the United States Environmental Protection Agency (US-EPA; (www.epa.gov/ncea/pdfs/dioxin/). As part of this evaluation the COT asked for COC advice on the evidence concerning human cancer risks. The COT asked COC for a view on the approach suggested by the EPA as outlined in its draft risk assessment of dioxins.

2. The COC considered the available epidemiological and experimental data in 1993 when the Committee concluded "...that there was insufficient evidence for a causal link, but it would be prudent at present to regard TCDD as a possible human carcinogen." This was a similar conclusion to that reached by the WHO International Agency for Research on Cancer (IARC) in 1987 when TCDD was classified in group 2B (ie possibly carcinogenic to humans). The IARC undertook a further review of the literature in 1997 and concluded that TCDD should be considered as a definite human carcinogen (ie Group 1 carcinogen) [2]. The Committee reviewed the IARC monograph in 1998 and, specifically, the critical epidemiology studies on TCDD cited in the monograph, ie those investigations which considered individuals whose exposure to TCDD occurred under industrial situations and was documented to be substantially higher than background exposures from environmental sources of TCDD[3-16]. The Committee also considered the literature on animal studies and investigations of the carcinogenic mechanism of TCDD in animals as cited in the monograph and a number of papers on the toxicological mechanisms of TCDD[17-27]. Members considered that TCDD was a potent carcinogen in laboratory animals. However, the information from the most heavily occupationally exposed cohorts suggested there was, at most, only a weak carcinogenic effect in these individuals. The Committee thus concluded, in 1998, that there were insufficient epidemiological and toxicological data on TCDD to conclude a causal link with cancer in humans, but that it would be prudent to consider TCDD as a "probable weak human carcinogen".

Review of epidemiology studies published since 1998

3. Epidemiology studies on the association between exposure to TCDD and other dioxins and cancer published since 1998 are predominantly updates of cohort investigations previously considered by the COC. The Committee agreed that, in general, these publications presented analysis of more data and had improved statistical power compared to previous studies [28-33]. Although the studies were all of cohorts with exposures to mixtures of chemicals, the authors had attempted to model past exposure to TCDD and to investigate dose-response. An attempt had been made in some studies to make allowance for confounding factors (eg smoking) by making

internal comparisons but in no case were individual smoking data available. It was noted that the approach taken in most of the studies to assess dose-response using back-extrapolation of TCDD or TEQ* levels in blood to estimate the body-burdens at the time of occupational exposure was generally the most appropriate approach that could be taken, particularly if the half-life of TCDD used in such calculations had been adjusted for body fat and age.

4. In reviewing the industrial cohort studies, Members agreed that the assessment of cancer incidence undertaken by Flesch-Janys et al, 1999 [28], on a possible association with breast cancer and by Lynge, 1998 [32], provided very limited data because of limitations due to small size, low power or inadequate exposure estimation. Members agreed that no definite conclusions could be reached on the basis of the ecological study of cancer incidence near to a municipal incinerator in France [33]. The Committee considered that the results from the updates of the cancer mortality studies using the Hamburg [29], NIOSH (US National Institute for Occupational Safety and Health) [30], and Dutch [31] cohorts provided evidence for an excess total cancer mortality in exposed individuals in these cohorts of 13%-50%. The dose-response analyses, using estimated TCDD doses, showed significant results for total cancer mortality in all three studies. The highest increase in lung cancer mortality of 50% was documented in the Hamburg cohort [29]. However, trend tests using the Hamburg cohort for lung cancer using TCDD or TEQ quartiles as estimates of exposure were not statistically significant. The Committee concluded that the back-extrapolation of exposure undertaken for the Hamburg and Dutch cohorts had been adequately undertaken (albeit on a minority of workers) but expressed reservations about the adequacy of the exposure estimate derived for the NIOSH cohort.

5. A 20-year mortality follow-up of the Seveso cohort had recently been published [34]. Members noted that this cohort provided valuable information on the association between exposure to TCDD and cancer since the accident had resulted in exposure to TCDD and not a mixture of dioxins, and the exposed group included both men and women. In addition, the follow-up and documentation of this study were excellent with over 99% of the cohort traced. The authors reported a 10% increase in risk of total cancer mortality in males but not in females. Among males, there was a 30% increase in mortality from respiratory cancer. There were also significant increases in risk of mortality from lymphohaematopoietic cancers in both sexes (males 70%, females 80%). The risk of Hodgkin's disease was elevated in the first 10 years of follow-up whilst risk of non-Hodgkin's lymphoma and myeloid leukaemia were increased after 15 years. Members noted that lymphohaematopoietic cancers had not been identified in the industrial cohorts and commented that it would be important to continue to monitor the literature for evidence of these particular cancers associated with exposure to dioxins.

6. Overall the Committee agreed that the epidemiological data provided limited evidence of carcinogenicity. Since a positive association had been observed between exposure to TCDD and an increase in relative risk for total cancer mortality, a causal interpretation of these data was considered credible, but bias or confounding could not be ruled out. Members commented that the data were still too inconsistent to draw conclusions with regard to lung cancer. The Committee concurred with the view expressed in the draft EPA risk assessment that cancer risk attributable to dioxins related to lifetime exposures and there was therefore no reason to anticipate that children were at any different risk to adults.

Review of Quantitative Risk Assessment undertaken by EPA using epidemiology studies.

7. The Committee noted that the approach taken by the EPA in its draft risk assessment was consistent with the general approach outlined by the agency in its proposed guidelines for carcinogen risk assessment [35]. In brief, dose-response data (based on estimated or calculated body-burdens of TCDD or TEQs) derived from the available industrial cohort epidemiological studies had been used to estimate the ED01 (dose level giving rise to 1% response). A linear extrapolation from the ED01 to zero had been used to estimate risk at background body-burdens.

8. The Committee considered that modelling of dose-response data from the industrial cohort epidemiology studies was limited by the variable quality of the exposure estimations (ie extrapolation from a sub-cohort, or use of work history to estimate exposures in members of cohort for whom no biological monitoring data were available) and the uncertainties associated with back-extrapolating estimates of body burden. Members considered that the data from the NIOSH cohort were not adequate for dose-response modelling as blood/adipose tissue data on TCDD/TEQs were not available and thus inclusion of data from this study in the summary dose-response model limited the value of this particular analysis undertaken by the EPA. The Committee agreed that the linear extrapolation for ED01 to estimate risks at background body-burdens was not acceptable in that the predicted kinetic profile of TCDD and other dioxins following occupational exposure predominantly via the skin over several decades was considerably different to that of background exposure via the diet. In addition, the available mechanistic data suggest a complex multi-step process involving receptor binding which is more likely to be consistent with a threshold-related response.

9. In conclusion, the Committee agreed that the review of cancer epidemiology studies and risk characterisation of cancer undertaken by EPA as part of its review of TCDD and related compounds was a detailed and valuable scientific assessment but the derivation of ED01 and the slope factor and risk at background exposure levels were not appropriate for risk assessment.

