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

Committee on TOXICITY

Committee on MUTAGENICITY

Committee on CARCINOGENICITY

Annual Report 2003

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Contents

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

About the Committees

This is the thirteenth 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 2003 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.

In common with other independent advisory committees the members are required to follow a Code of Conduct which also gives guidance on how their 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 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/ouradvisors/toxicity

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

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

In 2003 the Committee took another major step forward in improving the openness and transparency of its working by permitting observers to attend committee meetings. So far, the numbers attending have been very small, but an important principle has been established. I welcome this decision, which has allowed interested parties to more fully understand the Committee's deliberations. More information on this process is described elsewhere in this report.

The Committee has provided advice on a number of chemicals that may be present in various foods, including metals such as arsenic, mercury and nickel, fluorine, iodine, brominated flame retardants, terphthalic acid, the enzyme chymosin and the bulk sweetener erythritol. The Committee published its Report on Phytoestrogens and Health and established a new working group to consider Variability and Uncertainty in Toxicology. Other generic issues discussed include guidelines for exposure assessment and the use of physiologically-based pharmacokinetic models.

The Food Standards Agency has occasionally needed to seek urgent advice on a food safety issue. These related to the use of 2,4-dinitrophenol as a dietary supplement, detection of fumonisins in maize meal, use of an unauthorised dye in chilli powder, and preliminary data on nickel leaching from kettles. Advice given between meetings is followed by a paper to the full committee at the subsequent meeting and is described in a separate section of this annual report.

This is my second annual report as COT chairman. I was sorry to lose the wisdom and accumulated experience of a number of members who completed their terms of office, but also pleased to welcome new members bringing new perspectives and expertise to the committee. I am grateful to Professor Rowlands for agreeing to take on the position of vice-chairman after Professor Aggett retired from the committee.

Finally, I would like to add my sincere thanks and appreciation of the work of the administrative and scientific secretariats without whose excellent work the Committee would not be able to function.

Professor I A Hughes (Chairman)
MA MD FRCP FRCP(C) FRCPH F Med Sci.

COT evaluations

Arsenic in food: results of the 1999 Total Diet Study

1.1 In 2002 the Food Standards Agency completed a survey of the total and inorganic arsenic levels in samples from the 1999 Total Diet Survey (TDS), which was carried out between 1999 and 2002. The COT was asked to comment on the survey and assess if the levels of arsenic in the diet posed a risk to human health.

1.2 The COT noted that inorganic arsenic is genotoxic and a known human carcinogen and therefore exposure should be as low as reasonably practicable (ALARP). The COT also noted the limited evidence to support the commonly held assumption that organic arsenic is less toxic than inorganic arsenic. Overall, the COT concluded that there are no relevant tolerable intakes or reference doses by which to assess safety of either inorganic or organic arsenic. The low concentrations of inorganic arsenic appeared to be consistent with dietary exposure being ALARP, but refinements to increase the sensitivity of the analysis would be welcome.

1.3 Fish was the major contributor to dietary exposure to arsenic, and the predominant form of arsenic in fish is organic. The COT considered that the limited evidence indicated that the dietary exposure to organic arsenic identified in this survey was unlikely to constitute a hazard to health. The average population dietary exposure to total arsenic was lower than that estimated for previous years, providing reassurance that exposure to total arsenic through food is not increasing.

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

Brominated flame retardants in fish from the Skerne-Tees rivers system

1.5 In 2003, the COT considered the results of a survey investigating the concentrations of brominated flame retardants (BFRs) in brown trout and eels from the Skerne-Tees river system. The Committee was asked to assess the toxicological properties of selected BFRs in order to advise on any health implications of the estimates of dietary exposure.

1.6 BFRs are structurally diverse chemicals used in plastics and other materials to enhance their flame retardant properties. The chemicals that were analysed for in the survey were those considered to be representative of the polybrominated biphenyl ethers (PBDEs) congeners used and produced in the Skerne-Tees area and hexabromocyclododecane (HBCD).

1.7 The COT concluded that the toxicological databases for the PBDEs and HBCD were insufficient to allow establishment of tolerable daily intakes. A Margin of Exposure (MoE) approach was therefore used in the risk assessment.

1.8 Members considered that comparison of the worst case estimated intakes from consumption of a single portion of eels or trout per week from the Skerne Tees with the available toxicological data indicated that these intakes were unlikely to represent a risk to health. However, in view of the uncertainties surrounding the toxicological database and exposure assessments, this conclusion was considered tentative.

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

Enzyme submission – Chymosin preparation derived from GM *Aspergillus niger var. awamori*

1.10 Chymosin is an enzyme preparation that has previously been granted clearance by the COT. In December 2000, the manufacturer sought clearance of a modified purification and recovery procedure for this enzyme preparation. The COT has raised a number of questions relating to the comparison of the product with the material used in the toxicity studies and the purity and specifications of the product (2000 Annual Report, page 16).

1.11 In May 2003, the manufacturer provided additional information in response to the previous COT request intended to demonstrate comparability between the new material and that used in the toxicity studies, the performance of the chromatography process used and product uniformity. A number of concerns were identified: the lack of any direct comparison with the material used in the original toxicity studies, a lack of data demonstrating that no new or potentially hazardous impurities were introduced by the new process, a lack of data on the performance of the column over its full lifetime, the lack of data on epichlorhydrin levels in the final product and information on the current and intended usage of the product. Members considered that the data provided did not adequately address the concerns that had been raised and were therefore unable to give full approval for the new processing procedure.

1.12 In December 2003, the manufacturer submitted further information to the COT to address the points raised. Representatives from the manufacturer were also in attendance at the meeting to answer any additional questions. On this occasion, the COT were satisfied that the company had provided sufficient information to address their concerns and agreed full approval for chymosin as produced by the new process. However, the COT requested information on the effect of column regeneration on the process, in order to determine the lifetime of the column.

Erythritol

1.13 Erythritol is a polyol which has potential uses as a sweetener and as a binding agent, thickener, bulking agent, sequestrant, flavour enhancer and freezing point depressant in a variety of foods and/or beverages. The COT was asked to consider the acceptability of the use of erythritol in beverages. Excessive ingestion of polyols causes laxative effects and for this reason other polyols are not currently authorised for use in beverages as intakes would potentially be much higher than from foods. However, higher intakes of erythritol than other polyols can be ingested before laxation ensues.

1.14 The COT considered laxation to be an unpleasant side effect that should be avoided in adults. Children were of more concern as they are more susceptible to dehydration. The laxation caused was considered to be a local physiological effect caused by excessive bolus ingestion of the polyol. The COT considered that for adults it was not appropriate to express the NOEL for laxative effects on a per kg bodyweight basis as the length of the colon does not vary between adults of different bodyweights. However, children would be expected to be more susceptible due to the shorter length of the GI tract.

1.15 In order for the COT to be able to determine a NOEL the committee would require information on the relative sensitivity of young children to adults. It was suggested that it may be possible to obtain this information from post-market surveillance of foods and beverages (where authorised) containing erythritol or other polyols. The COT concluded that it was not acceptable at present to use erythritol in beverages.

Fluorine in the 1997 Total Diet Study

1.16 Fluorine, bromine and iodine in the 1997 Total Diet Study was originally considered by the COT in 2000. Consideration of any potential effects of dietary fluorine intakes was deferred as fluoride (the ionic form of fluorine found in food) was being considered by the Expert Group on Vitamins and Minerals (EVM). The COT considered the draft EVM review of fluoride in 2001, but agreed that it would be more appropriate to wait until the EVM had finalised its view.

1.17 The EVM subsequently agreed that fluoride was not within its remit and has thus not completed a risk assessment for fluoride. Following the EVM's conclusions the COT considered the available data on fluorine and health outcomes and data on intakes from the 1997 Total Diet Study and from non-dietary sources.

1.18 The COT noted that the most sensitive effect of fluorine (as fluoride) in humans was dental fluorosis, a mottling of the tooth enamel, which occurs in children below the age of 8 years. The COT established a NOAEL for aesthetically significant dental fluorosis of 0.05 mg/kg bw/day. It was noted that the results of the Total Diet Study indicated that a small proportion of children may be at risk of moderate dental fluorosis due to dietary exposure to fluoride. The COT noted that the integrity of teeth with mild or moderate dental fluorosis was not affected, but recommended that more research was needed to determine the impact of the cosmetic effect of dental fluorosis on affected individuals, and on any possible long-term health effects in people with dental fluorosis. No adverse effects other than mild to moderate dental fluorosis were expected to be associated with dietary fluorine intakes in the UK.

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

Hexachlorobutadiene contamination at Weston Quarries

1.20 In 2000 the COT was asked for advice after environmental contamination with hexachloro-1,3-butadiene (HCBD) was found around a disused waste dump in a quarry in Weston, Runcorn (2000 Annual Report, page 20). The COT noted that the target organ for toxicity was the kidney and determined a NOAEL for non-carcinogenic effects of 0.2 mg/kg bw/day from animal studies. This was calculated to correspond to continuous inhalation of air containing 60 ppb HCBD. The COT advised that an air concentration of 0.6 ppb (incorporating an uncertainty factor of 100) would not be expected to cause health risks for non-carcinogenic effects.

1.21 The COM had advised that it would be prudent to consider HCBD to be an *in vivo* mutagen. However, the COT noted that continuous exposure to HCBD at a level of 0.6 ppb resulted in an intake 10,000 times lower than the minimal carcinogenic dose in animals. Therefore 0.6 ppb was recommended as a minimum risk level for risk management purposes.

1.22 In 2003 the COT considered new data, including an assessment of biochemical markers of renal glomerular and tubular toxicity in residents of houses in which HCBD had been detected at average concentrations above 0.6 ppb. The assessments were carried out within 2 months and at least 10 months after the residents were evacuated from their houses. Mean levels of kidney markers had decreased on the second round of testing for all but retinol binding protein. The mean levels of markers were all within the range that is considered normal by the Health and Safety Laboratory in workers. However, the data indicated that 19% of the individuals still had marker levels above the reference range at the second round of testing.

1.23 It was noted that the pattern of changes in individual marker levels was not consistent with the pattern of renal damage seen in animal studies, which could indicate that the apparent decreases were simply due to natural fluctuation. However, the COT considered that the data indicated that any sub-clinical effects on the kidney were now resolving and that this provided reassurance that people had been protected by the risk management action taken.

Iodine in cows' milk

1.24 The COT has reviewed iodine concentrations in cows' milk on a number of occasions since 1989. During these reviews the COT recommended there was a need for additional information on the different chemical forms of iodine which might be present in cows' milk (2000 Annual Report, page 17). In October 2002, the Committee considered the results of a research project on iodine speciation in milk. Following this discussion COT requested further information on possible sources of iodine in milk (2002 Annual Report, page 20).

1.25 Surveys have indicated a seasonal effect on iodine levels in milk. There is very limited information on the species of iodine present in cows' milk although studies have indicated that the predominant form is iodide. A study to determine the species of iodine present in cows' milk indicated that the majority of iodine eluted at a retention time comparable to that of iodide but did not definitively identify the species. Traces of iodine-containing substances with molecular weights ranging from 1 to $\geqslant$ 600 kDa were also detected but not identified. In human breast milk, 80% of the iodine present is reported to be iodide with the remaining 20% as organically bound iodine. There is no information in the literature as to the identification of these organic bound compounds.

1.26 It is generally assumed that following ingestion iodine and iodate are reduced to iodide in the gut. However, much of the toxicological information in the literature does not distinguish between different species.

1.27 Members noted that dietary iodine deficiency had been a significant problem in the recent past and because of this the intake of iodine from milk was generally considered beneficial. Members noted the additional information on iodine species in milk and concluded that there were no health concerns related to presence of iodine in milk at current levels. However, if in the future production methods altered and the iodine content of milk increased this might need to be re-evaluated.

Mercury in fish and shellfish – reconsideration of 2002 COT opinion

1.28 In 2002, the COT had considered the results of a survey of mercury in imported fish and shellfish and UK farmed fish and their products (see 2002 Annual Report, page 16). The COT considered that dietary exposure to mercury resulting from average and high level consumption of fish for which consumption data were available was not likely to result in adverse effects. It also commented on the number of portions of shark, swordfish, marlin or tuna that would not be expected to result in adverse effects on the developing fetus or infant. However, the COT noted uncertainties in the risk assessment and agreed that its conclusions should be reviewed following the JECFA evaluation of methylmercury in 2003.

1.29 In June 2003, JECFA revised the PTWI in order to protect against neurodevelopmental effects, and the COT therefore subsequently reviewed its 2002 statement.

1.30 The COT agreed that the 2003 JECFA PTWI of 1.6 μg /kg bw/week was sufficient to protect against neurodevelopmental effects in the fetus. The COT noted the lack of information on susceptibility of infants to methylmercury and agreed that, as a precautionary measure, the 2003 JECFA PTWI of 1.6 μg /kg bw/week should also be applied to infants. The Committee noted that there was no new evidence to indicate health risks other than neurodevelopmental effects at the 2000 JECFA PTWI of 3.3 μg /kg bw/week, and therefore this guideline could be applied to protect against non-developmental adverse effects in the general population.

1.31 The COT also considered research needs and noted the difficulty in extrapolating possible effects from methylmercury exposure associated with regular consumption of fish in the Seychelles Islands, to occasional consumption of fish containing higher concentrations of methylmercury in the UK. However, conduct of meaningful epidemiological studies, with adequate sized populations, would be difficult in the UK. Mechanistic studies could help to elucidate population groups more at risk. Development of analytical methodology to allow direct measurement of methylmercury would help to identify whether exceedances of the guidelines are of concern. Members also suggested that research integrating the risks with nutritional benefits could be of value. This could be considered further by the joint COT/SACN subgroup reviewing the risks and benefits of fish consumption.

1.32 The COT statement was revised to take into account the new PTWI, and is included at the end of this report. This 2003 COT statement supersedes the version in the 2002 Annual Report.

Metals and other elements in infant foods

1.33 The COT was informed of the results of a Food Standards Agency survey of metals in infant food. This survey was carried out to establish the concentrations of aluminium, antimony, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc in a representative range of commercial infant foods and formulae. Estimates of dietary exposure were calculated for each of the 12 elements using three different approaches to allow an assessment of the dietary exposure to each metal by infants aged 0-12 months.

1.34 The COT considered that consumption data based on the 1986 survey of British infants was likely to result in an underestimation of dietary exposure, but was useful as it allowed comparison with the previous survey of elements in infant foods. An approach based on manufacturers' feeding recommendations was probably an over estimate of dietary exposure and could be considered to be a worst case scenario. Members agreed that these two approaches should be used in assessing the results of the survey, however new studies to determine the patterns of consumption of foodstuffs in infants would be welcomed.

1.35 Members reviewed the concentrations of elements in the infant foods, and considered the potential toxicological effects of the estimated dietary exposures. Overall, Members agreed that the consumption of infant foods sampled in the survey would not result in the intake of such quantities of any of the analysed elements such as would give concern for the health of the infants. Future assessments would be more robust if information was made available on the actual species of metal present in the food and on the contribution of the metal concentrations in water used to reconstitute formula and dried foods.

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

Metals and other elements in the 2000 Total Diet Study

1.37 The COT was also informed of the results of a Food Standards Agency survey of aluminium, arsenic, cadmium, chromium, copper, lead, manganese, mercury, nickel, selenium, tin and zinc in the 2000 Total Diet Study (TDS). Estimates of dietary exposure had been calculated for each of the 12 elements using food consumption data taken from the National Food Survey and the National Diet and Nutrition Surveys (NDNS).

1.38 The COT reviewed the concentrations of elements in the food samples, and considered the potential health effects of the estimated dietary exposures. Whilst there were no specific concerns related to the estimated dietary exposures, Members agreed that in future surveys of elements in food, priority should be given to those of greatest toxicological concern such as arsenic, mercury and lead, and that speciation of metals such as mercury, arsenic and chromium would be helpful for the risk assessment.

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

Nickel leaching from kettle elements into boiled water

1.40 In January 2003, COT Members were asked to provide urgent advice on the health implications of a report on nickel leaching from kettle elements into boiled water, based upon results of a study commissioned by the Drinking Water Inspectorate (DWI). The study reported that more nickel leached into water boiled in kettles with exposed nickel-plated copper heating elements than when the water was boiled in kettles with exposed stainless steel elements or concealed elements. The study also reported that filtering the water in commercially available filter jugs increased the amount of nickel leaching from exposed nickel-plated copper heating elements.

1.41 Members were provided with the full DWI study report, a summary of the data drafted by the Secretariat, the draft risk assessment of nickel from the Expert Group on Vitamins and Minerals (EVM) and an opinion previously provided by a COT expert to support the EVM evaluation. Seven Members were able to provide written comments in the time available. A summary of the COT comments, drafted by the Secretariat and approved by the Chairman, is included at the end of this report.

1.42 The COT concluded that the results of the DWI report supported previous observations that boiling water in some types of kettle may result in elevated levels of nickel in the water. No other conclusions could be reached in the absence of statistical analysis of the data. In order to assess the risks associated with nickel in boiled water, more information was needed to derive exposure data based on water boiled under conditions similar to those used in homes, the work-place and catering establishments.

1.43 Following this assessment, the FSA advised people with nickel allergic dermatitis who were considering buying a new kettle, that they should either choose a flat-bed kettle or seek advice from retailers on which kettles have stainless steel elements.