Review of mechanism data

10. The Committee agreed that there is good evidence to assume that most of the toxic effects of dioxins were consequent to an initial binding to the Ah receptor (AHR) [36]. Most of the evidence on TCDD induced gene transcription related to the CYP1A1 gene. It was now clear that the sequence of events from binding to AHR to transcription was very complex involving other transcription factors, chaperones such as HSP90 and regulatory proteins such as ARA9. Heterodimerisation of AHR with (Ah Receptor Nuclear Transfer Factor) (ARNT) within the nucleus is essential for TCDD activated AHR to induce DNA binding and transactivation. Heterodimerisation can also occur with hypoxia inducible factor 1-a (HIF1-a) and AHR repressor [36,37]. There is also evidence that levels of these proteins may be regulated by cell type and activation and by stages of growth and differentiation. In addition there was some evidence to suggest that the phosphorylation status of AHR is important with regard to the mechanism of TCDD toxicity [38]. Overall, the data were consistent with a complex multi-step process involving receptor binding and thus a threshold interpretation of TCDD induced carcinogenicity. Members noted

that, although events leading up to gene transcription are quite well understood, there is very little information on how AHR induced gene-transcription leads to cancer. There was good in-vivo evidence to support the view that AHR was involved in the acute and chronic toxic effects of TCDD, including the fact that AHR null allele ("knockout") mice strains are very resistant to TCDD toxicity. However there was evidence from such strains of mice that, at very high doses, TCDD could produce toxic effects via other mechanisms. The biological significance of this is questionable since the doses required to produce these effects are higher than those tolerated in wild type animals. Overall, there are gaps in our understanding of how TCDD causes cancer. Unfortunately, the AHR knockout mice do not survive long enough to be used in a conventional cancer bioassay and it is therefore not possible to provide a clear answer on the role of AHR in TCDD-induced carcinogenesis.

General Discussion

11. The Committee reconsidered its 1998 statement and agreed that TCDD was a multi-site carcinogen in several species of laboratory animals. Members confirmed that, in addition to the limitations of the dose-response modelling of epidemiological data, which are discussed in the preceding sections of this statement, it was also inappropriate to undertake quantitative risk assessment for cancer by modelling the dose-response for tumour data in animals fed diets containing TCDD in view of the assumptions needed for extrapolation from high doses used in such studies to background environmental exposures and the uncertainties involved in inter-species extrapolation. Members agreed that the mechanism of carcinogenicity in animals was complex and it was not possible to make any detailed comment on the role of the AHR. Members thought that molecular studies of tumours from animals exposed to TCDD might be helpful with regard to identification of tumour promotion effects of TCDD. It was noted that prenatal treatment of rats with TCDD followed by postnatal treatment with the genotoxic carcinogen dimethylbenzanthracene resulted in an increased number of mammary gland adenocarcinomas compared to animals that had not been treated with TCDD. A proliferative effect of TCDD on the terminal end buds of mammary gland ductules was noted. The data were consistent with the hypothesis that TCDD has a tumour promoting effect [39].

12. The Committee confirmed that it was not possible to comment in detail on the role of AHR mediated gene transcription in humans with regard to cancer. Members were aware of the evidence for polymorphism of the AHR gene in humans [40-42], but agreed that the functional significance of these polymorphisms for risk of carcinogenicity had not been adequately investigated for any conclusions to be drawn at present.

13. Members considered that it was important to review all the available dose-response data from the epidemiology studies to determine whether there was an adequate margin of safety between reported dioxin body burdens associated with an increased risk of cancer in epidemiological studies and average background body burdens. Members noted that the nature and time-course for TEQ or TCDD body-burdens following average lifetime exposure, occupational exposure to dioxins for 20-30 years, or following exposure to TCDD after the Seveso accident were different and agreed with the evaluation of this aspect presented in the draft EPA risk characterisation document. In the case of background exposure via the diet, body burdens would gradually increase up to steady state levels at about 40 years of age. Occupational exposure would be associated with a substantially greater build up of

dioxin body burden to a peak level then gradual elimination of dioxins following cessation of occupational exposure. The time-course following the Seveso accident would have been characterised by a rapid rise up to a peak body burden followed by gradual elimination of TCDD back to background levels. It was therefore difficult to compare these different exposure profiles.

14. The Committee noted that the average body burdens of dioxin in the general population were estimated to be 1-2 orders of magnitude lower than in the critical industrial cohort studies as suggested in the draft EPA risk assessment. However, in terms of TCDD blood lipid concentrations, background levels were estimated to be 2-3 orders of magnitude lower than in the critical industrial cohort studies at the time of last exposure, but only one order of magnitude lower than the Seveso cohort [43]. Members agreed that, in view of the difficulties in selecting the appropriate metric of exposure, it was not possible to quantify the margin-of-safety risk assessment. However, Members noted that the excess cancer mortality reported in the heavily exposed industrial cohorts was small and commented that any increased risk of cancer at background levels of exposure is likely to be extremely small and not detectable by current epidemiological methods.

Conclusion

15. The COC agreed that TCDD should be regarded as a probable human carcinogen on the basis of all the available data. The Committee agreed that, although a precise mechanism for carcinogenesis in laboratory animals or humans could not be elucidated from the available information, the data (ie negative genotoxicity in standard assays, and evidence from studies of mechanisms) suggested that a threshold approach to risk assessment was likely to be appropriate. In this respect Members commented that any increased risk of cancer at background levels of exposure is likely to be extremely small and not detectable by current epidemiological methods.

*TEQ = 2,3,7,8-TCDD Toxic Equivalence of a mixture of dioxins. The method of calculation is derived by multiplying the Toxic Equivalency Factor (TEF) for each dioxin congener by its mass concentration and then the product is summed to produce the TEQ of the mixture. The TEF is a measure of the potency of each dioxin congener relative to 2,3,7,8-TCDD which has been internationally agreed by regulatory authorities.

July 2001

COC/01/S2 - July 2001

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CARCINOGENICITY OF 1,3-DICHLOROPROPAN-2-OL (1,3 DCP) AND 2,3-DICHLOROPROPAN-1-OL (2,3 DCP)

Introduction

1. The chloropropanols are a group of chemicals which include 3-chloro-1,2-propanediol (3-MCPD), 1,3-Dichloropropan-2-ol (1,3-DCP) and 2,3 dichloropropan-1-ol (2,3 DCP). Chloropropanols are contaminants of some foodstuffs and of polyamine flocculants used in the treatment of drinking water. Both the COC and COM have published statements on 3-MCPD[1,2].

2. The COM have reviewed mutagenicity data for 1,3 DCP and 2,3 DCP and concluded "that it would be prudent to regard 1,3-DCP and 2,3-DCP as potentially genotoxic in vivo and agreed that both compounds should be tested for genotoxicity in-vivo using the approach set out in the COM guidelines" [3].

1,3 DCP

3. Available toxicology, mutagenicity and carcinogenicity data for 1,3 DCP has been summarised by the Joint FAO/WHO Committee on Food Additives (JECFA) [4] although much of the key data remain unpublished. From a 13-week oral toxicity study, a NOAEL of 1mg/kg/day had been identified. Limited information on the metabolism of 1,3 DCP indicates that it may be metabolised to form epichlorohydrin, which may, via glycidol, be conjugated to form mercapturic acid derivatives [5]. In vitro investigations with hepatocyte cultures indicate also a pathway involving CYP2E1 to dichloroacetone (a directly acting cytotoxic compound) leading to glutathione depletion[6-10].

4. The results of mutagenicity studies with bacteria and mammalian cells show that 1,3-DCP is mutagenic in vitro. It has been suggested that the in-vitro genotoxicity of 1,3-DCP is due to the chemical formation of epichlorohydrin [11]. 1,3 DCP was negative in a SMART assay in Drosophila [3].

5. A 104-week toxicology and carcinogenicity study with 1,3 DCP in Wistar rats [12] was previously considered by COC in 1991. At the time COC concluded that 1,3 DCP was genotoxic and carcinogenic, although a formal committee statement was not issued. Additional information on the study is now available to the Committee, together with additional mutagenicity and metabolism data which have been reported since 1991.

Carcinogenicity study [12]

6. 1,3 DCP was administered in the drinking water to Wistar rats for 104 weeks. The study comprised four groups each containing 80 males and 80 females who received 1,3 DCP at concentrations of 0 mg/l (control), 27 mg/l (low dose), 80 mg/l (medium dose) or 240 mg/l (high dose). These concentrations were equivalent to 0, 3, 10 or 30 mg/kg bw/d for male rats and 0, 2, 6 or 19 mg/kg bw/d for female rats. Interim sacrifices of 10 animals per sex per group were carried out at weeks 26, 52 and 78 of the study, leaving 50 animals per group who were exposed to 1,3 DCP for the full 104

weeks. Parameters evaluated in the study included; mortality, body weight, feed consumption, haematology, urinalysis, clinical chemistry, organ weights and gross pathological and histopathological changes.

7. Mortality of high dose males and females was increased when compared with controls and other dose groups. No treatment related changes in appearance and behaviour were noted in any group, and mean food and water consumption values were similar for all groups throughout the study. Mean body weight gain of all groups showed no treatment-related changes until week 75 for males and week 79 for females when statistically significant and dose-related decreases in mean body weight were seen in the high dose group. The assessment of clinical biochemistry and urinalysis data suggested an hepatotoxic effect with space-occupying lesions in mid dose and high dose groups.

8. At 104 weeks all tissues were examined microscopically for neoplastic lesions in all rats of the control and high dose groups and in those animals in the low and mid dose groups who had died spontaneously or who were killed in extremis. In addition the following tissues were examined microscopically in all animals in low and mid-dose groups ; adrenal glands, oesophagus, kidneys, lungs, thyroid gland and tongue.

9. Non neoplastic lesions considered to be treatment related were observed as follows:

   i) In the liver,

      - An increased incidence of slight to moderate fatty change along with increased incidence of haemosiderin-containing Kupffer cells in the mid and high dose groups; this was considered to reflect metabolic disturbance of the liver caused by 1,3 DCP.
      - A dose-dependent increase in sinusoidal peliosis in treated animals ; peliosis may represent a pre-neoplastic stage of vascular hepatic neoplasia such as haemangiosarcoma
      - Eosinophilic foci in mid and high dose groups and glycogen -free foci in the high dose group

   ii) In the kidney, there was a high level of chronic progressive nephrosis (CPN) in all groups of male rats in the study, ranging from 40/50 in controls to 48/50 in high dose males.

   iii) In the thyroid, 1,3 DCP induced thyroid follicular cell hyperplasia in dose-related manner in both male and female rats.

   iv) Increased incidences of neoplastic lesions were observed in the liver, tongue and thyroid in both sexes and additionally in the kidney of male rats.

   v) In the liver, combined incidences of hepatocellular adenoma and carcinoma, showed a statistically significant dose-related increase ($p<0.001$) in both males and females. (eg males -controls 1/50, high dose 8/50; females - controls 1/50, high dose 41/50).

   vi) In the tongue, combined incidences of squamous cell papilloma and carcinoma showed a statistically significant dose-related increase ($p<0.001$) in both males and females. (eg males - controls 0/50, high dose 12/50; females - controls 0/49, high dose 11/49).

vii) In the thyroid combined incidences of follicular cell adenoma and carcinoma showed a statistically significant dose-related increase ($p<0.001$) in both males and females (eg males - controls 0/50, high dose 4/50; females - controls 1/49, high dose 5/49).

viii) In the kidney, combined incidences of renal tubular adenoma and carcinoma, showed a statistically significant dose-related increase ($p<0.001$) in males only (eg controls 0/50, high dose 9/50).

10. Regarding the onset of oncogenic lesions, the following findings were reported at the interim sacrifices :

at 26 weeks, hepatocellular adenoma (1/10 mid dose males) ;

at 52 weeks, liver carcinoma (1/10 high dose females) and,

at 78 weeks, hepatocellular carcinomas (7/10 high dose females ; 3/10 high dose males), lingual papilloma (1/10 mid dose males and 1/10 high dose males), and renal tubular adenomas (1/10 high dose males).

11. It was concluded that the spectrum of tumours observed in the 104-weeks rat study, particularly in the liver and tongue was evidence of a clear carcinogenic effect of 1,3 DCP. It was possible that the tumours in the male kidney could be associated with the high rate of chronic progressive nephropathy observed in the study and additionally, the thyroid follicular cell tumours could be associated with hyperplasia, a toxic finding commonly seen in male rats, although no specific mechanism data were available.

2,3 DCP

12. There are very few toxicological data for 2,3 DCP and carcinogenicity studies have not been carried out. Theoretically, 2,3 DCP could be metabolised to produce epichlorohydrin (and subsequently glycidol) and therefore has structural alerts for genotoxicity and carcinogenicity.

13. COM have recently considered 2,3 DCP [3] and while there is evidence of genotoxicity in-vitro, no studies have been performed in-vivo. COM concluded that it would be prudent to regard 2,3-DCP as potentially genotoxic in-vivo.

14. Although there are no carcinogenicity studies available for 2,3 DCP, IARC have recently evaluated the brominated analogue, 2,3 dibromo-propanol (2,3 DBP) [12] and considered that "there is sufficient evidence in experimental animals for the carcinogenicity of 2,3 dibromo propan-1-ol" Skin appplication of 2,3 DBP produced multisite tumours in both rats and mice. However, the Committee considered that no conclusions could be drawn from these studies in respect of the carcinogenicity of 2,3 DCP.

Conclusions

15. The Committee came to the following conclusions ;

i) It is prudent to assume that 1,3 DCP is a genotoxic carcinogen and that exposures to 1,3 DCP should be reduced to as low a level as technologically feasible

ii) It is prudent to assume that 2,3 DCP may posses genotoxic activity in-vivo. Although no carcinogenicity data are available, it would however be prudent to reduce exposures to 2,3 DCP to as low a level as technologically feasible

iii) Additionally, in view of the possible human exposure through drinking water and certain foods, the Committee recommended that relevant regulatory authorities should review the likely exposures of these compounds with the intention of achieving the above recommendations.