1.44 In December 2003, the COT reviewed the results of an additional study commissioned by the Scottish Executive. Members noted that the statistical concerns in the earlier study were addressed in the new study. However the extreme differences between the results of the laboratory and consumer phases of the study, and the uncertainty regarding the reasons for these differences, meant that it was not possible to estimate potential levels of dietary exposure. The COT therefore again could not conduct a risk assessment and considered it would be beneficial if the Secretariat met with the study sponsors to discuss the information required.

Terephthalic acid

1.45 In 2000, the Committee reviewed the health implications of the results of a survey of terephthalic acid (TPA) migration from can coatings into food (2000 Annual Report, page 24). In particular, the Committee was asked to give its views on the possibility that TPA might have endocrine disrupting activity. The Committee considered that the available toxicity studies were inadequate to exclude this possibility. It was therefore recommended that appropriate studies should be carried out to determine

whether TPA possesses endocrine disruptor activity. The Food Standards Agency directed industry to carry out this further toxicological work and to keep migration of TPA and isophthalic acid, from can coatings into food, to a minimum.

1.46 In June 2003, BP Chemicals Ltd submitted the report of a full multigeneration reproduction toxicity study on terephthalic acid. Members considered that the information provided in the report was sufficient to demonstrate that TPA does not have endocrine disrupting effects at 20000 ppm in the diet, the highest dose tested.

1.47 However, Members noted that reductions in kidney weights occurred at all doses of TPA. Therefore, the dose resulting from administration of 1000 ppm TPA in the diet appeared to be the lowest observable effect level (LOEL) for effects on the kidney. This LOEL was in the region of 100 mg/kg bw/day, which appears to be lower than the dose used by the Scientific Committee on Food in deriving the temporary TDI of 0.125 mg/kg bw/day. Histopathological changes in the urinary bladder and the kidney were reported at the highest dose (20000 ppm) but these organs had not been examined in the mid- and low dose groups (5000 and 1000 ppm respectively).

1.48 The COT considered that it was important to follow up the effects observed in the urinary bladder and the kidney and asked for information on the histopathology of these organs if they are available.

Urgent advice provided by COT

2,4-Dinitrophenol

1.49 The Food Standards Agency (FSA) was notified via the European Commission Rapid Alert System for Food and Feed that a Finnish body builder became seriously ill after taking some capsules purchased from a website, apparently based in the UK. Further information indicated that the individual had purchased 'fat-burner' capsules containing 2,4-dinitrophenol (2,4-DNP). This website marketed a variety of products aimed primarily at the bodybuilding community and 2,4-DNP was portrayed as a way of losing excess fat. 2,4-DNP is an industrial chemical with no approved human uses.

1.50 The Medicines and Healthcare products Regulatory Agency (MHRA) considered that 2,4-DNP was not a medicinal product. The MHRA identified other items on the website that would be a cause for concern and would require further investigations.

1.51 A risk assessment was undertaken based on literature searches identifying observed human effects and information provided by the Finnish authorities on levels of 2,4-DNP in the capsules.

1.52 DNP is a metabolic poison. Signs of acute poisoning include nausea, vomiting, restlessness, flushed skin, sweating, dizziness, headaches, rapid respiration, tachycardia, fever and cyanosis, possibly leading to coma and death. The lethal oral dose in humans is 1 to 3 g.

1.53 The capsules were advertised on the website as containing 200 mg DNP, but Finnish authorities have reported about 380 mg/capsule. There appears to be significant variability in the amount contained in the capsules. Based on the reported analytical findings taking 3 or 4 of these capsules at once could be lethal. Apparently, the toxicity runs a rapid course, such that death or recovery occurs within 24 to 48 hours of ingestion.

1.54 DNP is also associated with chronic effects; these include formation of cataracts and skin lesions, weight loss, cardiovascular effects and effects on bone marrow and the CNS. The US EPA has established a Reference Dose for chronic oral exposure for DNP of 0.002 mg/kg bw/day. This was based on reports of cataract formation when DNP was used medicinally (as a slimming pill), with a LOAEL of 2 mg/kg bw/day and a total uncertainty factor of 1000. This indicates that repeated consumption of just 140 mg/day in an average 70 kg man, i.e. less than half of one capsule, could result in harmful effects.

1.55 The COT Chairman was consulted on the risk assessment and agreed with the conclusion that there were both short and long term health risks associated with 2,4-DNP.

1.56 The FSA considered that 2,4-DNP was not suitable for human consumption and advised consumers not to take any product containing 2,4-DNP at any level. This advice was issued without compromising further investigations or prosecutions by other bodies.

Fumonisins in maize meal

1.57 In 2003, the Food Standards Agency requested advice on a risk assessment on fumonisins in maize meal. This was produced by the Secretariat in consultation with the COT chairman, and is included at the end of this report.

1.58 Fumonisins are mycotoxins produced by the fungi *Fusarium verticilloides* and *Fusarium proliferatum*. The three most common fumonisins, B1, B2 and B3 usually occur together, with the fumonisin B1 generally found in the highest amount and fumonisin B3 present in small amounts, if at all. The toxins are predominantly found as contaminants of maize and maize products, although they have, on occasions, been found in other cereals and cereal products albeit at low levels. Fumonisins have been shown to be both hepatotoxic and nephrotoxic in rodents after short term exposure and there is evidence for carcinogenicity in the liver and kidney after long term exposure. Fumonisins have been implicated as a possible factor in the increased incidence of oesophageal cancer in some populations.

1.59 The Agency is conducting an on-going survey of mycotoxins in maize-based foodstuffs. During this survey two maize meal products were found to contain fumonisins that exceed the proposed European maximum level of 500 μg/kg for fumonisin B1 + fumonisin B2. The affected products were withdrawn from sale.

1.60 The risk assessment, as agreed by the COT chairman, concluded:

- *The levels of fumonisins found were not a concern for the health of those consuming the affected products already sold, given the low levels of consumption of maize meal. However, if consumers were concerned they were advised to dispose of the affected products.*

Sudan I found in chilli powder

1.61 Sudan I, a dye that is not permitted for use in food, was found in some food products manufactured in the UK that contain chilli powder imported from India. The source of the Sudan I was determined to be adulterated chilli powder.

1.62 There is evidence that Sudan I is carcinogenic in rodents and genotoxic *in vitro* and *in vivo*. Following consultation with the chairmen of the COT, COC and COM, it was agreed that, although there are some incomplete and possibly equivocal results, it is prudent to assume that Sudan I is a genotoxic carcinogen and that dietary exposure should be as low as reasonably practicable (ALARP).

1.63 Based on this risk assessment and the fact that Sudan I is not a permitted food additive, the Agency has been working with the local authorities and industry to ensure that products containing Sudan I do not enter or are removed from the food chain. The Agency has also issued press releases informing consumers of the products contaminated with Sudan I, and advising people who have bought any of these products not to eat them.

1.64 The companies originally identified as being responsible for supplying the contaminated chilli have had their licences to trade suspended. In addition, new legislation has been introduced within the European Community (2003/460/EC). It states that all hot chilli and hot chilli products imported into the Community in whatever form, intended for human consumption, should be accompanied by an analytical report provided by the importer or food business operator concerned demonstrating that the consignment does not contain Sudan I. The legislation also states that Member States shall carry out random sampling and analysis of hot chilli and hot chilli products at import or already on the market.

1.65 The risk assessment, agreed in consultation with the COT, COM and COC chairmen, is at the end of this report.

Committee procedures and working groups

Horizon scanning

1.66 Most of the Committee's work is based on the need for advice from Government Departments, particularly the Food Standards Agency and Department of Health, and thus tends to be reactive. Members have agreed that they wish to have an annual horizon scanning agenda item to discuss topics that are likely to be of interest/concern in the future. A list of future topics is displayed on the COT website, allowing interested parties an opportunity to provide additional information.

1.67 Members were provided with an update on the literature relating to toxicogenomics and proteomics, subsequent to the COT/COC/COM joint symposium in October 2001. Members commented that a considerable amount of data is being generated, and although these may be of value in hazard identification, the relevance to risk assessment is still uncertain. The data therefore did not warrant revision of the conclusions reached at the joint meeting. However, Members found the paper a useful means of keeping track of current data and methodology and considered that an annual update of the advances reported in the literature, especially with regard to validation of techniques and bioinformatics, would be helpful.

1.68 Other topics suggested under horizon scanning were:

- Adverse trends in the development of the male reproductive system;
- Proposal for a working group on uncertainty factors in toxicology.

1.69 These topics were taken forward in subsequent discussions and are discussed in more detail elsewhere in this report. In addition, Members were reminded that they may inform the Secretariat at any time of any substances for which the toxicological data require evaluation, or of other topics of emerging importance.

Phytoestrogens and health

1.70 In 1999, the COT established a Working Group to advise on the health implications of dietary phytoestrogens through review of published scientific research and the Food Standards Agency phytoestrogen research programme.

1.71 The COT had discussed the Working Group's draft report at its meetings in February and July 2002 and the report had been issued for public consultation between October and December 2002. The Working Group had received 47 submissions, which included a comprehensive review by an independent expert in the field. Additionally, at the request of the Working Group, the Scientific Committee on Nutrition (SACN) had provided an opinion on the sections of the report relating to soy-based infant formula. The Working Group on Phytoestrogens met in January 2003 to consider the submissions and as a result the report had been modified.

1.72 In February 2003, the Committee endorsed the report, subject to agreed changes. The final report was published in May 2003 and is available at http://www.food.gov.uk/multimedia/pdfs/phytoreport0503.

Procedure for holding COT meetings in open session

1.73 In December 2002, the COT agreed in principle to move to open meetings during 2003 but wished to discuss and agree a more detailed protocol for holding meetings in open session. This protocol

was intended to minimise adverse impacts on the ability of the Committee to function effectively and to provide adequate security for members and officials. The protocol was produced in line with the Committee's existing Code of Practice on openness. It covers all aspects of the advertising of meetings and the application process, any restrictions on attendees and limitations on attendance. The protocol describes the circumstances where discussion is not possible in public and arrangements to discuss these in closed session. Members specified that every agenda should include an opportunity for observers to ask questions. The protocol was agreed by COT in February and all COT meetings since April have been held in open session.

1.74 The protocol is intended to be adjusted in light of experience and to be rapidly modified to adopt best practice. There have already been minor amendments in regard to attendance by the media to allow these arrangements to better fit their time-scales.

1.75 The draft protocol is being discussed by COM and COC with a view to those committees following the same procedures for holding meetings in open session. Following discussion and agreement by COT, COM and COC, an appendix summarising the protocol would be added to the Code of Practice on Openness and publication in a future Annual Report.

Variability and uncertainty in toxicology

1.76 The COT agreed to establish a new Working Group to consider variability and uncertainty in toxicology, and alternative approaches to dealing with these in the risk assessment process. The Food Standards Agency will find the considerations of the Working Group helpful in allowing it to interpret the health implications of exceedances of safety guidelines by particular subsets of the population.

1.77 The terms of reference of the Working Group are:

- To review the evidence of the bases and range of variability in adverse response to chemicals.
- To consider sources of uncertainty in hazard characterisation.
- To consider the appropriateness of uncertainty factors customarily used to extrapolate toxicological data from animals to humans.
- To consider the appropriateness of uncertainty factors customarily used to allow for variation within the human population, including specific subgroups such as children.
- To consider other methods which might be used in setting acceptable or tolerable intakes for food chemicals.
- To consider how to express the level of confidence in the risk assessment.

Risk assessment strategies

Guidelines for Exposure Assessment Practice for Human Health

1.78 The Interdepartmental Group on Health Risks from Chemicals (IGHRC) is an informal group of representatives of UK government departments, agencies and research councils (see http://www.le.ac.uk/ieh/ighrc/ighrc.html). One aim of the IGHRC is to produce guidance documents on the approaches of different government departments and agencies to risk assessment (see also paragraph 1.87). In July 2003 the COT considered a draft of the third of these guidance documents, entitled "IGHRC report: guidelines for exposure assessment practice for human health effects of chemicals".

1.79 The document is aims to serve as an outline of exposure assessment for individuals without specialist knowledge of the area, who use the assessments either for risk assessment or risk management purposes. The document also provides an overview of differences in the ways exposure assessments are carried out by different departments and agencies and addresses different exposure scenarios (food, environment, etc.). The document does not aim to provide a comprehensive review of exposure assessment methodology or explicit details of how each stage in the process is carried out.

1.80 The COT considered the document provided a useful guide, and commended the stepwise approach and inclusion of case studies. Additional information needs were: the relative importance of different routes of exposure, perhaps illustrated by a case study on routes of exposure to a pesticide or a solvent in the domestic situation, information on literature searching and evaluation, and discussion of bias in sensitivity and uncertainty analysis.

1.81 The document is to be revised in the light of comments from the COT and other consultees and will be published in due course.

Physiologically-based pharmacokinetic modelling

1.82 Pharmacokinetics describes the relationship between exposure and the concentration-time profile of a chemical within the body. This relationship is usually expressed as an equation based on an abstract representation of the body as one or more boxes (compartments). The equation or model used is essentially empirical and may bear little relation to the physiological processes involved. Physiologically based pharmacokinetic modelling (PBPK) is a method based on physiological principles rather than observed data, which provides greater understanding of what actually occurs following exposure to a chemical. The concept of PBPK modelling was first described by Thorsten Teorell in 1937. However, at that time the lack of computing power to solve the resulting mathematical equations meant that the approach was impracticable.

1.83 Because PBPK modelling allows for the underlying physiological processes and the physico-chemical properties of the chemical administered it facilitates prediction of events in humans from animal data and helps to explain differences in the behaviour of different chemicals. PBPK modelling permits

prediction of chemical concentrations at specific target sites and can incorporate different exposure scenarios, disease states or changes with age and co-administration of other chemicals. Some PBPK models can simulate population response to exposure to a chemical by producing a distribution of outputs.

1.84 PBPK models are based on three main elements: physiological parameters, chemical specific parameters and design of the model. The physiological parameters define each tissue or organ by its structure, size, blood flow, and functionality. Overlaid onto these in the model are the chemical specific parameters: binding within blood (e.g. to proteins, red cells), tissue affinity (binding, partitioning), membrane permeability, and sensitivity to enzymic modification. The complexity of the model can be varied according to the information required. In a simplified model, tissues with similar physiological properties are considered as a single tissue.

1.85 The majority of work on development and validation of PBPK models has occurred in the development and selection of pharmaceutical candidates. However, there is increasing interest in using PBPK modelling as a tool in risk assessment in North America and the EU. The COT held a one-day meeting to discuss PBPK modelling and key issues regarding its use in risk assessment. COT recognised that PBPK modelling could be a useful approach in the risk assessment of chemicals where sufficient data existed but that there were difficulties with validation of PBPK models for non-pharmaceutical chemicals.

1.86 The COT statement is at the end of this report.

Uncertainty Factors: Their Use in Human Health Risk Assessment by UK Government

1.87 In December 2001, the COT considered a first draft of this document (2001 Annual Report, page 17), which is part of the IGHRC programme of work (see also paragraph 1.78). The document outlines the risk assessment process, highlighting areas of toxicological uncertainty, describing current approaches to dealing with uncertainties, approaches used in UK government regulatory decision-making and those used in other countries. It also includes a brief historical perspective and listed some of the recent advances in dealing with toxicological uncertainty.

1.88 The 2001 document had been revised in the light of comments from the COT and the four other expert advisory Committees consulted. The proposed final version was presented to COT in April 2003 to ensure no major points needed to be addressed prior to publication.

1.89 The COT noted that the document was a useful compilation of the *status quo* but raised a number of points that needed clarification in order to maintain a consistently high standard. The finalised report was published in October 2003 and is available at http://www.le.ac.uk/ieh/ighrc/ighrc.html

Ongoing work

Adverse trends in the development of the male reproductive system

1.90 In February 2003 the COT was invited to consider whether it was an appropriate time to review the available evidence for adverse trends in development of the male reproductive system and possible contribution of chemical exposure to these trends. The Committee noted that the subject had been reviewed extensively, including a comprehensive recent (2002) review by the International Programme on Chemical Safety (IPCS). In contrast to effects in wildlife, the Committee concluded that current evidence has not provided convincing evidence that exposure to endocrine disrupting chemicals has adversely affected the human male reproductive system.

1.91 However, the COT agreed there is a need for new approaches to consider possible causes of adverse trends in reproductive health. This includes the need to consider all chemicals, not just those reported to have endocrine disrupting activities, that may be involved in the reported decline in male reproductive health; and changes in lifestyle factors, which are not within the remit of the COT.

1.92 The Committee concluded that the evidence of adverse trends in human male reproductive health should be reviewed, before considering possible causes, including lifestyle factors and the role of chemicals in general. Since much of this would be outside of the remit of the COT, it was recommended that a scientific meeting be held to review the evidence, involving experts from the relevant medical and scientific disciplines.

1.93 In addition, the COT expected to issue a brief statement. However, the statement has not been finalised pending publication of a new and pertinent report.

Phosphorus, parathyroid hormone and bone metabolism

1.94 In December 2003, the COT was asked to consider the data on phosphorus, parathyroid hormone, calcium balance and bone health, in order to allow the FSA to formulate appropriate consumer advice in relation to food supplements containing phosphorus. Advice has been received from experts in bone health, and the COT discussion will continue in 2004.

Tryptophan and Eosinophilia Myalgia Syndrome

1.95 In 1990 the COT endorsed a ban on the addition of tryptophan to foods, including dietary supplements. This followed reports of a new epidemic illness in the US known as the Eosinophilia-Myalgia Syndrome (EMS), which was associated with the consumption of L-tryptophan supplements. EMS was a serious disorder which affected over 1500 people in the US and caused at least 37 deaths. Several cases of EMS also occurred in the UK.

1.96 In late 2002 the Institute for Optimum Nutrition submitted a document to the Food Standards Agency which claimed that the cause of EMS was now known to be a contaminant in the trytophan produced by one manufacturer and that there was no need for the continuing ban on the addition of tryptophan to foods. The COT was asked to consider the available data and advise on the risk to health of tryptophan, particularly in food supplements. Additional information needs were identified and the discussion will be continued in 2004.

Statements of the COT

Statement on arsenic in food: results of the 1999 Total Diet Study

Statement on brominated flame retardants in fish from the Skerne-Tees rivers system

Statement on fluorine in the 1997 Total Diet Study

Updated statement on a survey of mercury in fish and shellfish

Statement on a survey of metals in infant food

Statement on metals and other elements in the 2000 Total Diet Study

Statement on physiologically based pharmacokinetic modelling

Statement on arsenic in food: results of the 1999 Total Diet Study

Introduction

1. The Food Standards Agency (FSA) has recently completed a Total Diet Study (TDS) of total and inorganic arsenic levels in food, which was carried out between 1999 and 2002. The Committee was asked to comment on the survey and assess if the levels of arsenic in the diet posed a risk to human health.

Toxicology of arsenic

2. Arsenic is a metal with complex chemistry that can form a number of inorganic and organic compounds. It can exist in many oxidation states, the most common being the tri- and pentavalent forms. A variety of inorganic arsenic compounds such as arsenates (AsO_4^{3-}, pentavalent arsenic) and arsenites (AsO_3^{3-}, trivalent arsenic) are found in water and at low levels in food.

3. Inorganic arsenic compounds (arsenite and arsenate) are well absorbed by the oral route in humans with absorption values reported to be from 50% to >95%[1]. They are metabolised by methylation and then excreted in the urine with a half-life of 3 to 5 days[1]. Inorganic arsenic is clastogenic in *in vitro* and *in vivo* assays and some evidence suggests clastogenicity in humans[2,3,4]. Arsenic in drinking-water (primarily inorganic, as arsenate and to a lesser extent arsenite) was evaluated as "carcinogenic to humans" (Group 1) on the basis of "sufficient evidence" for an increased risk for cancer of the urinary bladder, lung and skin[4]. Increased risks of lung and bladder cancer and of arsenic-associated skin lesions and other skin changes (such as hyperkeratosis and pigmentation changes) have been reported to be associated with ingestion of drinking-water at concentrations from approximately 30 μg arsenic/litre[5]. Chronic exposure to arsenic in drinking water has also been associated with peripheral vascular diseases such as blackfoot disease, cardiovascular diseases and possibly with diabetes and reproductive effects[5].

4. Organic arsenic compounds such as arsenobetaine and arsenocholine are found in fish and shellfish. Most arsenic in fish (>90%) is in the form of arsenobetaine which is also the main form found in crustaceans and bi-valve molluscs[6], the remainder is arsenocholine and a small amount of inorganic arsenic (usually <1%). Fish is the main source of arsenic in the diet; arsenobetaine is therefore the main form of arsenic present in food.

5. The fate of organic arsenic has not been clearly defined in experimental animals or in humans. In general organoarsenicals are thought to be less extensively metabolised than inorganic arsenic and more rapidly excreted[5]. Yamauchi *et al.*[7] calculated biological half-lives after administration of organoarsenicals to hamsters, reporting a 6.1 hour half-life for arsenobetaine. In humans, exposure to arsenobetaine through consumption of plaice resulted in 69 to 85% of the arsenobetaine being excreted unchanged in the urine within 5 days[8]. In a study of women volunteers who consumed fish containing arsenobetaine, Lehmann *et al.*[9] observed rapid elimination of the arsenobetaine from blood with a half-life of approximately 7.1 hours during the first 2 to 10 hours following ingestion. Between 10 and 48 hours elimination from the blood was slower with a half-life of approximately 63 hours.

6. There are no data on tissue distribution of arsenic in humans following ingestion of organic arsenic present in fish and seafood. Following intravenous administration of arsenobetaine, the highest tissue concentrations were found in kidney, liver and pancreas of mice and rats[7], and in the liver, kidney, spleen, muscle, skin and brain of rabbits and hamsters[7, 10]. Limited data indicate that organic arsenic compounds such as arsenobetaine and arsenocholine are not converted to inorganic arsenic *in vivo*[5].

7. Despite the limited database, the organic forms of arsenic are generally assumed to be less toxic than the inorganic compounds[1, 11]. There are no adequate studies of toxicity in man or animals from the consumption of organoarsenicals in seafood. In the one toxicity study available, weanling rats were fed diets containing fish, which provided a dose of approximately 3 mg/kg bw/day organic arsenic for 42 days. No treatment-related toxic effects were reported in the limited range of endpoints studied[12]. Limited data indicate that arsenobetaine and arsenocholine are not genotoxic in mammalian cells *in vitro*[5].

Arsenic in fish

8. There does not appear to be any particular type of fish that contains higher levels of arsenic and bio-magnification in aquatic food chains has not been observed[5]. In the last multi-element survey of fish (1998), levels of total arsenic in the most commonly consumed fish (cod, haddock, salmon, tuna) in the UK were in the range of 1.9 mg/kg – 8.4 mg/kg fresh weight, with a mean of 4.6 mg/kg[13].

Previous Evaluations

JECFA

9. In 1983 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) proposed a Provisional Maximum Tolerable Daily Intake (PMTDI) for inorganic arsenic of 2 μg/kg body-weight (bw) per day[14]. JECFA noted the epidemiological evidence of an association between overexposure of humans to inorganic arsenic from drinking-water and an increased cancer risk; 0.2 mg As/L was associated with a 5% increase in the lifetime risk of skin cancer. At that time JECFA also noted that skin cancer did not occur in the absence of other toxic effects of arsenic. The available epidemiological evidence allowed the tentative conclusion that arsenicism could be associated with water supplies containing an upper arsenic concentration of 1 mg/L or greater, and that a concentration of 0.1 mg/L may give rise to presumptive signs of toxicity. The chemical species of arsenic present in the drinking-water were not clearly determined but JECFA concluded it was reasonable to consider them to be inorganic arsenic. Assuming a daily water consumption of 1.5 litres, JECFA concluded that intakes of 1.5 mg/day of inorganic arsenic were likely to result in chronic arsenic toxicity and daily intakes of 0.15 mg may also be toxic in the long term to some individuals. However, the rationale for the PMTDI was unclear.

10. JECFA reviewed its evaluation in 1989, and established a Provisional Tolerable Weekly Intake (PTWI) for inorganic arsenic of 15 μg/kg bw/week based on the previous PMTDI of 2 μg/kg bw/day[11]. JECFA acknowledged that there was a narrow margin between the PTWI and intakes reported to have toxic effects in epidemiological studies, but again did not provide clear justification for the value of the PTWI.

11. In 1989 JECFA also considered organic arsenic present in seafood, and commented that further investigations of the type and levels of organic arsenic compounds naturally occurring in marine products and further animal studies on these specific compounds would be highly desirable[11]. The available data were not sufficient to set a PTWI for organic arsenic. JECFA noted reports of populations who consume large quantities of fish resulting in intakes of organic arsenic of about 50 μg/kg bw/day, with no subsequent reports of ill health effects[11]. However no information was provided on what possible effects were investigated in these fish eating populations, and there have been no specific epidemiological studies to determine if there are any health effects associated with this level of organic arsenic intake.

WHO drinking water guidelines

12. In 1993 the WHO established a provisional guideline value for arsenic in drinking water of 10 μg/L[15], which was described as the 'practical quantification limit'. This concentration was considered to be associated with an estimated excess lifetime skin cancer risk of 6×10^{-4} (or 6 additional cases per 10000 people). WHO noted that a similar value could be derived by assigning a 20% allocation of the JECFA PTWI to drinking water. The drinking water guideline value is currently under review. The draft text, which is open for consultation, proposes that the guideline value of 10 μg/L should be retained, whilst noting the uncertainty in the risk assessment and the practical difficulties in removing arsenic from drinking water[16].

COT

13. The COT last considered arsenic in food in 1995 when it reviewed the results of the 1991 TDS[17]. The estimated upper bound dietary intake of total arsenic from the 1991 TDS was 0.067 mg/day, approximately 1 μg/kg bw/day in a 70 kg adult. The Committee concluded that "since almost all of the estimated dietary intake of arsenic is expected to consist of organic compounds which are of low toxicity compared to inorganic arsenic compounds, it is unlikely to constitute a hazard to health. We would like, however, to see specific estimates of intakes of inorganic and organic compounds of arsenic, both for the general population and for particular groups with greater than average intakes of arsenic, such as adults consuming relatively large amounts of fish and shellfish."

Contaminated land guideline values

14. In 2001, the Committee endorsed a toxicological approach to setting guideline values for hazardous chemicals in contaminated soil. This approach defines the possibility of establishing an "Index Dose" for a genotoxic carcinogen, based on an accepted exposure standard, such as a drinking water standard. The Index Dose is applied to a single source of contaminant and is defined as a level at which the risk is considered minimal, but there is a requirement that exposure from each individual source should be as low as reasonably practicable. An Index Dose of 0.3 μg/kg bw/day was recommended for inorganic arsenic[1], based on the EU/WHO drinking water guideline of 10 μg/L, assuming consumption of 2L water per day.

The 1999 Total Diet Study (TDS)

15. In response to the previous request of the COT, analysis of samples from the 1999 TDS was carried out to investigate levels of both inorganic and total arsenic in foods. One hundred and nineteen different categories of food were collected from 24 towns throughout the UK and made into 20 composite food groups. The proportion of each food in a food group reflects its importance in the average UK diet (largely based on an average of three years previous consumption data from the National Food Survey).

16. Each of the 24 samples of the 20 food groups were analysed in duplicate for total and inorganic arsenic using both direct nebulisation inductively coupled plasma-mass spectrometry (ICP-MS) and hydride generation ICP-MS for total arsenic, and high resolution ICP-MS for inorganic arsenic. The limits of detection were 0.01mg/kg for inorganic arsenic in all food groups and ranged from 0.0005 to 0.004 mg/kg for total arsenic in different food groups. Specific organic arsenic compounds were not measured.

Results of the Total Diet Study

17. The full survey results are published in a Food Surveillance Information Sheet[18]. Total arsenic was detected in all samples of the carcass meat, offal, fish and "other vegetables" food groups and in some samples of each the other food groups. In approximately one quarter of all the samples analysed, the concentration of total arsenic was below the limit of detection. The highest levels of total arsenic were found in fish (mean 3214 μg/kg, range 1106-8423 μg/kg), poultry (mean 73.1 μg/kg, range <2.1-167 μg/kg) and the miscellaneous cereals food groups (mean 13 μg/kg, range <2.1-26 μg/kg). The mean total arsenic concentrations in all the other food groups were below 10 μg/kg. These data are similar to those from the 1994 and 1997 TDS[19,20].

18. Inorganic arsenic was detected in 20 of the 24 fish samples, 10 of the 24 miscellaneous cereals samples and 3 of the 24 poultry samples. The upper bound mean concentrations of inorganic arsenic in fish, poultry and miscellaneous cereals were 15.9, 12.5 and 11.6 μg/kg, respectively. The mean concentration of inorganic arsenic in fish was less than 0.5% of total arsenic. The concentrations of total arsenic in all other food groups were below the limit of detection for inorganic arsenic (i.e. < 10 μg/kg) and therefore inorganic arsenic was not measured because it was assumed that it would not be detectable.

19. Estimates of dietary exposure to arsenic (total and inorganic) for consumers of all age groups (toddlers to elderly) are summarised in Table 1, expressed as a range from lower bound to upper bound. For adult consumers the mean and high level estimates of dietary exposure to total arsenic were 1.3 and 4.4 μg/kg bw/day, respectively. The mean total arsenic level was lower than that of the previous TDS[19] (2.0 μg/kg bw/day), but the 97.5 percentile was the same (4.4 μg/kg bw/day). Intake estimates for children were higher than those for adults, as would be expected from their higher food consumption expressed relative to body weight. Intake data from previous surveys are not available for population groups other than the adults. The data indicate that fish was the major contributor to dietary exposure to total arsenic providing 4.6 μg/kg bw/day for the high level adult consumers of fish.

20. The upper bound estimate of intake of inorganic arsenic was calculated assuming that all the arsenic was inorganic in those food groups where the total arsenic content was below the limit of detection for inorganic arsenic. The upper bound mean estimates of inorganic arsenic intake ranged from 0.07 to 0.2 μg/kg bw/day for different consumer groups, upper bound high level estimates were 0.13 to 0.34 μg/kg bw/day. The miscellaneous cereals food group was the major contributor to inorganic arsenic, providing up to 0.064 μg/kg bw/day for the high level adult consumer.

21. Population mean exposures were calculated using the mean concentrations of arsenic in each food group and the average consumption of each food group based on data from the National Food Survey (NFS) of household food purchases. The upper bound population mean exposure to total arsenic was 0.83 μg/kg bw/day, which is slightly lower than for the 1991, 1994 and 1997 TDS (1.0 –1.1 μg/kg bw/day)[19,20,21]. The upper bound population mean exposure to inorganic arsenic was 0.09 μg/kg bw/day. No previous data are available for dietary exposure to inorganic arsenic.

Table 1: Estimated dietary exposure to arsenic (total and inorganic) for mean and high level consumers.

Survey Population Group	Estimated total dietary exposure to arsenic (μg/kg body-weight/day)			
	Mean		97.5th Percentile	
	Total arsenic[a]	Inorganic arsenic[b]	Total arsenic[a]	Inorganic arsenic[b]
Adults[c]	1.33	0.018 – 0.082	4.37	0.043 – 0.14
Toddlers (1.5 – 4.5 years)[d]	2.43 – 2.46	0.049 – 0.2	11.31 – 11.34	0.11 – 0.34
Young people aged 4-18[e]	1.60 – 1.61	0.033 – 0.13	6.65 – 6.66	0.076 – 0.25
Elderly (free living)[f]	1.60 – 1.61	0.017 – 0.073	5.33 – 5.34	0.041 – 0.13
Elderly (institutionalised)[f]	1.44 – 1.46	0.022 – 0.089	4.62 – 4.64	0.047 – 0.15
"Vegetarians"[g] (including fish eaters)	1.24 – 1.25	0.019 – 0.071	6.98 – 6.99	0.047 – 0.13

Notes:

a. Exposures to total arsenic have been estimated from the upper and lower bound mean concentrations, which assume non-detectable concentrations were the limit of detection and zero, respectively. Where the difference between the lower bound and upper bound mean concentrations is very small, rounding of the data leads to a single value.

b. The upper end of the range for inorganic arsenic was calculated assuming that all the arsenic was inorganic in food groups where the total arsenic was below the limit of detection for inorganic arsenic. The lower end of the range was calculated using the lower bound mean concentrations of inorganic arsenic in fish, poultry and miscellaneous cereals. For those food groups where the total arsenic level was lower than the limit of detection for inorganic arsenic, it was assumed that none of the arsenic was inorganic.

c. Dietary and Nutritional Surveys of British Adults. (1990)[22]

d. National Diet and Nutrition Surveys Children Aged 1½ -4½ years. (1995)[23]

e. National Diet and Nutrition Survey: young people aged 4-18 years. (2000)[24]

f. National Diet and Nutrition Survey: People aged 65 years and over. (1998)[25]

g. Vegetarians Dietary Survey: Technical Report on Weighed Intake Diary Data. (1990) (individuals describing themselves as vegetarians, some of whom ate fish):[26]

Arsenic in drinking water

22. A significant proportion of inorganic arsenic intake comes from drinking water. The current statutory limit for arsenic in drinking water is 50 μg/L, although this will be reduced to 10 μg/L in December 2003. Information provided by the Drinking Water Inspectorate for England and Wales (DWI), Department for Environment Northern Ireland (DOENI) and the Drinking Water Quality Regulator for Scotland (DWQRfS) suggests that the level of arsenic in drinking water for the majority of the population in the UK does not exceed the incoming standard of 10 μg/L. A consumption level of 2 L/day is commonly assumed for drinking water, this would contribute up to 20 μg/day or (0.28 μg/kg bw/day for a 70kg adult) to total arsenic exposure. Data available from a 1995 national survey of tap water consumption indicate that children aged 0-5 years consume an average of about 0.5 L/day, which would contribute 0.34 μg/kg bw/day for a 14.5 kg toddler aged 1.5-4.5 years[27]. However, for a significant proportion of the population, arsenic levels in water are considerably lower than the incoming standard of 10 μg/L, therefore exposure from drinking water would be far less.

COT evaluation

23. Having reviewed the previous evaluations of arsenic, the Committee concluded that there are no relevant tolerable intakes or reference doses by which to assess the safety of either inorganic or organic arsenic in the diet. The JECFA PTWI for inorganic arsenic was established in 1989 using an approach that would not now be considered appropriate in view of the evidence of genotoxicity and carcinogenicity. The index dose was proposed as a risk management tool in support of guideline values for hazardous chemicals in contaminated land, and is also not appropriate for evaluation of dietary exposure. The Committee therefore concluded that exposure to inorganic arsenic from all sources should be as low as reasonably practicable (ALARP).

24. Very few data are available on clearance and toxicity in animals and humans of the forms of organic arsenic found in fish. The general assumption that organic arsenic is less toxic than inorganic arsenic is therefore based on an extremely limited database, which is not adequate to establish tolerable intakes.