May 2001

COC/01/S1 - May 2001

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STATEMENT ON THE EVIDENCE FOR AN INCREASE IN MORTALITY RATES FROM INTRAHEPATIC CHOLANGIOCARCINOMA IN ENGLAND AND WALES 1968-1998.

Introduction

1. 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 1979-1998. A preliminary investigation suggested that the increase in mortality from liver cancer may in part be due to an increase in mortality from intrahepatic cholangiocarcinoma.[1] Intrahepatic cholangiocarcinoma is a relatively rare tumour in the UK and the prognosis for patients with this tumour is poor.[2] It is therefore necessary to review any evidence for an increase in the incidence of this tumour in order to establish whether any documented increase in mortality is real or artefactual. For example, changes in classification of tumour type, changes in the collection or coding of mortality data, or the introduction of improved diagnostic procedures for cholangiocarcinoma, such as endoscopic retrograde cholangiopancreatography (ERCP), could account for the observed increase in incidence of this tumour. However, should a genuine increase in the mortality from intrahepatic cholangiocarcinoma in England be established, it would be important to consider the aetiological factors that might be responsible for such an increase and any preventative action that could be taken.

Background to COC consideration

Evidence for an increase in mortality rate from intrahepatic cholangiocarcinoma in England and Wales 1968-1998.

2. In November 1999, the Committee reviewed a prepublication copy of a draft paper by Taylor-Robinson et al, which presented a detailed analysis of mortality data from 1968 to 1986 for tumours of the liver, and of the pancreas, obtained from the Office of National Statistics. Members heard a presentation of these data from Professor Howard Thomas and colleagues at the Division of Medicine, Imperial College of Science, Technology and Medicine. 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. The tumours considered in the report were:

ICD- 9 155	(all primary liver tumours)
ICD-9 155.0	(hepatocellular carcinoma, HCC)
ICD-9 155.1	(intrahepatic cholangiocarcinoma)
ICD-9 155.2	(liver unspecified)
ICD-9 156	(all extrahepatic biliary system tumours)
ICD-9 156.0	(gall bladder tumours)
ICD-9 156.1	(tumours of the extrahepatic bile duct)

ICD-9 157 (pancreatic tumours)

3. ASMRs per 100,000 population and, for intrahepatic cholangiocarcinoma, age-specific mortality rates (AspMR) per 100,000 population (using four age bands: 20-44y, 45-64y, 65-74y and 75y+) were calculated. Mortality data were considered to be a general indicator of incidence because prognosis from cancer in the liver is poor. Detailed evaluation of the data on intrahepatic cholangiocarcinoma was undertaken to ascertain whether the observed increase in mortality from this tumour represented a true increase in the numbers of this rare form of cancer.

4. The authors noted that in 1978 there was a total of 95 deaths in England and Wales reported from intrahepatic cholangiocarcinoma whereas there were 736 cancer deaths attributed to this tumour in 1996. The increase in 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.

5. In June 2001 the COC saw a revised version of the paper by Taylor- Robinson et al[3] (and a subsequent correction[4]) which included additional data on mortality from intrahepatic carcinoma for 1997-1998. ASMR per 100,000 population increased to 1.37 and 1.12 in 1998 for males and females, respectively, bringing the total number of deaths for that year to 864. AspMR per 100,000 population increased from 1968 to 1996, on average, 12-fold in ages 45 and over, with larger increases in older ages and women.

6. In 2001, the Committee also saw two publications reporting increases in the reported incidence and mortality rates for intrahepatic cholangiocarcinoma in the US[5,6]. The ASMR per 100,000 population in the US was reported to have increased from 0.07 in 1973 to 0.69 in 1997; and the estimated incidence rates per 100,000 from 0.13 in 1973 to 0.67 to 1997[6].

COC consideration

7. The Committee observed that the exceptionally large increase in ASMR for intrahepatic cholangiocarcinoma documented in the paper by Taylor-Robinson et al (i.e. 13.7 fold in males, 22.4 fold in females over the period 1968-1998) was a highly unusual finding. The Committee noted that a number of potential confounding factors had been considered by the authors, such as changes in criteria for ICDisease codes (ICD revisions 8 and 9), the introduction of ERCP as a new diagnostic technique for facilitating precision in the location of site for several cancers of the hepatobiliary system in the mid to late 1970s, and the potential for diagnostic transfer from tumours of the pancreas, gallbladder and extrahepatic biliary tree. The Committee commented that the reported increase in mortality from intrahepatic cholangiocarcinoma appeared to coincide with the introduction of ERCP as a new diagnostic technique. Members considered that the interpretation of the apparent increasing trend in ASMR for intrahepatic cholangiocarcinoma was complicated in that the increase extended beyond the period required for learning and correctly applying this diagnostic technique. However, Members considered that a small diagnostic transfer from secondary pancreatic cancer to intrahepatic cholangiocarcinoma, occurring as a result of increasing use of new diagnostic techniques, could account for the observed results either totally or in part. Members also considered that diagnostic transfer from secondary adenocarcinoma in the liver, which is relatively common and may be indistinguishable from intrahepatic

cholangiocarcinoma histologically, might be another explanation. Members also noted that, in both England and Wales and in the US, there had been a steady decrease in reported mortality rates from extrahepatic biliary system tumours as the reported mortality rates of intrahepatic cholangiocarcinoma had risen and considered that diagnostic transfer from extrahepatic to intrahepatic biliary system tumours could account, in part, for the reported increase in the latter. The Committee considered that the ASpMR data, which indicated higher increases in the rate of intrahepatic cholangiocarcinoma in older age groups, supported the hypothesis of diagnostic transfer. It was to be expected that cancer would be more common in older patients. In the past, investigation of cancer in the liver tended to be less extensive in older than younger patients, which may have led to less precise diagnosis and the highest level of underreporting of intrahepatic cholangiocarcinoma in older age groups. Changes in clinical practice had made it increasingly more likely that older patients would receive a precise diagnosis and thus it was in these age groups that the greatest rate of increase in recorded cases of intrahepatic cholangiocarcinoma might be anticipated.