25. Interpretation of the data is restricted by the limited sensitivity of the analyses, particularly for inorganic arsenic. Nevertheless, the data confirm that fish is the major contributor to arsenic in the diet, and the predominant form of arsenic in fish is organic, most of which is likely to be arsenobetaine. Overall, inorganic arsenic contributed less that 10% of the total dietary exposure to arsenic. Whilst the low analytical sensitivity results in considerable uncertainty in the estimates of dietary exposure to inorganic arsenic, the large number of food samples with inorganic arsenic concentration below the limit of detection appears to be consistent with dietary exposure being ALARP.

26. It has previously been noted that some fish eating populations are exposed to organic arsenic at levels up to 50 μg/kg bw/day and there is no evidence to suggest that these populations suffer ill-effects as a result. In the absence of information on the toxicological properties of organic arsenic in fish, it is not possible to define the nature of potential ill-effects that should be investigated in such populations.

27. The adult consumer and population mean exposures to total arsenic appeared slightly lower than for previous surveys. Although this decrease may be due to refinements in the methodology used to measure total arsenic over the years, it offers reassurance that the level of arsenic in food is not increasing.

Conclusions

28. We *consider* that there are no relevant tolerable intakes or reference doses by which to assess safety of either inorganic or organic arsenic in the diet. Inorganic arsenic is genotoxic and a known human carcinogen. We therefore *conclude* that exposure to inorganic arsenic should be as low as reasonably practicable (ALARP).

29. We *note* the low sensitivity of the method used to measure inorganic arsenic. The large number of food samples with inorganic arsenic concentration below the limit of detection appears to be consistent with dietary exposure being ALARP. However we would welcome any refinements to the methodology that would increase the sensitivity of the analysis.

30. We *note* that fish is the major contributor to dietary exposure to arsenic, and the predominant form of arsenic in fish is organic.

31. We *note* that the general assumption that organic arsenic is less toxic than inorganic arsenic is based on an extremely limited database. However there is no evidence that exposure to organic arsenic through high levels of fish consumption has resulted in harmful effects, which indicates that the dietary exposure to organic arsenic identified in this survey is unlikely to constitute a hazard to health.

32. We *note* that the average population dietary exposure to total arsenic is lower than that estimated for previous years providing reassurance that exposure to total arsenic through food is not increasing. No data are available on trends in dietary exposure to inorganic arsenic.

May 2003

COT statement 2003/01

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COT statement on brominated flame retardants in fish from the Skerne-Tees rivers system

Introduction

1. The Food Standards Agency (FSA) has recently completed a survey to determine the concentrations of brominated flame-retardants (BFRs) in brown trout and eels from the Skerne-Tees river system. The Committee was asked to assess the toxicological properties of selected BFRs in order to advise on any health implications of the estimates of dietary exposure.

Background

2. Brominated flame-retardants (BFRs) are structurally diverse chemicals used in plastics, textiles and other materials to enhance their flame-retardant properties. Some BFRs, including polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are mixed into polymers rather than being chemically bound to them and can leach out of the products/materials in which they are used and into the environment.

3. PBDEs are produced by direct bromination of diphenyl ether. There are 209 individual PBDE congeners, each of which is identifiable by a unique congener number. Three commercial PBDE flame-retardants, pentabromodiphenyl ether (pentaBDE), octabromodiphenyl ether (octaBDE) and decabromodiphenyl ether (decaBDE) have been available in the UK. The commercial PBDEs are not pure products but a mixture of various diphenyl ethers with varying degrees of bromination.

4. The actual composition of the commercial products varies with supplier and is considered to be commercially sensitive information. However, example compositions of PBDEs have been published (see Table 1). These figures are broadly representative of the commercial products currently supplied. The commercial products are usually named on the basis of the principal PBDE congener e.g. pentaBDE. Trade-name nomenclature may also incorporate a number, which is related to the performance characteristics (e.g. flame retardant properties) of the commercial mixture rather than the constituent congeners[1].

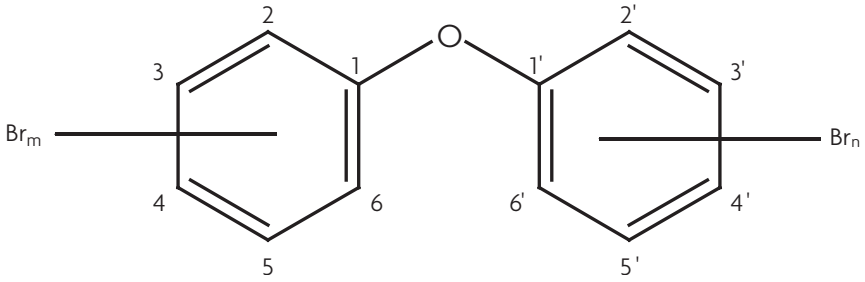
Table 1. Relative congener distribution for penta- and octaBDE

Commercial product	% congeners in commercial product						
	tetra	penta	hexa	hepta	octa	nona	deca
PentaBDE[1]	24 – 38	50 – 62	4 – 12	–	–	–	–
OctaBDE[2]	–	–	⩽12	⩽45	⩽33	⩽10	–
DecaBDE[3]	–	–	–	–	–	⩽3	⩽97

5. The commercial PBDEs have recently been evaluated under the EU Existing Substances Regulations. As a result of their potential to bioaccumulate in the environment, the EU has agreed to ban the marketing and use of penta- and octaBDE from 1 July 2004. However, for some time after this date there will still be existing PBDE-containing products in use.

6. There are limited data available on the potential of decaBDE to bioaccumulate in the environment and it is not currently included in the EU prohibition. In addition, it was not measured in the Skerne Tees survey. However, information on decaBDE has been included in this statement for completeness and as a basis for future evaluations because decaBDE will be the only PBDE product commercially available when the ban on penta- and octaBDE comes into force. The chemical structure of PBDEs is given in Figure 1.

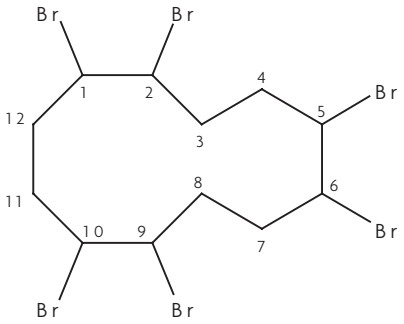
Figure 1. Chemical structure of PBDEs



Where (m) plus (n) equal between 1 and 10 bromine atoms.

7. HBCD is synthesised through bromination of cyclododecatriene. It is commercially available in the UK as a mixture of three stereoisomers α, β and γ. There is currently no proposal to ban HBCD in the EU[4]. The chemical structure of HBCD is given in Figure 2.

Figure 2. Chemical structure of HBCD



Toxicology of PBDEs and HBCD

8. Completed EU risk assessments are available for pentaBDE[1] and octaBDE[2], and draft risk assessments are available for decaBDE[3] and HBCD[4]. Unless otherwise indicated, the following summary is based on the information provided in these risk assessments. The COT also considered new studies published subsequent to the final literature searches for the EU risk assessments. A more detailed summary of the toxicological data is available at http://www.food.gov.uk/multimedia/pdfs/tox14.pdf. Throughout this statement the terms pentaBDE, octaBDE, decaBDE and HBCD refer to the commercial mixtures of brominated flame-retardants whereas individual congeners are denoted by inclusion of the specific congener number e.g. PBDE-99.

PBDEs

9. There is limited information on the toxicokinetics of penta-, octa- and decaBDE. Studies in laboratory animals have indicated that penta- and octaBDE are absorbed following oral administration however, the extent of absorption is unknown[1,2]. DecaBDE is not well absorbed after oral administration (<10%)[3].

10. The primary route of excretion for all PBDEs is considered to be the faeces, although it is unclear how much of the PBDE present in the faeces represents unabsorbed material. In rats, following oral administration, the majority of pentaBDE was detected unchanged in the faeces[1]. Limited information indicates that decaBDE is metabolised to lesser brominated phenolic products[3]. There is no information on the metabolism of octaBDE. Elimination of pentaBDE from rat adipose tissue is slow ($t_{1/2}$ = 25-47 days) indicating that it has the potential for bioaccumulation[1]. There is no information on the elimination of octa- or decaBDE in animals, or on the bioaccumulation or the route of elimination of PBDEs in humans.

11. There is no information on PBDE levels in adipose tissue from the UK. However, PBDE-47 (2,2',4,4'-tetraBDE), PBDE-99 (2,2',4,4',5-pentaBDE), PBDE-100 (2,2',4,4',6-pentaBDE), PBDE-153 (2,2',4,4',5,5'-hexaBDE) and PBDE-154 (2,2',4,4',5,6'-hexaBDE) have been detected in human breast adipose tissue in the US. The sum of total PBDEs detected was 86 ng/g fat[5]. PBDE-47 has also been detected in adipose tissue in Sweden (1.0-98.2 ng/g lipid)[6].

12. In Sweden, there has been an increase in total PBDE levels in samples of human milk over a 25 year period from 1972-1997. The predominant congener was PDBE-47 (2,2',4,4'-tetraBDE) which accounted for 62% of the total (2.28 ng/g lipid). PBDE-99 (2,2',4,4',5-pentaBDE) accounted for 13% (0.48 ng/g lipid) and PBDE-153 (2,2',4,4',5,5'-hexaBDE) accounted for a further 8% of the total (0.46 ng/g lipid). The remaining 17% was accounted for by other tri-, tetra-, penta- and hexa congeners[7]. Data from North America also indicate an increase in total PBDE levels in breast milk. In 1992, samples of breast milk from the Canadian milk bank contained less than 50 ng PBDE/g fat. In 1997, concentrations of approximately 150 ng PBDE/g fat were detected in samples from women in New York State and 200 ng PBDE/g fat was detected in samples from Austin and Denver in 2000[8]. A recent abstract reported a mean concentration of total PBDE of 6.6 ng/g lipid in breast milk sampled from women in the UK in 2001-3[9].

13. Repeat dose studies of commercial pentaBBDE in rodents have identified the liver as a key target organ, with effects seen at doses of 2 mg/kg bw/day and greater. Based on a study in which a commercial pentaBDE product was administered to rats in the diet for 30-days (0-1 mg/kg bw/day) with no treatment related changes, the EU risk assessment concluded, the no observable adverse effect level (NOAEL) for pentaBDE was 0.45 mg/kg bw/day[1]. This value was derived based on the content of 50-62% pentaBDE in the commercial product and assuming a maximum oral absorption of 90%, by analogy with other polyhalogenated diaromatic compounds. Applying similar correction factors to the doses at which liver toxicity has been observed indicates a LOAEL of 0.9 mg/kg bw/day.

14. For octaBDE, a LOAEL of 7.2 mg/kg bw/day was identified for histopathological liver changes in the rat[2]. A NOAEL for liver changes following administration of octaBDE has not been established. The repeat dose toxicity of decaBDE is low. The EU risk assessment reported a NOAEL of 1,120 mg/kg bw/day for liver changes seen in the carcinogenicity study in rats (paragraph 19)[3].

15. In short term gavage studies in rats commercial mixtures of pentaBDE (10-300 mg/kg bw/day), PBDE-47 (2,2',4,4'-tetraBDE; 18 mg/kg bw/day) and a commercial mixture of octaBDE (10-100 mg/kg bw/day) have been shown to increase the metabolism of model substrates for drug metabolising enzymes, in a manner consistent with induction of cytochrome P450 isozymes of the CYP1A and CYP2B subfamilies and UDP-glucuronosyl transferase. This induction was associated with perturbation of thyroid hormones, liver enlargement and histopathological changes in the thyroid[1,10,11,12]. DecaBDE was not found to induce a similar spectrum of changes in CYP subfamilies and thyroid hormones[10,13]. In this study the effect of decaBDE was investigated in rats at doses up 100 mg/kg/day for 4 consecutive days. This dose is an order of magnitude below the LOAEL for lesions in the liver and thyroid in the carcinogenicity studies and as such, may have been insufficient to produce an effect in short-term studies.

16. Penta-BDE has not been shown to be mutagenic in four studies in *S. typhimurium* and one study in *S. cerevisiae*. A cytogenetic study in human peripheral blood lymphocytes also gave negative results. In a single study in *S. typhimurium*, a commercial pentaBDE referred to as Tardex 50 (10-10000 μg/plate) was shown to increase point mutations by 3-fold at the highest concentration in the absence, but not in the presence, of metabolic activation. This single positive result was considered in the EU risk assessment to be a chance finding[1]. PentaBDE has not been tested for genotoxicity *in vivo*.

17. Commercial octaBDE preparations were not mutagenic in four studies in *S. typhimurium* and one in *S. cerevisiae* in the presence and absence of metabolic activation. One preparation referred to as Muster 82 showed weak mutagenic activity in *S. typhimurium* without activation. No information is available on the composition of this preparation. Commercial octaBDE did not induce unscheduled DNA synthesis in human fibroblasts, sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) cells or chromosomal aberrations in human peripheral blood lymphocytes. OctaBDE has not been tested for genotoxicity *in vivo*[2].

18. Commercial decaBDE preparations of known purity (97-98%) were not mutagenic in *S. typhimurium* or *E. coli* in the presence and absence of rat metabolic activation. Two preparations referred to as Muster 83 and Muster 88 had positive effects in strains TA 98, 100 and 1535 with and without activation, but not in strains TA 1537 or 1538. No information is available on the composition of these preparations. In studies reported by the NTP, decaBDE was not mutagenic in the mouse lymphoma assay and did not induce SCE or chromosomal aberrations in CHO cells. In a reproductive study with dietary administration of decaBDE (3-100 mg/kg/day), there was no increase in chromosomal aberrations in the bone marrow cells of parent rats or of the offspring at weaning[3].

19. There are no carcinogenicity data for pentaBDE and octaBDE. The EU risk assessment noted that the evidence for carcinogenicity of decaBDE was considered to be equivocal. In a 103-week feeding study in mice there was an increase in hepatocellular adenomas or carcinomas at the lowest dose (3200 mg/kg bw/day), but not at the higher dose (6650 mg/kg bw/day). Thyroid gland follicular cell adenomas were reported in male mice at both doses. There was no evidence of carcinogenicity in female mice. In a 103-week feeding study in rats there was a dose dependant increase in neoplastic liver nodules, which was significantly greater than control in low dose males (1120 mg/kg bw/day) and in both sexes at the high dose (2240 mg/kg bw/day)[3].

20. In a developmental study in rats, oral administration of commercial pentaBDE on days 6-15 of gestation resulted in no adverse fetal effects at the doses up to 200 mg/kg bw/day[1]. Commercial preparations of octaBDE have been shown to cause fetal toxicity and malformations at doses below those causing maternal toxicity in rats and rabbits. The EU risk assessment identified 2 mg/kg bw/day as the lowest NOAEL for octaBDE, from a study in rabbits in which slight fetotoxicity was observed at 5 mg/kg bw/day[2]. A commercial preparation of decaBDE did not show developmental effects following dietary administration in the diet to rats at doses up to 100 mg/kg bw/day for 60 days prior to mating until weaning[3].

21. The PBDE congeners PBDE-47 (2,2',4,4'-tetraBDE) and PBDE-99 (2,2',4,4',5-pentaBDE) have been reported to cause neurobehavioural changes in NMRI mice following a single oral dose administered on post natal day (PND) 3, 10 or 19. Neurobehavioural effects were detected at 60 and 120 days of age[14,15,16]. In addition, perinatal exposure of CD1 mice to PBDE-99 resulted in neurobehavioural effects detected at 60 days[17]. The main neurobehavioural effect of PBDE-99 was delayed habituation behaviour, which was found to occur at the lowest doses tested (0.6 mg/kg[17]; and 0.8 mg/kg[15,16]).

22. Limited data on the interaction of a range of PBDE congeners with the arylhydrocarbon (Ah) receptor suggest that those measured have much lower potency than the dioxins and co-planar PCBs[18,19]. *In vitro* studies have suggested that a range of PBDEs have endocrine disrupting activity mediated via the oestrogen receptor[19,20].

HBCD

23. All toxicological studies with HBCD were conducted using the commercial mixture. Studies in laboratory animals have shown that, following oral administration, HBCD can be detected in the adipose tissue, liver and muscle. Longer-term exposure shows HBCD has the potential to bioaccumulate. The α-isomer has been found to accumulate more than the β- and γ-isomers. The extent of metabolism of the commercial HBCD is unknown. Following oral administration, the majority of HBCD was detected unchanged in the faeces, although it is unclear how much of this was unabsorbed material. There is no information on the toxicokinetics of HBCD in humans[4].

24. Repeat dose studies of HBCD have identified the liver as a key target organ. Increased liver weights and disturbances in thyroid hormones were observed at 100 mg/kg bw/day which was the lowest dose tested[4].

25. HBCD was not mutagenic in one study using *S. typhimurium* and did not induce chromosomal aberrations in human peripheral blood lymphocytes in the presence and absence of metabolic activation. HBCD caused a slight but significant increase in somatic recombinations in a non-standard assay using two Chinese hamster cell lines containing duplication mutations in the *hprt* gene. The relevance of this is unclear. *In vivo*, there were no significant increases in the frequency of micronuclei in mouse bone marrow cells. In an 18-month lifetime study of dietary administration to mice (available to the EU rapporteur in summary form only) there were no treatment related increases in tumour incidence at HBCD doses of 13-1300 mg/kg bw/day)[4].

26. Administration of HBCD to pregnant rats at dietary doses up to 750 mg/kg bw/day, or gavage doses up to 1000 mg/kg bw/day did not result in fetal toxicity or teratogenicity[4]. HBCD has also been investigated for neurodevelopmental effects[21]. A single dose of HBCD resulted in changes in spontaneous behaviour. However, this information was only available as an abstract, the doses resulting in these effects were unclear and therefore the data could not be used in the risk assessment.

COT evaluation of the toxicological properties of PBDEs and HBCDs

27. There are deficiencies in the toxicological database of the PBDEs and HBCD and these uncertainties need to be reflected in the evaluation. The majority of studies reviewed were relatively old and, although conducted to the standards of the time would not meet current requirements for study design and reporting. In addition, the duration of the longest studies undertaken with pentaBDE were similar to the reported half-life and the resulting tissue concentrations would only reach half of their maximal value by the end of the study.

28. The limited data on the toxicokinetics of the PBDEs suggest differences in absorption and excretion of individual compounds. Data on the genotoxicity, carcinogenicity and reproductive toxicity of PBDEs and HBCD are also limited. As the EU is introducing prohibitions on the use of some PBDEs, it is unlikely that new studies will be undertaken to address the deficiencies in the toxicological databases.

29. The toxicity studies have generally been conducted using commercial mixtures of PBDEs and HBCD. The composition of the material used in many of the studies was unclear and likely to differ from the mixture of congeners measured in food and the environment. Although a similar pattern of biological effects was reported for different mixtures, extrapolation of findings between mixtures should be treated with caution.

30. Whilst some Ah receptor mediated activity could be measured with PBDEs, the potency was low and the most sensitive end-points of toxicity were unlikely to be mediated by this mechanism. It has also been suggested that PBDEs have endocrine disrupting activity mediated via the oestrogen receptor, although the current data are limited.

31. The liver is a target organ for the PBDEs, with pentaBDE being the most toxic, and decaBDE the least. OctaBDE has been found to exhibit reproductive toxicity, whereas pentaBDE and decaBDE did not produce adverse effects in routine developmental studies.

32. Non-routine studies suggest that the most sensitive endpoints are neurodevelopmental. Two of the congeners that are present in commercial pentaBDE, have been shown to cause neurobehavioural effects in adult mice following administration of a single postnatal oral dose. Octa- and decaBDE, and the congeners commonly found in them, have not been investigated using this protocol.

33. HBCD is also hepatotoxic. It has not shown evidence of developmental toxicity in routine studies. One study, available in abstract form only, indicates that it might produce neurodevelopmental effects but there is insufficient detail to use the data in risk assessment.

34. Table 2 shows the NOAELs and LOAELs for the key effects of the PBDEs and HBCD. Based on the available data, pentaBDE appears to be the most toxic of the PBDEs, with a NOAEL of 0.45 mg/kg bw/day for liver effects following repeat dosing and a LOAEL of 0.6 mg/kg bw/day for neurodevelopmental effects following a single post-natal dose. For HBCD, the LOAELs are 100 mg/kg bw/day for liver effects following repeat dosing and 0.9 mg/kg bw/day for neurodevelopmental effects following a single post-natal dose.

35. It is anticipated that the human perinatal blood-brain barrier would be as permeable to PBDE as that of the mouse neonatal blood-brain barrier. There is no single neurodevelopmental stage of the human brain that is directly comparable with the mouse, as different brain parts develop at different rates in the two species. These studies involved administration at postnatal days 3 and 10, at which age the mouse brain probably models the human brain from 1 month pre-natal to 1 month post-natal. There is a lack of data on levels in breast milk, which might be a major source at the critical time for neurodevelopmental toxicity.

Table 2. NOAELs and LOAELs for PBDEs or HBCD

BFR	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Target	Comments
Commercial pentaBDE[1]	0.45	0.9	Liver	No chronic studies available
PBDE-99 (2,2',4,4',5-pentaBDE)[15]	ND	0.6	Neurodevelopment	
Commercial octaBDE[2]	2 ND	5 7.2	Reproduction	No neurodevelopmental data
Commercial decaBDE[3]	ND	1,120	Liver	No neurodevelopmental data
Commercial HBCD[4]	ND	100	Liver	Chronic data seen in summary form only

ND: NOAEL was not determined in the study identifying the LOAEL

36. In view of the inadequacies in the toxicological database and the absence of identifiable no-effect levels, it was not possible to determine a tolerable daily intake (TDI). The Committee therefore decided to take a Margin of Exposure (MoE) approach in which the estimated human exposures are compared with the relevant NOAEL or LOAEL identified from the animal studies. Had it been possible to establish a TDI from a NOAEL, uncertainty factors (UFs) would be required to allow for inter-and intraspecies differences in toxicokinetics and toxicodynamics (100) and limitations in the database such as study duration and gaps in the data (up to 10). Combining these uncertainty factors suggests a target MoE of 1000 for liver toxicity of penta-BDE, above which risks to health would not be expected. A NOAEL has not been identified for the neurodevelopmental effects of penta-BDE and therefore an additional UF of 3-10 would be required for extrapolation from a LOAEL to a NOAEL. This suggests a target MoE of 3,000-10,000. Similarly, a NOAEL has not been identified for the hepatic effects of HBCD, indicating a target MoE of 3,000-10,000. The exposure is not expected to represent a risk to health if the calculated MoE exceeds the target MoE.

PBDEs and HBCDs in fish from the Skerne Tees survey and in the 2001 Total Diet Study (TDS)

37. In 1999, a study sponsored by the Department of the Environment, Transport and Regions measured the concentration of PBDEs in rivers and estuaries downsteam of potential sources. The maximum concentrations of PBDEs were detected in the livers of fish (1294 μg/kg wet wt) and sediment (239 μg/kg dry wt) from the Skerne-Tees river system and the Tees estuary at Newton Aycliffe, which is downstream from the Great Lakes Chemical Company[22]. The Great Lakes Chemical Company is known to have manufactured both penta- and octaBDE at this site until the late 1990's, and still produces HBCD. DecaBDE was never manufactured at the site but may have been distributed via this site. The concentrations detected in the fish livers and sediment were only measured at one time-point and it is important to note that the trout in this river are restocked annually indicating these figures may not accurately represent the actual levels of contamination in the river.

38. Following these reports, the Food Standards Agency conducted a survey to determine the concentrations of congeners known to be components of commercial penta- and octaBDE in brown trout and eels from the Skerne-Tees river system. The Great Lakes Chemical Company is also known to have manufactured HBCD and thus, it was included in the survey. The chemical formula of the each congener and the BFR to which each congener corresponds is given in Table 3. A preliminary assessment of dietary exposure based on analysis of food samples from the 2001 Total Diet Study (TDS) was also conducted.

39. A survey was commenced in late 2001 to determine the concentrations of PBDE and HBCD in brown trout and eels from the Skerne-Tees river system. The samples examined included control samples of trout and eels from the Skerne and eels from the Tees. Samples were obtained from the river Skerne and river Tees at eight easily accessible locations that were upstream and downstream of the Great Lakes Chemical Company and at the confluence of the two rivers (see Figure 3). The PBDE congeners analysed in the survey were selected as a representative sample of those produced and used in the Skerne-Tees area. The chemical formula of each congener and the commercial PBDE to which each congener corresponds is given in Table 3.

40. At the time of this survey no data were available on other sources of dietary exposure to PBDEs and HBCDs in the UK. Therefore, a survey of the concentrations of PBDE congeners in food samples from the 2001 Total Diet Study (TDS) was commissioned in 2002. Single composite food group samples were formed by homogenising individual foods groups (excluding beverages) from 24 locations. These composite samples were analysed for the same range of PBDE congeners as the fish survey. It had been planned to additionally analyse these samples for decaBDE, however since none of the congeners measured were detectable this analysis was not undertaken.

Table 3. PBDE and HBCD congeners measured in the FSA survey

Congener	Chemical formula	Component
PBDE 28	2, 4, 4'-TriBDE	Possible component of commercial pentaBDE
PBDE 47	2, 2', 4, 4'-TetraBDE	Known substantial component of commercial pentaBDE
PBDE 99	2, 2', 4, 4', 5-PentaBDE	Known substantial component of commercial pentaBDE
PBDE 100	2, 2', 4, 4', 6-PentaBDE	Known substantial component of commercial pentaBDE
PBDE 153	2, 2', 4, 4', 5, 5'-HexaBDE	Possible component of commercial pentaBDE and known component of commercial octaBDE
PBDE 154	2, 2', 4, 4', 5, 6'-HexaBDE	Possible component of commercial pentaBDE and commercial octaBDE
α-HBCD	1R, 2S, 5R, 6R, 9S, 10S-HBCD	Known component of commercial HBCD
β-HBCD	1R, 2R, 5R, 6S, 9R, 10S-HBCD	Known component of commercial HBCD
γ-HBCD	1S, 2S, 5S, 6S, 9S, 10S-HBCD	Known component of commercial HBCD

R & S indicate the stereo position of the bromine on the HBCD molecule

Analytical methodology

41. The analytical method involved solvent extraction of the fat component of composite samples from the TDS or a portion of flesh removed from fish or eels. The extracts contained BFRs and other compounds that were separated from the dissolved fat component by adsorption chromatography. The PBDEs were measured using gas chromatography coupled to a mass spectrometric detector (GC-MS).

42. For HBCD, a simplified clean-up stage was performed which consisted of shaking another aliquot of the crude extract with concentrated sulphuric acid. The acid destroys the fats but leaves HBCD compounds intact. The temperatures used to separate PBDEs during GC analysis can affect the structures of the isomers and result in conversion between the three HBCD isomers. Therefore HBCD was determined using a liquid chromatography mass spectrometry (LC-MS) method.

43. The production of composite samples from the individual foods that compr Due to their lipophilicity BFRs are most likely to be detected in fatty foods ise a particular food group in the TDS may result in dilution of the overall fat content and thus may reduce the ability to detect these substances.

Dietary exposure to PBDEs and HBCD from Skerne Tees trout and eels

44. Table 4 shows the concerntration ranges of total PBDEs and HCDB in trout and eels taken from those test sites where the highest concentrations were found and from the control sites. The most contaminated trout were caught at the Haughton Road site, which was the closest river Skerne location downstream of the Great Lakes Chemical Company. No eels are caught at this site, and the most contaminated eels were caught from the next river Skerne downstream location at Oxenfield Bridge.The sum of the concentrations of individual PBDE congeners detected in the edible portion varied from 12 to 14 μg/kg freshweight in trout and was 53 μg/kg freshweight in the eel at control sites. At test sites, the sum of the concentrations varied from 59 to 197 μg/kg freshweight in trout and 164 to 288 μg/kg freshweight in eels.

Table 4. Estimated average dietary intake of PBDE and HBCD following consumption of trout or eels from the Skerne-Tees river system

BFR and sampling	Species	No. of samples	Concentration range (μg/kg freshweight	Maximum intake (μg/portion)[c]	Maximum intake (mg/kg bw/day)[d]
PBDE (Ricknall Grange) [a]	Trout	5	12-14	1.6	0.003
PDBE (Haughton Rd) [b]	Trout	7	59-197	24	0.056
PBDE (Ricknall Grange) [a]	Eels	1	53	3.7	0.0088
PBDE (Oxenfield Bridge) [b]	Eels	5	164-288	20.2	0.048
HBCD (Ricknall Grange) [a]	Trout	5	21-119	14	0.034
HBCD (Haughton Road) [b]	Trout	7	159-6758	810	1.9
HBCD (Ricknall Grange) [a]	Eels	1	159	11.1	0.027
HBCD (Oxenfield Bridge) [b]	Eels	3	570-9432	660	1.57

a Control site for Skerne River

b Test site showing the highest concentration of PBDEs or HCBD in trout or eels

c Portion sizes were assumed to be 120g trout or 70g eels, as cited in MAFF Food Portion Sizes[23]

d Average daily intake was calculated assuming consumption of one portion of trout or eels per week and an adult bodyweight of 60kg

45. For HBCD the concentration in the edible portion of trout varied from 21-119μg/kg freshweight and was 159 μg/kg freshweight in the eel at control sites. At test sites, the sum of the concentrations was 159 to 6758 μg/kg freshweight in trout and 570 to 9432 μg/kg freshweight in eels. The trout population of the Skerne Tees River system is restocked annually and no information was available to ascertain the ages of the fish sampled and whether concentrations increased in older fish. Detailed results will be published in a Food Surveillance Information Sheet.

46. The estimated average intake from consumption of one weekly portion (120g) of trout at the maximum levels detected were 0.056 μg/kg bw/day of PBDEs and 1.9 μg/kg bw/day of HBCD. The estimated average intake from consumption of one weekly portion (70g) of eels, were 0.048 μg/kg bw/day of PBDEs and 1.57 μg/kg bw/day of HBCD.

47. There are no commercial fisheries in the river and consumption would be limited to fish caught by anglers.

Total dietary exposure to PBDEs and HBCD

48 None of the congeners measured was present at concentrations exceeding the limit of detection (LOD) in any of the composite TDS samples analysed. Estimated intakes were therefore calculated from the upper bound concentrations (assuming that PBDE and HBCD were present at the LOD in all foods) together with consumption data from the 2000 National Diet and Nutrition Survey[24]. Dietary exposure for mean adult consumers was estimated to be $\leq$0.047 μg/kg bw/day for the sum of the PBDE congeners measured and ≤0.010 μg/kg bw/day for the sum of HBCD isomers. The actual intakes might be very much lower.

COT evaluation of dietary exposure to PBDEs and HBCD

49. The concentrations of PBDEs and HBCD detected varied widely in the fish sampled from the test sites and also from those sites considered to be control sites. In the absence of information to the contrary, it is assumed that the fish will move freely around the Skerne-Tees river system and therefore concentrations in the fish taken from one site are not representative. It is therefore not possible to identify an average concentration of PBDEs or HBCD which could be used to estimate intake for an individual regularly consuming fish caught from an individual site. Therefore worst-case intake estimations have been calculated from the highest measured concentration at any site. It is unlikely that this worst case intake would be achieved on a regular basis.

50. The PBDE congeners measured in the survey were selected as major representative components of penta- and octaBDE. Toxicity data are not available for the individual congeners. Concentrations of the individual congeners have therefore been summed for comparison with the toxicity data on the commercial PBDE mixtures. Studies on the commercial PBDEs indicate that pentaBDE is the most toxic. Comparison of the estimated intakes of the sum of the measured PBDE congeners with the reported effect levels for pentaBDE provides a precautionary approach because some of the congeners are expected to be less toxic.

51. The most sensitive effect of pentaBDE and HBCD was considered to be neurodevelopmental. The LOAEL for pentaBDE (600μg/kg bw/day) for this effect was obtained from studies in which the test material was administered by a single oral dose to mice on postnatal days 3 or 10. Limited evidence suggests that HBCD also has this effect. Human infants of comparable developmental stage (up to 1 month) would not eat fish and so would not be directly exposed to PBDE or HBCD from fish. Although pentaBDE is known to pass into breast-milk, the available data are not sufficient to estimate the potential exposure to the breast-fed infant resulting from consumption of contaminated fish by the mother. There is a need for data on levels of PBDEs and HBCD in breast-milk in the UK, in order to determine whether the breast-fed infant is at risk of neurodevelopmental effects arising from consumption of contaminated fish. The available studies on reproductive toxicity have not included investigation of neurobehavioural effects, and therefore there are no data of relevance to exposure by

pregnant women. Overall, it was considered not possible to calculate a relevant MoE with respect to neurodevelopmental effects.

52. For older children and adults eating trout or eels contaminated with PBDEs and HBCD, the liver toxicity is the most relevant and sensitive effect on which to base a risk assessment.

53. The worst-case estimated intake of total PBDEs from consuming one portion of trout per week from the Skerne-Tees river system was 0.056 μg/kg bw/day indicating a MoE of approximately 10,000 compared with the NOAEL of 450 μg/kg bw/day for liver effects of pentaBDE in rats. The MoE for intake of PBDEs from consuming one portion of eels per week would also be about 10,000. Since these MoEs are larger than the target MoE of 1000 for pentaBDE, these intakes are unlikely to pose a health risk.

54. The worst case estimated intake of total HBCD from consuming one portion of trout per week was 1.9 μg/kg bw/day indicating a MoE of approximately 50,000 compared with the LOAEL of 100,000 μg/kg bw/day for liver effects of HBCD in rats. The MoE for intake of HBCD from consuming one portion of eels per week would be about 60,000. Since these MoEs are larger than the target MoE of 3,000-10,000 for HBCD, these intakes are unlikely to pose a health risk.

Conclusions

55. We *conclude* that the uncertainties and deficiencies in the toxicological databases for PBDEs and HBCD prevent establishment of tolerable daily intakes. A Margin of Exposure (MoE) approach has therefore been used in this risk assessment.

56. We *consider* that the most sensitive endpoint for the PBDEs appears to be neurodevelopmental effects resulting from a single oral administration to neonatal mice at a developmental stage comparable to infants up to one month of age, and limited data indicate that HBCD could also have this effect. It is reassuring that infants of this age do not eat fish and therefore are not directly exposed to PBDEs from this source.

57. We *note* the uncertainty in the relevance of the neurodevelopmental effects for exposure to the fetus or breast-fed infant following maternal consumption of fish containing high levels of PBDEs or HBCD. This results from the lack of neurodevelopmental studies with exposure during pregnancy and the lack of information on concentrations in breast milk that could result from consumption of fish by the mother.