8. The Committee noted that little was known about the aetiology of intrahepatic cholangiocarcinoma. Tumours of the intrahepatic bile ducts are common in South East Asia but are relatively rare in Western populations.[7] The most recent information from the Office of National Statistics confirms this. In 1998 there were 1921 deaths from liver cancer in England and Wales (i.e. 1.41% as a proportion of deaths from all malignant tumours) and 86 deaths from intrahepatic cholangiocarcinoma (i.e. 0.63% as a proportion of deaths from all malignant tumours). The causes of intrahepatic cholangiocarcinoma have not been fully elucidated and there are only three known risk factors. Infestation by liver flukes (distomes *Clonorchis sinensis* and *Opisthorchis viverrini*) is associated with these tumours and geographical distribution of intrahepatic cholangiocarcinoma coincides with endemic areas for infestation with such parasites. Primary sclerosing cholangitis is also associated with an increased risk of intrahepatic biliary tract carcinoma but this is likely to account for only a small number of the total cases of intrahepatic cholangiocarcinoma. Thorium dioxide (Thorotrast), which was used in the 1930-1940s as a radiological contrast agent in medicine, is also known to induce cholangiocarcinoma in humans. [7,8]

COC Conclusions

9. The Committee noted that a substantial increase in the reported rate of mortality from intrahepatic cholangiocarcinoma had been documented in both England and Wales and in the US over the last 30 years or so and concluded that changes in diagnostic standards over time could contribute to this increase. It was therefore important to undertake additional investigations before a definite conclusion could be reached about whether there had been real increase in the incidence of this tumour. The Committee recommended that:

   i) an integrated pathological and clinical review of cases was needed to ascertain the accuracy of diagnosis of intrahepatic cholangiocarcinoma and, in particular, the potential for diagnostic transfer from secondary adenocarcinoma in the liver, for example, from carcinoma of the pancreas to intrahepatic cholangiocarcinoma.

ii) further evaluation of time-trends for intrahepatic cholangiocarcinoma in other countries , particularly with reference to the introduction of improvements in diagnostic imaging, would be helpful.

iii) consideration should be given to examination of information on the diagnosis of intrahepatic cholangiocarcinoma in a number of specialist liver units before and after the introduction of better diagnostic imaging techniques.

iv) whether or not the recorded increase in incidence of intrahepatic cholangiocarcinoma was artefactual, it was clearly higher than was previously believed to be the case. Given the poor prognosis from this cancer, it was important to improve our understanding of its aetiology

v) the topic should be kept under review.

December 2001

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2001 Membership of the Committee on the Carciongenicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

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

Head of Department of Environmental and Occupational Medicine, University of Newcastle

MEMBERS

Professor C Cooper BSc PhD DSc
Head of Molecular Carcinogenesis Section, Institute of Cancer Research, Haddow Laboratories

Professor D Forman BA PhD Hon MFPHM
Professor of Cancer Epidemiology, Academic Unit of Epidemiology and Health Services research, School of Medicine, University of Leeds

Professor D Harrison BSc MB ChB MD FRCPath FRCP(Edin)
Professor and Head of Department of Pathology, University of Edinburgh Medical School

Ms Denise Howel BSc MSc Cstat FIS
Senior Lecturer in Epidemiological Statistics, Department of Epidemiology and Public Health, University of Newcastle

Dr Sandra Jane Kennedy BSc PhD FRCPath CBiol FIBiol
Director of Pharmacoproteomics and Pre-Clinical Development, Oxford GlycoSciences plc

Ms M Langley BA
Lay Member

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Professor of Genetics, School of Biological Sciences, University of Wales, Swansea

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Professor of Environmental Carcinogenesis, Institute of Cancer Research

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Professor of Biochemical Pharmacology, University of Southampton

Dr Ruth Roberts BSc PhD
Head of Cell Biology Research, Syngenta Central Toxicology Laboratory

Professor D E G Shuker BSc ARCS PhD DIC Cchem FRSC
Department of Chemistry, The Open University

Professor G T Williams BSc MD FRCP FRCPath
Department of Pathology, University of Wales College of Medicine

SECRETARIAT

J M Battershill BSc MSc (Scientific)

Diane Benford BSc PhD (Scientific – Food Standards Agency)

K N Mistry (Administrative)

R J Fielder BSc PhD Dip RCPath

Frances D Pollitt MA Dip RCPath

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	Oxford Glycosciences Unilever	Salary Share Holder	NONE	NONE
Ms M Langley	Please copy from COM		NONE	NONE
Prof J M Parry	Compass Catering JIB Insurance National Power Smith Kline 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	NONE	NONE

Member	Personal Interest		Non-Personal Interest	
	Company	*Interest*	*Company*	*Interest*
Prof A G Renwick OBE	International Sweeteners Association Targacept Novartis	Consultant Consultant Consultant	Smith Kline Beecham Pfizer	Research Support Grant
Dr R Roberts	AstraZeneca Ely-Lily P & O Zeneca	Share Holder Share Holder Share Holder Salary	Baxter Healthcare European Council for Plastics and Intermediate (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 Nationalplc 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:

Department of Health

Food Standards Agency

Department for the Environment, Food and Rural Affairs

Department of Transport, Local Government and the Regions

Department of Trade and Industry

Health and Safety Executive

Pesticide Safety Directorate

Veterinary Medicines Directorate

Medicines Control Agency

Medical Devices Agency

Home Office

Scottish Executive

National Assembly for Wales

Northern Ireland Executive

Other Government Departments and Agencies

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 page 148);
- 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 at pages 149 to150.

(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 e.g.:
  - (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, Mutageneticity, or Carcinogenicity of Chemicals in Food, Consumer Products and the Environment.

In this Code 'the Secretariat' means the Secretariat of the COT.

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 CMO and, through the CMO, the Government on a wide range of matters concerning chemicals in food, consumer products and the environment.

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

3. In discussing these proposals (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 generic 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 para 11-13 below).

Procedures for handling confidential information

Background

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

12. 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

***a priori*:** The formulation of a hypothesis before undertaking an investigation or experiment.

Acceptable Daily Intake (ADI) Estimate of the amount of a substance in food or drink, expressed on a body weight basis (e.g. mg/kg bodyweight), that can be ingested daily over a lifetime by humans without appreciable health risk.

Acute: Short term, in relation to exposure or effect.

Acute toxicity: Effects that occur over a short period of time (up to 14 days) immediately following exposure.

Adduct: A chemical grouping which is covalently bound (see covalent binding) to a large molecule such as DNA (qv) or protein.

Adenoma: A benign neoplasm arising from a gland forming epithelial tissue such as colon, stomach or respiratory tract.

Adverse effect: Change in morphology, physiology, biochemistry, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences.

Ah receptor: The Ah (Aromatic hydrocarbon) receptor protein regulates some specific gene expressions associated with toxicity. The identity of the natural endogenous chemicals which bind to the Ah receptor is unknown. Binding to the Ah receptor is an integral part of the toxicological mechanism of a range of chemicals, such as chlorinated dibenzodioxins and polychlorinated biphenyls.

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

Allele: Alternative form of a gene.

Allergen: Substance capable of stimulating an allergic reaction.

Allergy: The adverse health effects that may result from the stimulation of a specific immune response.

Allergic reaction: an adverse reaction elicited by exposure to a previously sensitised individual to the relevant antigen.

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.

Apoptosis: A form of active cell death resulting in fragmentation of the cell into membrane-bound fragments (apoptotic bodies). These are usually rapidly removed *in vivo* by

engulfment by phagocytic cells. Apoptosis can occur normally during development, but is often triggered by toxic stimuli.

Base pair (bp): Two complementary nucleotide (qv) bases joined together by chemical bonds.

Bias: In the context of epidemiological studies, an interference which at any stage of an investigation tends to produce results that depart systematically 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.