58. We *note* that consumption of fish from the Skerne Tees is unlikely to be widespread since there are no commercial fisheries in the area. However given the variability in BFR levels observed in this limited survey, it is not possible to exclude higher intakes in a small number of anglers or others eating their fish.

59. We *consider* that comparison of the worst case estimated intakes from consumption of a single portion of eels or trout per week from the Skerne Tees with the available toxicological data indicates that these intakes are unlikely to represent a risk to health. However, in view of the uncertainties surrounding the toxicological database and exposure assessments, this conclusion should be considered tentative.

60. PentaBDE and octaBDE are being phased out in 2004, which offers some reassurance that exposure to these compounds is unlikely to increase significantly. Concentrations of deca-BDE and HBCD should continue to be monitored, particularly in fatty foods.

COT statement 2003/04

October 2003

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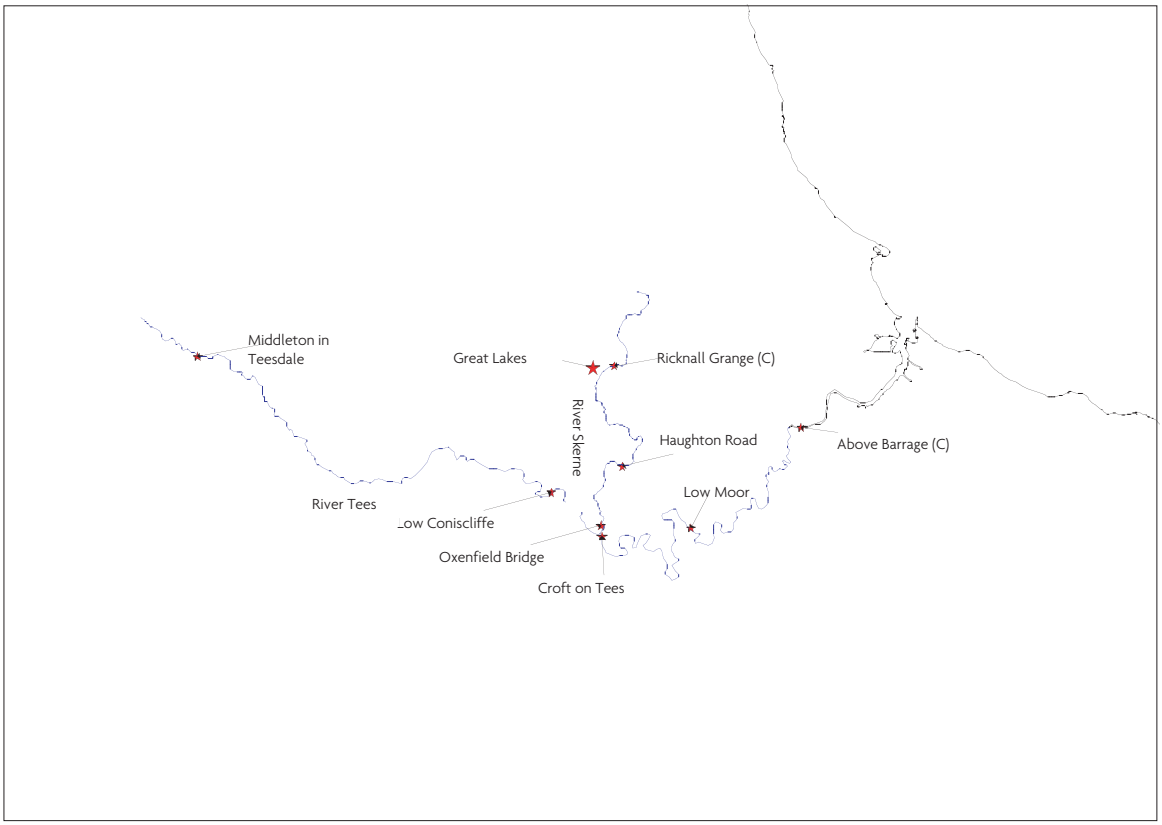
Figure 3. Sampling locations for the survey for brominated flame-retardants in fish from the Skerne-Tees River system



The sites shown on the map are the sites where samples of fish and eels were taken from. The sampling sites were upstream and downstream of the Great Lakes Chemical Company and at the confluence of the two rivers. Control samples (c) were obtained from the River Skerne at Ricknall Grange and from the River Tees at above the Tees Barrage. It was only possible to sample both trout and eels at the Oxenfield Bridge and Croft-on-Tees sampling sites.

Statement on fluorine in the 1997 Total Diet Study

Introduction

1. In 2000 the COT considered the results of a study conducted by the Food Standards Agency in which samples collected in the 1997 Total Diet Study (TDS) were analysed for the presence of fluorine, bromine and iodine. The Committee concluded that the total dietary intakes of bromine and iodine estimated from the survey were unlikely to pose a risk to health[1]. However, consideration of fluorine was deferred as the toxicity of this trace element was due to be considered by the *ad hoc* Expert Group on Vitamins and Minerals (EVM). The final report of the EVM was published in May 2003. The EVM concluded that fluoride was not within its remit as food fortification with fluoride is carried out as a public health measure. Determination of maximum levels of supplementation therefore has to take place within the context of local exposure and involves a consideration of risks and benefits, which was not in the terms of reference of the EVM. The EVM report is available at http://www.food.gov.uk/science/ouradvisors/vitandmin/.

Background

2. Fluorine is a trace element which is ubiquitous in the environment and is present at low levels in all plants and animals. The analytical method used in this survey did not distinguish between different forms of fluorine. Elemental fluorine is a highly reactive gas, and the ionic form fluoride is present in food. Therefore this statement considers the toxicity of fluoride, and uses the term fluoride throughout for ease of clarity.

3. Based on studies in animals, fluoride was considered by a WHO expert committee to be necessary for animal life[2]. However, although low intakes of fluoride in humans are associated with increased incidences of dental caries and general weaknesses of bones and teeth, a true fluoride deficiency state has not been documented. Human requirements have therefore not been determined.

4. Fluoride has a well-documented beneficial effect in protecting against dental caries[3]. Systemic exposure to fluoride during the pre-eruptive development of teeth results in its incorporation into the enamel matrix of the tooth, forming an enamel which is more resistant to acid decay. Post-eruption, fluoride has a beneficial topical effect, apparently by reducing enamel demineralisation and promoting remineralisation, and by inhibiting plaque acid-producing bacteria. For these reasons many dental products and some public water supplies are artificially fluoridated.

Toxicity of fluoride

Dental fluorosis

5. The most sensitive effect of excessive fluoride exposure in humans is considered to be dental fluorosis, a developmental defect of the tooth enamel. Dental fluorosis is caused by the over-incorporation of fluoride into the dental enamel to the effect that the composition and structure of the enamel are altered.

6. Dental fluorosis may be classified, using Dean's classification, as very mild, mild, moderate or severe. Pictures of the various forms of dental fluorosis can be viewed on pages 32-34 of the book 'Health Effects of Ingested Fluoride', by the US National Research Council[4], which is available to view electronically at http://books.nap.edu/books/030904975X/html. In its mildest forms, dental fluorosis presents as a barely visible white mottling of the teeth, which may not be apparent to the affected individual and is not considered to be aesthetically significant. The dental integrity of mild to moderately fluorosed teeth is not affected, and they may be more resistant to acid decay than non fluorosed teeth[5]. Moderate and severe effects of dental fluorosis include more noticeable white mottling, yellow/brown staining and pitting of the enamel[6].

7. Data from epidemiological studies suggest that the enamel tissue is most susceptible to fluoride-induced changes during the third or fourth years of life for the permanent anterior teeth and at 22-26 months for the maxillary central incisors, which is when the enamel is in the transitional or early maturation stages[7]. The maxillary central incisors are the two teeth most visible when smiling and therefore fluoride-induced changes in these teeth would be of most concern. The pre-eruptive maturation of the permanent teeth is completed by the age of 8 years, and children over the age of 8 years and adults are not susceptible to dental fluorosis.

8. Epidemiological studies by Dean (1942)[8] showed that in populations with drinking water containing about 2 mg/L fluoride, less than 5% of the population had moderate dental fluorosis. According to the US Food and Nutrition Board, total fluoride intakes from food and water in these populations were estimated as 0.08-0.12 mg/kg bw/day[5]. Fluoride-containing dental products and supplements were not available at the time. In populations with water containing close to 1 mg/L fluoride, a low prevalence of very mild to mild dental fluorosis (10-12%) and no cases of moderate dental fluorosis were observed[8] (estimated average total fluoride intake, 0.05 mg/kg bw/day[5]). An intake of 0.05 mg/kg bw/day is therefore assumed to be a no observed adverse effect level (NOAEL) for moderate dental fluorosis.

9. The prevalence of aesthetically significant fluorosis (moderate or severe) is estimated to be 3-4% in areas of the UK where the drinking water is artificially fluoridated and 0.5-1% where it is not artificially fluoridated[3].

Skeletal fluorosis

10. Symptomatic or clinical skeletal fluorosis is a condition characterised by skeletal abnormalities and joint pain. It is caused by pathological bone formation due to the mitogenic action of fluoride on osteoblasts. In its more severe forms, skeletal fluorosis causes kyphosis, crippling and invalidism. Secondary neurological complications in the form of myelopathy, with or without radiculopathy, may also occur.

11. Clinical skeletal fluorosis is endemic in regions of the world which have high fluoride levels in the water (up to 18 mg/L in 15 states of India) and hot, dry climates. In such climates clinical skeletal fluorosis has been associated with consumption of water containing fluoride levels as low as 1.5 mg/L[9]. However, studies conducted in the US in the 1950s indicate that in more temperate climates, no cases of clinical skeletal fluorosis were associated with fluoride levels up to 4 mg/L in drinking water[10]. The reason for the difference is uncertain, but it is likely to be largely due to the increased consumption of water in hot, dry climates. Dietary differences and fluoride exposures from other sources may also have contributed to the difference. There is no evidence of clinical skeletal fluorosis arising from exposures in the UK.

Other skeletal effects

12. A number of studies have investigated possible links of fluoride exposure, primarily through fluoridation of water, with fracture risk. Some studies reported a protective effect of increased fluoride exposure and others an increase in fracture incidence. In a recent meta-analysis of studies on bone fracture frequency and water fluoridation, no significant associations were found, except for studies of 10 years or longer, which showed a protective effect of water fluoridation on fracture risk[11].

13. A number of studies in humans and animals have investigated a possible relationship between fluoride intake and incidence of osteosarcoma because fluoride accumulates in bone and has a mitogenic action on osteoblasts.

14. The Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) reviewed the mutagenicity of fluoride in 1990. The COM concluded that although *in-vitro* mutagenic effects were seen at relatively high concentrations, the activity was considered to be indirect and unlikely to occur at low concentrations. All well-conducted *in-vivo* mutagenicity tests were negative. The COM therefore concluded that the consumption of fluoridated water would not constitute a mutagenic hazard to man. This view was endorsed in 1995 when some additional studies were considered[12].

15. The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) considered the available epidemiology and animal bioassay data in 1990, including the US National Toxicology Program (NTP) 2 year carcinogenicity study in F344/N rats and B6C3F1 mice, with drinking water concentrations up to 175 mg/L sodium fluoride (79 mg/L fluoride ion)[13]. The COC concluded that there was no evidence to indicate any carcinogenic risk to humans from exposure to fluoride[14]. There have been no data published since then to warrant seeking a further view from the COC.

Renal toxicity

16. Fluoride nephrotoxicity has been investigated in a number of animal species but the observed effects have generally been subtle, e.g. dilatation of the renal tubules and increased diuresis. No renal effects were observed in the 2-year NTP studies in rats and mice given drinking water containing 79 mg/L fluoride, providing intakes of approximately 7.9 and 15.8 mg/kg bw/day, respectively[13].

17. No renal disorders have been identified in humans in areas of endemic dental and skeletal fluorosis. One report exists of a single case of renal failure occurring in an individual who consumed, over a long period of time, large amounts of a mineral water containing 8.5 mg/L fluoride. Osteosclerosis was also diagnosed and the effects were attributed to the fluoride. However, it was not possible to calculate the fluoride intake of this individual[15].

Cardiac effects

18. Research conducted in China has shown that the percentage of patients with abnormal electrocardiograms (ECG) increased with increasing severity of skeletal fluorosis symptoms[16]. The relevance of this to fluoride exposure in the UK is unclear.

Reproductive effects

19. Fluoride has been shown to be toxic to the male reproductive system following administration to rats at 9 mg/kg bw/day for 29 days (as sodium fluoride, given by oral gavage). Effects on testis, prostate and seminal vesicle weights, reduced plasma testosterone levels, reduced epididymal sperm counts and reduced testicular Δ5,3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase activities have been reported. Histological findings included dilatation of seminiferous tubules and reduced numbers of mature luminal spermatozoa[17]. However, other studies in rats and rabbits have shown no adverse effects at higher doses (8.1-9.5 and 27 mg/kg bw/day in rats and 18 mg/kg bw/day in rabbits) given in the drinking water[18,19].

20. In humans, high fluoride intakes and symptoms of skeletal fluorosis have been associated with decreased serum testosterone levels[20]. The relevance of this observation to fluoride exposure in the UK is also unclear.

Neurotoxicity and neurobehavioural effects

21. Nine adult male Long-Evans rats were exposed for up to 52 weeks to drinking-water reported to contain 2.1 mg/L sodium fluoride (0.95 mg/L fluoride, or about 0.06 mg fluoride/kg bw/day)[21]. Although the administered fluoride is likely to have been insignificant in comparison to the fluoride content of the normal rat diet (which was not assessed), the authors reported statistically significant differences from a control group for brain aluminium, neuronal cell injury, and cerebral IgM and immunoreactivity for beta-amyloid. This study was seriously flawed by a high incidence of intercurrent infections[22] and mortality, which may have contributed to the findings.

22. The behavioural effects of pre-natal exposure to sodium fluoride have been studied in two experiments in Sprague-Dawley rats[23]. Pregnant dams were administered sodium fluoride by 9 subcutaneous injections of 0.13 mg/kg bw (0.06 mg fluoride per kg bw) in order to produce high peak plasma fluoride levels. In the first experiment, 1 or 2 injections per day were given to 7 dams on gestation days 14-18 inclusive. In the second experiment, 3 injections per day were given to 9 dams on gestation days 17-19 inclusive. Plasma fluoride levels were measured in pups at 3 and 9 weeks of age, and did not differ from levels in controls. Behavioural testing on the pups was undertaken at 9 weeks of age, leading to three measures of spontaneous behaviour (initiations, total time, and time structure). The only statistically significant difference from matched controls was for behavioural time structure, in males, in the GD 17-19 study.

23. These authors applied the same behavioural tests in a study of Sprague-Dawley rats provided with drinking-water containing fluoride at 0, 75, 100 or 125 mg/L from weaning at age 3 weeks, for 6-20 weeks[23]. At 75 mg/L (corresponding to a daily fluoride intake of about 3.8 mg/kg body weight), there was no statistically significant difference from controls. At 100 mg/L (corresponding to a daily fluoride intake of about 5 mg/kg body weight), differences in behaviour were found in females; no data on males at this dose were presented. The concentration of 125 mg/L (corresponding to a daily fluoride intake of about 6.3 mg/kg bw) was associated with reduced weight gain (8-17% reduction) and differences in behaviour, in both males and females. In a further study[23], adult male and female Sprague-Dawley rats were given drinking-water containing 100 mg/L fluoride for 6 weeks, from age 12 weeks; differences in behaviour were found in females but not in males.

24. Dose-related reductions in cell size and number of neurons in the hippocampus and dentate gyrus were noted in a study of adult female Swiss albino mice given drinking-water containing sodium fluoride at 0, 30, 60 and 120 mg/L for 30 days (5 animals per dose)[24]. It is not clear whether the concentrations were stated as fluoride or sodium fluoride; if the latter, they correspond to fluoride concentrations of 0, 13.6, 27 and 54.3 mg/L, and estimated fluoride intakes of approximately 0, 2.7, 5.4 and 10.9 mg/kg bw per day). Adverse effects on motor co-ordination, swim endurance and maze skill were noted at the top dose but not at the lower doses.

25. No abnormalities were seen in the brain (frontal cortex and basal ganglia, parietal cortex and thalamus, cerebellum and pons) pituitary gland, and spinal cord, in F344N rats given up to 300 mg/L sodium fluoride or in B6C3F1 mice given up to 600 mg/l sodium fluoride in drinking-water for 6 months[13]. No abnormalities were seen in these tissues, or in sciatic nerve, in these strains of rat and mouse given up to 175 mg/L sodium fluoride in drinking-water for 2 years[13]. These studies did not include routine examination of the hippocampus, or specific neurobehavioural tests.

The 1997 Total Diet Study

26. The Total Diet Study (TDS) forms part of the Food Standards Agency's surveillance programme for chemicals in food. Analyses for metals and other elements are generally carried out every three years. However, 1997 was the first year since 1980 in which fluoride had been considered.

27. A total of 400 samples were collected from retail outlets in 20 locations throughout the UK. Each of the samples was prepared or cooked according to normal domestic practice at a central location. The samples were combined into 20 composite food groups, the proportion of each food in a food group reflecting its importance in the average UK diet (largely based on an average of three years previous consumption data from the National Food Survey). The fluoride present in each sample was diffused as hydrogen fluoride at room temperature in the presence of perchloric acid saturated with hexamethyldisiloxane. The released fluoride was absorbed into a trapping layer of sodium hydroxide, which was then dried, dissolved in water and the fluoride content determined by ion exchange chromatography[25].