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

Bioinformatics: The science of informatics as applied to biological research. Informatics is the management and analysis of data using advanced computing techniques. Bioinformatics is particularly important as an adjunct to genomics research, because of the large amount of complex data this research generates.

Biomarker: Observable change (not necessarily pathological) in an organism, related to a specific exposure or effect.

Body burden: Total amount of a chemical present in an organism at a given time.

Bradford Hill Criteria: Sir Austin Bradford-Hill established criteria that may be used to assist in the interpretation of associations reported from epidemiological 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 disease increases likelihood that the association is causal.
- Temporality – The association must demonstrate that exposure leads to disease. The relationship of time since first exposure, duration of exposure and time since last exposure are all important in assessing causality.
- Biological gradient – If an association reveals a biological gradient or dose-response 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 the disease?

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

C. elegans: *Caenorhabditis elegans*, a nematode or roundworm, the first animal to have its genome completely sequenced and all the genes fully characterised.

Cancer: Synonym for a malignant neoplasm – that is, a tumour (qv) that grows progressively, invades local tissues and spreads to distant sites (see also tumour and metastasis).

Candidate gene: A gene that has been implicated in causing or contributing to the development of a particular disease.

Carcinogenesis: The origin, causation and development of tumours (qv). The term applies to benign as well as malignant neoplasms and not just to carcinomas (qv).

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

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 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 active 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, retrospective study) A comparison is made of the proportion of cases who have been exposed to a particular hazard (e.g. a carcinogen) with the proportion of controls who have been exposed to the hazard.

Cell 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.

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

Chromosome: In simple prokaryotic organisms, such as bacteria and most viruses, the chromosome consists of a single circular molecule of DNA containing the entire genetic material of the cell. In eukaryotic cells, the chromosomes are thread-like structures, composed mainly of DNA and protein, which are present within the nuclei of every cell. They occur in pairs, the numbers varying from one to more than 100 per nucleus in different species. Normal somatic cells in humans have 23 pairs of chromosomes, each consisting of linear sequences of DNA which are known as genes (qv).

Chronic effect: Consequence which develops slowly and has a long-lasting course (often but not always irreversible).

Chronic exposure: Continued exposures occurring over an extended period of time, or a significant fraction of the life-time of a human or test animal.

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

Clearance: Volume of blood or plasma, or mass of an organ, effectively cleared of a substance by elimination (metabolism and excretion) in a given time interval. Total clearance is the sum or the clearances for each eliminating organ or tissue.

Clone: A term which is applied to genes, cells, or entire organisms which are derived from - and are genetically identical to - a single common ancestor gene, cell, or organism, respectively. Cloning of genes and cells to create many copies in the laboratory is a common procedure essential for biomedical research.

Coding regions: those parts of the DNA that contain the information needed to form proteins. Other parts of the DNA may have non-coding functions (e.g. start-stop, pointing or timer functions) or as yet unresolved functions or maybe even 'noise'.

Codon: a set of three nucleotide bases in a DNA or RNA sequence, which together code for a unique amino acid.

Cohort: A defined population that continues to exist through time.

Cohort study: (Synonyms - follow-up, longitudinal study) The study of a group of people defined at a particular point in time (the cohort), who have particular characteristics in common, such as a particular exposure. They are then observed over a period of time for the occurrence of disease. The rate at which the disease develops in the cohort is compared with the rate in a comparison population, in which the characteristics (e.g. exposure) are absent.

Complementary DNA (cDNA): cDNA is DNA that is synthesised in the laboratory from mRNA by reverse transcription. A cDNA is so-called because its sequence is the complement of the original mRNA sequence.

Confounding variable *(synonym - confounder) An extraneous variable that satisfies BOTH of 2 conditions: (1) it is a risk factor for the disease under study (2) it is associated with the study exposure but is not a consequence of exposure. For example cigarette smoking is a confounding variable with respect to an association between alcohol consumption and heart disease. Failure to adjust for a confounding variable results in distortion of the apparent magnitude of the effect of the exposure under study. (In the example, smoking is a risk factor*

for heart disease and is associated with alcohol consumption but is not a consequence of alcohol consumption.)

***Congeners**: Related compounds varying in chemical structure but with similar biological properties.*

Covalent binding: Chemical bonding formed by the sharing of an electron pair between two atoms. Molecules are combinations of atoms bound together by covalent bonds.

Cytochrome P450 (CYP): An extensive family of haem-containing proteins involved in enzymic oxidation of a wide range of endogenous and xenobiotic (qv) substances and their conversion to forms that may be more easily excreted. In some cases the metabolites produced may be reactive and may have increased toxicity. In other cases the substances may be natural precursors of hormones (e.g. steroids).

Cytogenetic: Concerning chromosomes, their origin, structure and function.

Deletion: A chromosomal aberration in which a proportion of the chromosome is lost. Deletions may range in size from a single nucleotide (qv) to an entire chromosome. Such deletions may be harmless, may result in disease, or may in rare cases be beneficial.

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 (qv).

DNA probe: A piece of single-stranded DNA, typically labelled so that it can be detected (for example, a radioactive or fluorescent label can be used), which can single out and bind with (and only with) another specific piece of DNA. DNA probes can be used to determine which sequences are present in a given length of DNA or which genes are present in a sample of DNA.

DNA repair genes: Genes which code for proteins that correct damage in DNA sequences. When these genes are altered, mutations may be able to accumulate in the genome, ultimately resulting in disease.

***Dominant lethal assay**: See Dominant Lethal mutation.*

Dominant lethal mutation: A dominant mutation that causes death of an early embryo.

Dose: Total amount of a substance administered to, taken or absorbed by an organism

Endocrine modulator (synonym – endocrine disruptor): A chemical, which can be naturally occurring or man-made, that causes adverse health effects in an organism, as a result of changes in hormonal function.

Endonuclease: An enzyme that cleaves its nucleic acid substrate at internal sites in the nucleotide sequence.

Epidemiology: Study of the distribution and the aetiology of disease in humans.

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 or increased blood flow in the skin.

***Erythrocyte**: Red blood cell.*

Estrogen: Sex hormone or other substance capable of developing and maintaining female characteristics of the body.

Exogenous: Arising outside the body.

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

Fluorescence In-Situ Hybridisation: A technique which allows individual chromosomes and their centromeres to be visualised in cells.

Fetotoxic: Causing toxic, potentially lethal effects to the developing fetus.

Forestomach: (See glandular stomach).

Full gene sequence: the complete order of bases in a gene. This order determines which protein a gene will produce.

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 (qv).

Gene expression: The process by which the information in a gene is used to create proteins or polypeptides.

Gene families: Groups of closely related genes that make similar products.

Gene product: The protein or polypeptide coded for by a gene.

Genetic engineering: Altering the genetic material of cells or organisms in order to make them capable of making new substances or performing new functions.

Genetic polymorphism: a difference in DNA sequence among individuals, groups, or populations (e.g. a genetic polymorphism might give rise to blue eyes versus brown eyes, or straight hair versus curly hair). Genetic polymorphisms may be the result of chance processes, or may have been induced by external agents (such as viruses or radiation). Changes in DNA sequence which have been confirmed to be caused by external agents are generally called “mutations” rather than “polymorphisms”.

Genetic predisposition: susceptibility to a disease which is related to a polymorphism, which may or may not result in actual development of the disease.

Genetically modified organism(GMO): An organism which has had genetic material inserted into, or removed from, its cells.

Genome: All the genetic material in the chromosomes of a particular organism; its size is generally given as its total number of base pairs.

Genomic DNA: The basic chromosome set consisting of a species-specific number of linkage groups and the genes contained therein.

Genomics: The study of genes and their function.

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

Genotype: The particular genetic pattern seen in the DNA of an individual. “Genotype” is usually used to refer to the particular pair of alleles that an individual possesses at a certain location in the genome. Compare this with phenotype.

Glandular stomach: The stomach in rodents consists of two separate regions - the forestomach and the glandular stomach. Only the glandular stomach is directly comparable to the human stomach.

Half-life: Time in which the concentration of a substance will be reduced by half, assuming a first order elimination process.

Hazard: Set of inherent properties of a substance, mixture of substances or a process involving substances that make it capable of causing adverse effects to organisms or the environment.

Hepatic: Pertaining to the liver

Hepatocyte: The principal cell type in the liver, possessing many metabolising enzymes (see 'metabolic activation').

Hepatotoxic: Causing toxicity to the liver.

Human Genome Project: An international research effort aimed at discovering the full sequence of bases in the human genome, led in the UK by the Wellcome Trust and Medical Research Council.

Hyperplasia: An increase in the size of an organ or tissue due to an increase in the number of cells.

Hypertrophy: An increase in the size of an organ or tissue due to an increase in the volume of individual cells within it.

Idiosyncrasy: Specific (and usually unexplained) reaction of an individual to e.g. a chemical exposure to which most other individuals do not react at all. General allergic reactions do not fall into this category.

In situ hybridisation (ISH): Use of a DNA or RNA probe to detect the presence of the complementary DNA sequence in cloned bacterial or cultured eukaryotic cells.

In vitro: A Latin term used to describe effects in biological material outside the living animal (literally “in glass”)

In vivo: A Latin term used to describe effects in living animals (literally “in life”).

Incidence: Number of new cases of illness occurring during a given period in a specific population.

Inducing agent: A chemical which, when administered to an animal, causes an increase in the expression of a particular enzyme. For example, chlorinated dibenzodioxins are inducing agents which act via the Ah-receptor (qv) to induce cytochrome P450 (qv) CYP1A1.

Intraperitoneal: Within the abdominal cavity.

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.

kilobase (kb): A length of DNA equal to 1000 nucleotides.

Knockout animals: Genetically engineered animals in which one or more genes, usually present and active in the normal animal, are absent or inactive.

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, but is being superseded by more refined methods.

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

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: A type of white blood cell that plays central roles in adaptive immune responses.

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'.

Messenger RNA (mRNA): the DNA of a gene is transcribed (see transcription) into mRNA molecules, which then serve as a template for the synthesis of proteins.

Meta-analysis: In the context of epidemiology, a statistical analysis of the results from independent studies, which aims to produce a single estimate of an effect.

Metabolic activation: Metabolism of a compound leading to an increase in its activity, whether beneficial (e.g. activation of a pro-drug) or deleterious (e.g. activation to a toxic metabolite).

Metabolic activation system: A cell-free preparation (e.g. from the livers of rats pre-treated with an inducing agent (qv)) added to *in vitro* tests to mimic the metabolic activation typical of mammals.

Metabolism: Chemical modification of a compound by enzymes within the body, for example by reactions such as hydroxylation (see cytochrome P450), epoxidation or conjugation. Metabolism may result in activation, inactivation, accumulation or excretion of the compound.

Metabolite: Product formed by metabolism of a compound.

Metabonomics: Techniques available to identify the presence and concentrations of metabolites in a biological sample.

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 chromosomal 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.

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. 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.

Mitogen: A stimulus which provokes cell division in somatic cells.

Mitosis: The type of cell division which occurs in somatic cells when they proliferate. Each daughter cell has the same complement of chromosomes 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.

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

Murine: Often taken to mean “of the mouse”, but strictly speaking means of the Family Muridae which includes rats and squirrels.

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

Mycotoxin: Toxic compound produced by a fungus.

Neoplasm: See 'tumour'.

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

Nephrotoxicity: Toxicity to the kidney.

Neurobehavioural: Of behaviour determined by the nervous system.

Neurotoxicity: Toxicity to the nervous system.

No observed adverse effect level (NOAEL): The highest administered dose at which no adverse (qv) effect has been observed.

Non-genotoxic: See 'carcinogens'.

Nucleic acid: One of the family of molecules which includes the DNA and RNA molecules. Nucleic acids were so named because they were originally discovered within the nucleus of cells, but they have since been found to exist outside the nucleus as well.

Nucleotide: the "building block" of nucleic acids, such as the DNA molecule. A nucleotide consists of one of four bases - adenine, guanine, cytosine, or thymine - attached to a phosphate-sugar group. In DNA the sugar group is deoxyribose, while in RNA (a DNA-related molecule which helps to translate genetic information into proteins), the sugar group is ribose, and the base uracil substitutes for thymine. Each group of three nucleotides in a gene is known as a codon. A nucleic acid is a long chain of nucleotides joined together, and therefore is sometimes referred to as a "polynucleotide."

Null allele: inactive form of a gene.

Odds ratio (OR): The odds of disease in an exposed group divided by the odds of disease in an unexposed group.

Oedema: Excessive accumulation of fluid in body tissues.

Oestrogen: (See estrogen)

Oligonucleotide: A molecule made up of a small number of nucleotides, typically fewer than 25.

Oncogene: A gene which is associated with the development of cancer (see proto-oncogene).

Organochlorine: A group of chemical compounds, containing multiple chlorine atoms, that are usually of concern as environmental pollutants. Some organochlorines have been manufactured as pesticides or coolants and others arise as contaminants of manufacturing processes or incineration.

Pharmacokinetics: Description of the fate of drugs in the body, including a mathematical account of their absorption, distribution, metabolism and excretion (see toxicokinetics).

Pharmacogenomics: The science of understanding the correlation between an individual patient's genetic make-up (genotype) and their response to drug treatment. Some drugs work well in some patient populations and not as well in others. Studying the genetic basis of patient response to therapeutics allows drug developers to design therapeutic treatments more effectively.

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

Phytoestrogen: Any plant substance or metabolite that induces biological responses in vertebrates and can mimic or modulate the actions of endogenous estrogens usually by binding to estrogen receptors.

Plasmid: A structure composed of DNA that is separate from the cell's genome (qv). In bacteria, plasmids confer a variety of traits and can be exchanged between individuals- even those of different species. Plasmids can be manipulated in the laboratory to deliver specific genetic sequences into a cell.

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 (polymerised). An example is the polymer glycogen, formed from linked molecules of the monomer glucose.