Results of the Total Diet Study

28. The full results of the survey were published in a Food Surveillance Information Sheet[25]. The highest mean fluoride concentrations were found in fish (1.9 mg/kg) and beverages (1.1 mg/kg). The fluoride content of beverages largely reflects the fluoride content of the water used in their preparation. However, tea contains higher amounts as fluoride is selectively taken up from the soil by the tea plant. The high fluoride levels in fish are thought to originate mainly from the skeleton, as fluoride accumulates in the bones of fish and some canned fish contains small bones.

Dietary exposure to fluoride

29. The TDS data were used to estimate dietary exposure to fluoride. Using food consumption data from the National Food Survey, which is updated every year based on household food purchases and so reflects changes in consumption patterns, the mean population intake of fluoride was estimated to be 1.2 mg/person/day (0.02 mg/kg bw/day for an average 60 kg person). Dietary exposure to fluoride was last estimated in 1984, when the mean population intake, calculated from concentrations of fluoride determined in selected food samples from the 1978, 1979 and 1980 Total Diet Studies, was estimated to be 1.8 mg/person/day. However, due to changes in the TDS design since 1981 and the limited number of samples that were used to estimate the intake in 1984, a direct comparison between the 1997 TDS and this earlier estimate cannot be made.

30. Mean and high level consumer intakes of fluoride for adults and children were estimated using consumption data from the National Diet and Nutrition Survey (NDNS) and are shown in Table 1. The highest intakes were for the 4 to 6 years age group, for whom high level dietary exposure was 0.06 mg/kg bw/day.

Table 1: Estimated dietary exposure to fluoride by children and adult consumers

Population group	Dietary exposure (mg/kg bw/day)	
	Mean	97.5th percentile
1½ to 4½ years[a]	0.023	0.053
4 to 6 years[b]	0.031	0.060
7 to 10 years[b]	0.024	0.047
11 to 14 years[b]	0.017	0.037
15 to 18 years[b]	0.015	0.034
Adults (19+ years)[c]	0.016	0.033

Notes:

a. Food consumption data from the NDNS: children aged 1½ to 4½ years[26]

b. Food consumption data from the NDNS: young people aged 4 to 18 years[27]

c. Food consumption data from the 1986/87 Dietary and Nutritional Survey of British Adults[28]

Other sources of exposure

31. Drinking water is a notable source of fluoride. The regulatory limit for fluoride in the UK public water supply, defined by The Water Supply (Water Quality) Regulations 1989, is 1.5 mg/L. Most public water supplies contain less than 0.7 mg/L fluoride. However, 10% of the UK water supply is artificially fluoridated to a level of 1.0 mg/L as a public health measure to protect against dental decay.

32. The regulatory limit for fluoride in spring water and bottled drinking water, defined by the Natural Mineral Water, Spring Water and Bottled Drinking Water Regulations 1999, is 1.5 mg/L, the same as for tap water. There is currently no regulatory limit on the amount of fluoride that natural mineral water may contain; however, an EC directive specifying a limit of 5 mg/L has been proposed, to apply from 1 July 2004 onwards. In a recent survey of 25 brands of bottled waters purchased in the UK, the maximum fluoride concentration identified was 0.37 mg/L[29].

33. Estimated fluoride intakes from water are 0.062, 0.033, 0.023 and 0.021 mg/kg bw/day for children aged 7 months to 4 years, 5 to 11 years, above the age of 12 years and adults, respectively. This assumes consumption of 0.8, 0.9, 1.3 and 1.5 L/day[30, 31] of water containing 1 mg/L fluoride and average body weights of 13, 27, 57 and 70 kg[30, 31], respectively for these age groups. Table 2 indicates total possible intakes from the diet and drinking water combined.

Table 2: Total possible fluoride intakes (mg/kg bw/day) from the diet and drinking water combined assuming water fluoride concentrations of 0.7 and 1.0 mg/L and mean or 97.5th percentile dietary intake

Population group	Fluoride concentration of drinking water 0.7 mg/L Mean intake (mg/kg bw/day)	97.5th %ile intake (mg/kg bw/day)	1 mg/L Mean intake (mg/kg bw/day)	97.5th %ile intake (mg/kg bw/day)
1½ to 4½ years	0.066	0.096	0.085	0.115
4 to 6 years	0.054	0.083	0.064	0.093
7 to 10 years	0.047	0.070	0.057	0.080
11 to 14 years	0.033	0.053	0.040	0.060
15 to 18 years	0.031	0.050	0.038	0.057
Adults	0.031	0.048	0.037	0.054

34. Few data are available on dietary exposure of infants to fluoride. This is likely to vary greatly between breast-fed and formula-fed infants, particularly where the formula is reconstituted using water with high fluoride content. Breast milk contains only trace amounts of fluoride and has been reported to provide less than 0.01 mg/day. In a survey conducted in Australia, fluoride contents of infant formulae reconstituted using deionised water ranged from 0.031 mg/L to 0.532 mg/L[32]. Assuming an average infant weight of 7 kg and consumption of formula reconstituted with 750 mL of water containing 1 mg/L fluoride, the intake of fluoride would range from 0.11 to 0.16 mg/kg bw/day. It is not known how relevant these data are to infant formulae on the UK market, but the water would provide the major contribution.

35. Dental products such as toothpaste and mouthwash generally contain added fluoride. Most toothpaste brands contain approximately 1000 mg/kg fluoride. Low-fluoride toothpastes for children contain 400-526 mg/kg fluoride. Fluoridated mouthwashes typically contain 230 mg/kg fluoride; they are not recommended for use by children under the age of 6 years. Some of the toothpaste and mouthwash used will be ingested, especially by young children. The amount of toothpaste used varies considerably, as does the amount swallowed, but it has been suggested that children under the age of 4 use 0.2 – 0.5 g/day of toothpaste and swallow 50% on average[33]. If toothpaste containing 1000 mg/kg fluoride is used, fluoride intakes would be 0.1 to 0.25 mg/day (equivalent to 0.008 to 0.019 mg/kg bw/day, assuming an average body weight of 13 kg).

36. The use of fluoride supplements is recommended by the British Dental Association for infants and young children in areas where the water supply contains less than 0.7 mg/L water. The dosage recommended varies depending on age and the level of fluoride in the public water supply.

COT evaluation

37. The Committee considered that a study of pre-natal exposure to injected sodium fluoride in rats did not provide persuasive evidence of an effect on postnatal behaviour. Exposure of weanling and adult rats to fluoride in drinking-water at concentrations equivalent to doses of about 5 mg/kg bw/day was associated, in females only, with abnormalities in behaviour (not found at the lower dose of about 3.8 mg/kg bw/day); this dose was close to that which caused evident systemic toxicity (reduced weight gain) at about 6.3 mg/kg bw/day.

38. A study which found structural abnormalities in the brain of rats exposed to fluoride in drinking-water at concentrations equivalent to doses of about 0.06 mg/kg bw/day was seriously flawed by a high incidence of intercurrent infections and mortality. A brief account of a small short-term study in mice reported dose-related abnormalities in the hippocampus, at fluoride concentrations in drinking-water presumed to be equivalent to daily fluoride doses of about 2.7, 5.4 and 10.9 mg/kg bw, but behavioural abnormalities were found at the top dose only.

39. Neurotoxicity was not detected in rats and mice in well-conducted long-term studies which included fluoride concentrations in drinking-water higher than those used in the behavioural and neurotoxicity studies described above, but which did not include routine examination of the hippocampus, or specific neurobehavioural tests.

40. The Committee noted that the most sensitive effect in humans is dental fluorosis, which occurs in children under the age of 8 years. Mild and very mild forms of dental fluorosis are generally not considered to be aesthetically significant. Moderate and severe forms of dental fluorosis are characterised by more noticeable white mottling, yellow/brown staining and pitting of the enamel. The integrity of teeth with mild to moderate dental fluorosis is not affected, and the teeth may be more resistant to dental decay than non-fluorosed teeth. Research is needed to determine the impact of the cosmetic effect of dental fluorosis on the affected individual, in order to determine whether the effects should be considered to be adverse.

41. An intake of 0.05 mg/kg bw/day has been reported to be a NOAEL for moderate dental fluorosis. This intake level was associated with a low incidence (10-12%) of very mild to mild dental fluorosis, which is not usually considered to be aesthetically significant. The threshold dose at which fluoride causes moderate or aesthetically significant dental fluorosis is 0.1 mg/kg bw/day, based on studies in which less than 5% of populations exposed to intakes of fluoride in the range 0.08-0.12 mg/kg bw/day had moderate dental fluorosis.

42. Information on total fluoride intakes is limited. The data in Table 1 derived from the 1997 TDS show that dietary exposure of high level consumers aged 11/2 to 6 years exceeds the NOAEL of 0.05 mg/kg bw/day by up to 20%. Taking into account other sources of exposure such as water and dental products, it is likely that a significant proportion of children under the age of 8 years have a total fluoride exposure above the NOAEL. Some of these children may be at risk of mild to moderate dental fluorosis, particularly during the third and fourth years during formation of the permanent anterior teeth. Because of the imprecise information on total exposure, it is not possible to predict the proportion that would exceed the threshold of 0.1 mg/kg bw/day, at which 5% of the exposed population would be expected to develop moderate (aesthetically significant) dental fluorosis. However, data on the prevalence of dental fluorosis in the UK indicate it to be low.

43. Breast milk contains only trace amounts of fluoride and has been reported to provide less than 0.01 mg/day. Based on the results of an Australian survey of fluoride concentrations in infant formula, intake in formula-fed infants could exceed the threshold for aesthetically significant dental fluorosis. However, although dental fluorosis may occur in the primary teeth, this may not lead to dental fluorosis of the permanent teeth if fluoride intakes have decreased by the time of the development and maturation of the dental enamel of the permanent teeth. Therefore infants may be at lesser risk than children aged 3 to 4 years.

44. There is a lack of studies to follow up long-term health outcomes of children with dental fluorosis. However, on the basis of the available information, the most sensitive effect of fluoride in children above the age of 8 years and in adults is clinical skeletal fluorosis. In regions with temperate climates, clinical skeletal fluorosis is not seen in populations with water fluoride concentrations below 4 mg/L. Other possible adverse effects of fluoride are seen at doses higher than those required to cause clinical skeletal fluorosis. It therefore appears unlikely that clinical skeletal fluorosis or any other adverse effects would occur in the general population from typical total fluoride intakes in the UK.

Conclusions

45. We *note* that a small number of studies of sodium fluoride in rodents have variously suggested abnormalities in behaviour, and structural abnormalities in the brain. These findings cannot be fully assessed without confirmatory studies. We note that neurotoxicity was not observed in well-conducted long-term studies in rodents at higher doses (more than 50 times the NOAEL in humans).

46. We *note* that the most sensitive effect of fluoride in humans appears to be dental fluorosis, which occurs in children under the age of 8 years. A total fluoride intake of 0.05 mg/kg bw/day represents a NOAEL for moderate (aesthetically significant) dental fluorosis.

47. We *consider* the results of this survey indicate that during formation of the permanent teeth, a small proportion of children may be at risk of moderate dental fluorosis due to dietary exposure to fluoride. However, we *note* that the prevalence of moderate dental fluorosis in the UK appears to be low.

48. We *note* that fluoride intakes of formula-fed infants may exceed the NOAEL for dental fluorosis, but *consider* that infants are at lesser risk because the critical time for development of aesthetically significant dental fluorosis is during formation of the permanent teeth.

49. We *note* that the integrity of teeth with mild to moderate dental fluorosis is not affected, and that the teeth may be more resistant to dental decay than non-fluorosed teeth. However, we *recommend* that more research is needed to determine the impact of the cosmetic effect of dental fluorosis on the affected individual and on any possible long-term health outcomes in people affected by dental fluorosis.

50. We *note* that more information is needed on total fluoride exposure, including intakes from toothpastes and mouthwashes.

51. We *conclude* that, based on the current information available and the dietary intakes estimated from the 1997 TDS, no adverse effects other than mild to moderate dental fluorosis would be expected to be associated with fluoride intake from food, either in adults or in children, at the intake levels in the UK.

COT Statement 2003/03

September 2003

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Updated statement on a survey of mercury in fish and shellfish

Introduction

1. In 2002, the Committee reviewed the results of a Food Standards Agency (FSA) survey of the mercury levels in imported fish and shellfish and UK farmed fish and their products[1] and the provisional results of blood mercury levels in UK adults[2].

2. The Committee concluded that the Provisional Tolerable Weekly Intake (PTWI) of 3.3 μg/kg bw/week could be used in assessing methylmercury intakes by the general population. This PTWI was initially established by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) in 1972 and confirmed on a number of occasions up to the year 2000, However, the 2000 JECFA PTWI was not considered adequate to protect against neurodevelopmental effects. The EPA reference dose of 0.1 μg/kg bw/day (0.7 μg/kg bw/week) was therefore applied for women who are pregnant, or who may become pregnant within the following year, or for breast-feeding mothers. The COT also noted that its conclusions should be reviewed following the JECFA evaluation of methylmercury in 2003[3].

3. In June 2003, JECFA recommended that the PTWI for methylmercury should be reduced from 3.3 μg/kg bw/week to 1.6 μg/kg bw/week. The Committee has therefore reviewed its previous evaluation in the light of the new JECFA PTWI, also taking into account more recent data on fish consumption by adults. This statement on mercury in fish and shellfish supersedes COT statement 2002-04.

4. The FSA has asked a subgroup of members of the COT and the Scientific Advisory Committee on Nutrition (SACN) to provide combined advice on the risks and benefits associated with fish consumption. The advice expressed in this COT statement therefore aims to protect the populations who are most susceptible to the risks of methylmercury, without being over-protective of individuals at lesser risk.

Background

5. The toxicity of mercury is dependent on whether it is inorganic, elemental or organic (e.g. methylmercury). Methylmercury affects the kidneys and also the central nervous system, particularly during development, as it crosses both the blood-brain barrier and the placenta[4]. Both neuro- and nephrotoxicity have been associated with acute methylmercury poisoning incidents in humans, and neurotoxicity, particularly in the developing fetus, has been associated with lower level chronic exposures.

6. Exposure of the general population to mercury can occur via inhalation of mercury vapour from dental amalgam fillings (elemental), or through the diet (methylmercury and inorganic mercury)[5]. Methylmercury in fish makes the most significant contribution to dietary exposure to mercury, although smaller amounts of inorganic mercury are present in other food sources. All forms of mercury entering the aquatic environment, as a result of man's activities or from geological sources, are converted into methylmercury by microorganisms and subsequently concentrated in fish and other aquatic species. Fish may concentrate the methylmercury either directly from the water or through consuming other components of the food chain. Methylmercury has a half-life of approximately 2 years in fish; thus, large older fish, particularly predatory species, will have accumulated considerably more methylmercury than small younger fish.

Previous COT evaluation

7. The COT previously considered the results of a survey of metals and other elements in marine fish and shellfish[6] published by the Ministry of Agriculture, Fisheries and Food (MAFF) in 1998. The survey examined a number of fish and shellfish species landed in the UK or imported from overseas ports including cod, haddock, herring, mackerel, lobster, mussels, crab and shrimps and samples of cod fish fingers. The survey also produced estimates of the mean and 97.5th percentile dietary intakes of the elements surveyed.

8. The 1998 survey demonstrated that the levels of mercury in the fish and shellfish tested were low and that average and high level fish and shellfish consumers in the UK would not exceed the then current JECFA PTWI for methylmercury of 3.3 μg/kg bw/week, even assuming all the mercury in fish was in this form. The estimated mercury intake for the highest level consumer was 1.1 μg/kg bw/week including mercury intake from the rest of the diet. The main conclusion drawn from the survey was that "dietary intakes of the elements surveyed were below safe limits, where defined, and did not represent any known health risk even to consumers who eat large amounts of marine fish or shellfish".

International Safety Guidelines

Previous Joint FAO/WHO Expert Committee on Food Additives (JECFA) Evaluations

9. In 1972, JECFA established a PTWI of 5 μg/kg bw/week for total mercury, of which no more than two thirds (3.3 μg/kg bw/week) should be from methylmercury[7] The PTWI of 3.3 μg/kg bw/week for methylmercury was subsequently confirmed in 1989 and 2000[8,9]. The PTWI was derived from toxicity data resulting from poisoning incidents at Minamata and Niigata in Japan. In these incidents the lowest mercury levels associated with the onset of clinical disease in adults were reported to be 50 μg/g in hair and 200 μg/L in whole blood. Individuals displaying clinical effects, such as peripheral neuropathy, at these mercury levels were considered to be more sensitive than the general population, because there were a number of persons in Japan and other countries with higher mercury levels in hair or blood who did not experience such effects. However, the methods employed in determining the intake associated with toxicity, and the subsequent establishment of the PTWI are unclear.

10. In 1989, JECFA had noted that pregnant women and nursing mothers may be at greater risk than the general population of adverse effects from methylmercury. Therefore in its 2000 re-evaluation of methylmercury, JECFA paid particular attention to possible effects of prenatal and postnatal exposure, looking at large long-term prospective epidemiological studies conducted in the Seychelles Islands and the Faroe Islands. These studies attempted to identify the lowest dietary mercury exposure associated with subtle effects on the developing nervous system[10-13]. They followed the neurological development of the children by testing their learning and spatial abilities at a number of time-points during their childhood. A number of smaller studies were also considered.

11. JECFA compared the two main studies;

- The Faroe Islands cohort was tested up to the age of 7 years, whereas at the time of the JECFA evaluation, the Seychelles cohort had only been tested up to the age of 5.5 years.