Polymerase chain reaction (PCR): A method for creating millions of copies of a particular segment of DNA. PCR can be used to amplify the amount of a particular DNA sequence until there are enough copies available to be detected.

Polymorphism: (see genetic polymorphism)

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

Prevalence: The number of cases of a disease that are present in a population at a given time.

Primer: Short pre-existing polynucleotide chain to which new deoxyribonucleotides can be added by DNA polymerase.

Proteomics: The determination of the function of all of the proteins encoded by the organism's entire genome.

Proto-oncogene: One of a group of normal genes which are concerned with the control of cellular proliferation and differentiation. They can be activated in various ways to forms (oncogenes) which are closely associated with one or more steps in carcinogenesis. Activating agents include chemicals and viruses. The process of proto-oncogene activation is thought to play an important part at several stages in the development of tumours.

Receptor: A small, discrete protein in the cell membrane or within the cell with which specific molecules interact to initiate a change in the working of a cell.

Recombinant DNA: DNA molecules that have been created by combining DNA more than one source.

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.

Regulatory gene: A gene which controls the protein-synthesising activity of other genes.

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 or a population-based case control study. A relative risk of 2 means that the exposed group has twice the disease risk compared to the unexposed group

Renal: Relating to the kidney.

Reporter gene: A gene that encodes an easily assayed product that is coupled to the upstream sequence of another gene and transfected (qv) into cells. The reporter gene can then be used to see which factors activate response elements in the upstream region of the gene of interest.

Risk: Possibility that a harmful event (death, injury or loss) arising from exposure to a chemical or physical agent may occur under specific conditions.

RNA (ribonucleic acid): a molecule similar to DNA (qv), which helps in the process of decoding the genetic information carried by DNA.

Safety: Practical certainty that injury will not result from a hazard under defined conditions.

SCF: The European Commission's Scientific Committee on Food (formerly the Scientific Committee for Food).

Single nucleotide polymorphism (SNP): DNA sequence variations that occur when a single nucleotide in the genome sequence is altered. For example, a SNP might change the DNA sequence A**A**GGCTAA to A**T**GGCTAA. By convention, SNPs occur in at least 1% of the population.

Sister chromatid exchange (SCE): Exchange of genetic material between two sub-units of a replicated chromosome.

Suppressor gene: A gene which helps to reverse the effects of damage to an individual's genetic material, typically effects which might lead to uncontrolled cell growth (as would occur in cancer). A suppressor gene may, for example, code for a protein which checks genes for misspellings, and/or which triggers a cell's self-destruction if too much DNA damage has occurred.

Systematic review: A review that has been prepared using a documented systematic approach to minimising biases and random errors.

TDI: See 'Tolerable Daily Intake'.
Teratogen*: A substance which, when administered to a pregnant woman or animal, can cause congenital malformations (structural defects) in the baby or offspring.*

Threshold: Dose or exposure concentration below which an effect is not expected.

Tolerable Daily Intake (TDI): An estimate of the amount of contaminant, expressed on a body weight basis (e.g. mg/kg bodyweight), that can be ingested daily over a lifetime without appreciable health risk.

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. TEF systems have been published for the chlorinated dibenzodioxins, dibenzofurans and dioxin-like polychlorinated biphenyls, and for polycyclic aromatic hydrocarbons.

Toxicodynamics: The process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects.

Toxicogenomics: A new scientific subdiscipline that combines the emerging technologies of genomics and bioinformatics to identify and characterise mechanisms of action of known and suspected toxicants. Currently, the premier toxicogenomic tools are the DNA microarray and the DNA chip, which are used for the simultaneous monitoring of expression levels of hundreds to thousands of genes.

Toxicokinetics: The description of the fate of chemicals in the body, including a mathematical account of their absorption, distribution, metabolism and excretion. (see pharmacokinetics)

Transcription: the process during which the information in a length of DNA (qv) is used to construct an mRNA (qv) molecule.

Transcriptomics: Techniques available to identify mRNA from actively transcribed genes.

Transfer RNA (tRNA): RNA molecules which bond with amino acids and transfer them to ribosomes, where protein synthesis is completed.

Transfection: A process by which the genetic material carried by an individual cell is altered by incorporation of exogenous DNA into its genome.

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.

Translation: In molecular biology, the process during which the information in mRNA molecules is used to construct proteins.

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

- Tumours arising from epithelia (qv): *benign* - adenomas, papillomas*; malignant* - adenocarcinomas, papillary carcinomas.
- Tumours arising from connective tissues such as fat, cartilage or bone: *benign* - lipomas, chondromas, osteomas; *malignant* - fibrosarcomas, liposarcomas, chondrosarcomas, osteosarcomas.
- Tumours arising from lymphoid tissues are malignant and are called lymphomas (qv); they are often multifocal. Malignant proliferations of bone marrow cells are called leukaemias.

Benign tumours may evolve to the corresponding malignant tumours; examples involve the adenoma ® carcinoma sequence in the large bowel in humans, and the papilloma ® carcinoma sequence in mouse skin.

Tumour initiation: A term originally used to describe and explain observations made in laboratory models of multistage carcinogenesis, principally involving repeated applications of chemicals to the skin of mice. Initiation, in such contexts, was the first step whereby small numbers of cells were irreversibly changed, or initiated. Subsequent, separate events (see tumour promotion) resulted in the development of tumours. It is now recognised that these early, irreversible heritable changes in initiated cells were due to genotoxic damage, usually

in the form of somatic mutations and the initiators used in these experimental models can be regarded as genotoxic carcinogens (qv).

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

Uncertainty factor: Value used in extrapolation from experimental animals to man (assuming that man may be more sensitive) or from selected individuals to the general population: for example, a value applied to the NOAEL to derive an ADI or TDI. The value depends on the size and type of population to be protected and the quality of the toxicological information available.

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.

Volume of distribution: Apparent volume of fluid required to contain the total amount of a substance in the body at the same concentration as that present in the plasma, assuming equilibrium has been attained.

Xenobiotic: A chemical foreign to the biologic system.

Xenoestrogen: A 'foreign' compound with estrogenic activity (see estrogen).

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.

2000 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)

Guidance on a Strategy for testing of chemicals for Mutagenicity. Department of Health (2000)

If you require any further information about the work of the committees, or the contents of this report, please write to the committee's administrative secretary at the following address:-

COT Secretariat
Food Standards Agency
Room 511C
Aviation House
Kingsway
London WC2B 6NH

Tel 020 7276 8522

Fax 020 7276 8513

E-mail Keith.Butler@foodstandards.gsi.gov.uk

http://www.food.gov.uk/science/ouradvisors/toxicity/

COC/COM Secretariat
Department of Health
Room 692D
Skipton House
80 London Road
Elephant and Castle
London SE1 6LH

Tel 020 7972 5020

Fax 020 7972 5134

E-mail Khandu.Mistry@doh.gov.uk

http://www.doh.gov.uk/com/com.htm

http://www.doh.gov.uk/coc/coc.htm