- Exposure in the Seychelles was through consumption of a range of fish species with average mercury concentrations between 0.05 and 0.25 mg/kg. In the Faroe Islands, most of the population consumed fish at least three times a week and occasionally (approximately once per month) consumed pilot whale, which contains up to 3 mg/kg mercury. Pilot whale also contains high concentrations of polychlorinated biphenyls (PCBs), but a reanalysis of the data indicated that any effects seen in the Faroes cohort could not be attributed to confounding by the PCBs[14].

- The two studies used different methodology in assessing methylmercury exposure. The Seychelles study used maternal hair samples (approx. 9cm long), one taken shortly after birth to estimate methylmercury exposure during pregnancy and one taken 6 months later. The Faroe Islands study used cord blood and maternal hair (various lengths) taken at birth.

- The studies used different batches of tests to assess the effects of methylmercury on neurological development. The tests used in the Faroe Islands study examined specific domains in the brain (visual, auditory, etc.). The Seychelles study used tests of a more global nature, with each test examining a number of domains.

12. JECFA found that although the mean mercury exposures during pregnancy (assessed by maternal hair mercury) were similar*, the results of these two studies were conflicting. In the Faroes study, regression analysis showed an association between methylmercury exposure and impaired performance in neuropsychological tests, an association that remained even after excluding the results of children with exposures associated with greater than 10 μg/g maternal hair mercury. However in the Seychelles study regression analysis identified no adverse trends, but increased maternal hair mercury was associated with a small statistically significant improvement in test scores on several of the developmental outcomes. The investigators noted that this could be due to beneficial nutritional effects of fish. A secondary analysis was performed where the results were split into sub-groups based on the maternal hair mercury level. Test scores in children with the highest mercury exposures (12 – 27 μg/g maternal hair) were not significantly different from the test scores in children with lowest exposure (< 3 μg/g maternal hair).

* Seychelles: arithmetic mean 6.8 μg/g, range 0.5-26.7 μg/g; Faroes: geometric mean, 4.27 μg/g, the upper mercury level in maternal hair is not clear from the reported data but may be as high as 70 μg/g.

13. A smaller study carried out in New Zealand on 6 year-old children[15] used a similar batch of tests to the Seychelles study and had similar exposure to methylmercury, yet found methylmercury related detrimental effects on behavioural test scores. However there were possible confounding factors that may have influenced the results of the New Zealand study, such as the ethnic group and social class of the children studied.

14. Having considered all of the epidemiological evidence, JECFA concluded that it did not provide consistent evidence of neurodevelopmental effects in children whose mothers had hair mercury levels of 20 μg/g or less. Since there was no clear indication of a consistent risk, JECFA did not revise its PTWI, but recommended that methylmercury should be re-evaluated when the latest evaluation of the Seychelles study and other relevant data become available[9].

Environmental Protection Agency (EPA)

15. In 1997 the US EPA established a reference dose of 0.1 μg/kg bw/day for methylmercury[16]. This was based on a peak maternal hair mercury level during pregnancy of 11 μg/g, which was associated with developmental effects (e.g. late walking, late talking, mental symptoms, seizures) in children exposed *in utero* during a poisoning incident in Iraq in 1971.

16. In 2000, the US National Research Council (NRC) published a review of this EPA reference dose[17]. Following analysis of the data resulting from the available epidemiological studies, the NRC identified a benchmark dose lower confidence limit of 12 μg/g in maternal hair (corresponding to 58 μg/L in cord blood, assuming a ratio of hair:cord blood of 200:1). This was the lower 5% confidence limit of the lowest dose considered to produce a sufficiently reliable neurological endpoint (a 5% increase in abnormal scores on the Boston Naming Test†) in the Faroe Islands study. The NRC made a number of assumptions in deriving an estimate of methylmercury intake and included a composite uncertainty factor of 10, to account for interindividual variability and database insufficiencies, concluding that the reference dose of 0.1 μg/kg bw/day, as had previously been used by the EPA, was scientifically justifiable.

2003 JECFA Evaluation

17. At its 61st meeting in June 2003[18], JECFA reviewed the new data from the Seychelles Child Development Study[19], re-analyses of the Faroes and New Zealand studies, epidemiological data from a number of small scale cross-sectional studies, and additional epidemiological data on reproductive toxicity, immunotoxicity, cardiotoxicity and general medical status.

18. The 9-year neurodevelopmental evaluations from the Seychelles study were performed using neurodevelopmental tests which, in contrast to the earlier assessments, allowed a direct comparison with the results of the Faroes Islands Study. The new data from the Seychelles study were consistent with results obtained at younger ages and provided no evidence for an inverse relationship between

† The Boston Naming Test is a neuropsychological test that assesses an individual's ability to retrieve a word that appropriately expresses a particular concern, for example naming an object portrayed by a simple line drawing.

maternal methylmercury exposure and neurodevelopmental performance in infants. Additional analyses carried out on the Seychelles data from younger ages did not alter the conclusion that in the Seychelles population of frequent fish-consumers, no adverse effects of prenatal methylmercury exposure have been detected.

19. No new data were available from the Faroes Islands study. New analyses of the existing data did not support a role of occasional exposure to higher levels of methylmercury or polychlorinated biphenyls (PCBs) from consumption of whale-meat, in accounting for the positive associations in this study[14,20-22]. The additional epidemiological data from smaller cross-sectional studies on neurodevelopmental effects of methylmercury were reviewed. Because of the cross-sectional design and because adult hair mercury levels do not accurately reflect previous exposure during the critical period for neurodevelopmental effects, JECFA did not consider that the results from these studies could be used to form the basis of a dose response assessment.

20. JECFA noted that despite additional evidence of immunotoxicity, cardiotoxicity, and reproductive toxicity, neurotoxicity was still considered to be the most sensitive endpoint, and concluded that the PTWI should be based on studies of this endpoint. It was uncertainty about the possibility that significant immunotoxicity or cardiovascular effects could occur at levels below the neurodevelopmental benchmark dose that had led to the inclusion of an additional safety factor for database insufficiencies in the composite factor of 10 recommended by the NRC.

21. JECFA based its evaluation on the Seychelles and Faroe Islands studies. In the absence of a dose response analysis of the latest Seychelles data, the analysis of the data from younger ages was used since it was consistent with the latest data. Exposure associated with a maternal hair concentration of 15.3 μg/g mercury was identified as the no observed adverse effect level (NOAEL) for the Seychelles study[23]. A benchmark dose lower confidence limit (BMDL) of 12 μg/g mercury in maternal hair was determined from the Faroes data[24-27]. This was viewed as a surrogate for the NOAEL.

22. Averaging the NOAEL and the BMDL resulted in a composite maternal hair concentration of 14 μg/g mercury reflecting exposure that was without effects in these study populations. Dividing by the average hair:blood ratio of 250 allowed conversion of the 14 μg/g in hair to a maternal blood mercury level of 56 μg/L. A pharmacokinetic model appropriate to pregnancy was then used to convert the blood mercury level to a steady-state daily ingestion of methylmercury of 1.5 g/kg bw/day, which would be without appreciable adverse effects in the offspring of the Seychelles and Faroe Islands study populations. The model assumed a maternal blood volume of 7 L (9% of body weight) whereas the EPA used a value of 5 L and the NRC 3.6 L.

23. JECFA then applied a data-specific adjustment factor of 2 to allow for inter-individual variability in the hair:blood ratio, and a default uncertainty factor of 3.2 to account for inter-individual variability in the association between blood mercury concentration and intake. This resulted in a PTWI of 1.6 g/kg bw/week, which JECFA considered to be sufficiently protective of the developing fetus. A factor for inter-individual variability in toxicodynamics was not required because the PTWI was based on studies in the most sensitive subgroup.

24. In its review, JECFA found no additional information that would suggest that the general population is at risk of methylmercury toxicity at intakes up to the previous PTWI of 3.3 g/kg bw/week.

Survey of the mercury levels in fish

25. The 2002 FSA survey complemented the previous MAFF survey since it examined a wider range of fish, including imported exotic species of fish that have become more widely available on the UK market. These included shark, swordfish, marlin, orange roughy, red snapper and monkfish, as well as UK farmed fish such as salmon and trout[1].

26. Of the fish species covered by the survey, all but 3 species had mean mercury levels falling within the range 0.01–0.6 mg/kg of fish. This range is in line with the levels defined by European Commission Regulation 466/2001 as amended by European Commission Regulation 221/2002 (0.5 mg of mercury/kg for fish in general and 1.0 mg mercury/kg for certain larger predatory species of fish including shark, swordfish, marlin, tuna and orange roughy).

27. The 3 species with the highest mercury content were shark, swordfish and marlin. These fish had mean mercury levels of 1.52, 1.36, and 1.09 mg/kg respectively and were therefore above the levels defined in European Commission Regulation 221/2002. Fresh tuna contained mercury levels ranging from 0.141 to 1.50 mg/kg with a mean of 0.40 mg/kg (only one sample out of 20 exceeded 1 mg/kg, the maximum mercury concentration in the other 19 samples was 0.62 mg/kg), whereas canned tuna had a lower mean mercury level of 0.19 mg/kg.

Blood mercury levels in British adults

28. A report produced by the Medical Research Council Human Nutrition Research in March 2002 detailed the provisional blood total mercury data obtained from 1320 adults (aged 19-64 years) participating in the National Diet and Nutritional Survey (NDNS)[2].

29. The mean and 97.5th percentile blood mercury levels in the survey were 1.6 and 5.88 g mercury/L respectively. The highest blood mercury level found in the study was approximately 26 μg/L in an individual with a high fish intake. If the blood mercury level was at steady state, and assuming a body weight of 70 kg and a blood volume of 9% of the body weight, then using the same pharmacokinetic model employed by JECFA in its 2003 evaluation, this would correspond to a mercury intake of approximately 5.39 μg/kg bw/week (0.77 μg/kg bw/day).

30. Of the population covered by the survey, 97.5% had blood mercury levels indicating that their mercury intakes were within the 2003 JECFA PTWI of 1.6 μg/kg bw/week.

COT evaluation

31. The Committee discussed the possible risks associated with dietary exposure to methylmercury, in the light of the new JECFA PTWI and the information on intakes from fish and on blood mercury levels in the UK population.

Toxicokinetic considerations

32. Following ingestion, approximately 95% of methylmercury is absorbed through the gastrointestinal tract, and it is subsequently distributed to all tissues in about 30 hours with approximately 5% found in blood and 10% in the brain. The methylmercury concentration in red blood cells is approximately 20 times higher than that in the plasma. Methylmercury readily crosses the placental barrier. Fetal brain mercury levels are approximately 5-7 times higher than in maternal blood. Methylmercury readily accumulates in hair and the ratio of hair mercury level μg/g) to maternal blood mercury level g/L) is approximately 250:1. Based on comparisons to hair concentrations, cord blood concentrations are reported to be 25% higher than the concentrations in maternal blood[10].

33. The excretion process for methylmercury involves transfer of the glutathione-mercury complex into the bile, demethylation by gut microflora to the inorganic form, then elimination from the body in the faeces. The half-life of mercury in the body is approximately 70 days in adults, with steady state being reached in about one year. Significant amounts of methylmercury also pass into the breast milk of lactating women, resulting in a decreased mercury half-life of approximately 45 days[28].

34. Doherty and Gates[29] reported that the excretion rate of mercury in the suckling rodent is less than 1% of the adult excretion rate. Sundberg *et al.*[30] reported a low elimination of mercury in suckling mice until lactational day 17. This is probably because biliary secretion and demethylation by microflora (which lead to faecal excretion) do not occur in suckling animals. The role of these processes in suckling human infants is unknown [4].

35. The concentration of mercury in breast-milk is approximately 5% of the blood mercury concentration of the mother[29]. Amin-Zaki *et al.*[31] reported that in women exposed to high levels of methylmercury during the Iraqi poisoning incident, 60% of the mercury in breast-milk was in the form of methylmercury. Therefore it may be estimated that the concentration of methylmercury in the breast-milk is approximately 3% of the total mercury concentration in the blood. For an infant to be exposed to methylmercury at the new JECFA PTWI of 1.6 μg/kg bw/week, the mother would have to be exposed to the following methylmercury level:

    Methylmercury intake of infant: = 0.23 μg/kg bw/day

    Assuming a daily milk intake of 150 mL/kg bw
    Concentration of methylmercury in milk = 1.53 μg/L

    Assuming 3% methylmercury transfer from maternal blood to milk
    Maternal blood mercury level = 51.1 μg/L

    Using the pharmacokinetic model employed by JECFA in its 2003 evaluation, and assuming a maternal body weight of 65kg

    Maternal methylmercury intake = 1.36 μg/kg bw/day (9.5 μg/kg bw/week)

Susceptible populations

36. In its 2003 evaluation of methylmercury, JECFA established a PTWI of 1.6 μg/kg bw/week in order to protect against neurodevelopmental effects but found no information to indicate that the previous PTWI of 3.3 μg/kg bw/week was not sufficiently protective for groups not susceptible to neurodevelopmental effects. The COT has been asked to advise on safety guidelines for methylmercury that could be used in assessing risks associated with fish consumption. The Committee concluded that the previous JECFA PTWI of 3.3 μg/kg bw/week could be used for the general population.

37. In its 2002 statement, the Committee had used the EPA reference dose of 0.1 μg/kg bw/day (0.7 μg/kg bw/week) in considering dietary exposure of the subpopulations at risk of neurodevelopmental effects. Members therefore discussed the differences between the 2003 JECFA PTWI and the EPA reference dose. The major differences related to the use of default uncertainty factors in derivation of the EPA reference dose, whereas chemical-specific data had been incorporated into the JECFA PTWI. The 2003 JECFA evaluation also took into account data published since the EPA review. The Committee had previously noted that the EPA reference dose was precautionary and agreed that the 2003 JECFA PTWI of 1.6 μg/kg bw/week should be used to protect against neurodevelopmental effects in susceptible populations. This PTWI is only necessary for the neurodevelopmental endpoint and therefore does not apply to the general population.

38. Due to this approach of applying different guidelines for different population groups, the Committee has given particular consideration to determining which groups are at higher risk and can be considered to be susceptible populations.

39. The critical effect of methylmercury is on the developing central nervous system and therefore pregnant women are considered to be the most susceptible population because of the risk to the fetus. There have been no studies of the effects of exposure prior to becoming pregnant. However, because the half-life of methylmercury in the human body is approximately 70 days, steady state concentration is attained in approximately one year and a woman's blood mercury level at the time of becoming pregnant is dependent on the exposure to methylmercury during the preceding year. The Committee therefore agreed that women who may become pregnant within the next year should also be considered as a susceptible population.

40. The evidence regarding consideration of other susceptible populations is not conclusive. Animal experiments indicate that exposure via breast-milk has less serious consequences to the central nervous system than prenatal exposure. Spyker and Spyker[32] reported that the effects of prenatal exposure to methylmercury dicyandiamide on the survival and weight gain of the offspring were more severe than those seen with postnatal exposure, and were greatest when the methylmercury was administered late in the period of organogenesis. However, these results are not necessarily relevant to the health effects of concern in human exposure.

41. Data from a 5-year longitudinal study following the Iraq poisoning incident have suggested that some children exposed to methylmercury via breast-milk demonstrated delayed motor development[31]. The maternal blood mercury levels immediately following the incident were estimated by extrapolation to

be in the range of approximately 100 to 5000 μg/L. Mothers who showed signs and symptoms of poisoning (ataxia, dysarthria, visual disturbance etc.) tended to have the higher blood levels (3000 to 5000 μg/L) although some women with levels in this range were asymptomatic.

42. The affected infants all had blood mercury levels above those associated with the 2000 JECFA PTWI of 3.3 μg/kg bw/week, and most of them had blood mercury levels higher than the minimum toxic level for adults of 200 μg/L, defined by JECFA. There was no paralysis, ataxia, blindness or apparent sensory change and there were no cases of the severe mental destruction and cerebral palsy that had been seen in the prenatally exposed infants of Minamata. However, language and motor development of the children were delayed. The authors of the study concluded that breast-fed infants are at less risk than the fetus, since most of the brain development has already occurred and the effects seen in the breast-feeding infant are different from those seen in infants exposed prenatally and not as severe.

43. There is no evidence that chronic exposure to methylmercury via breast milk at levels below those observed in the Iraqi incident has any adverse effect on the neurophysiological/psychological development of the child. Data from the Faroe Islands study suggests that the beneficial effects of nursing on early motor development are sufficient to compensate for any adverse impact that prenatal exposure to low concentrations of methylmercury might have on these endpoints[33,34]. Grandjean *et al.*[33] looked at the relationship between seafood consumption and concentrations of contaminants in breast-milk in the Faroes Island population. Of 88 samples of breast-milk, three had a mercury level that would cause the infant to exceed the old PTWI for mercury.

44. There have been few studies of the effects of methylmercury on young children. Most information has come from the poisoning incidents in Minamata, Niigata and Iraq. In all of these cases the exposures were very high, and in Iraq, the exposure was acute. Methylmercury is excreted by children as efficiently as by adults[4]. In the incidents where children were exposed to methylmercury directly rather than prenatally, the damage seen in the brain was similar to that seen in adults: focal lesions of necrosis. The damage seen when the fetus is exposed is much more widespread[4].

45. The longitudinal study in the Seychelles has attempted to examine the effects of postnatal exposure to methylmercury[12]. This is complicated by the facts that in the Seychelles, the children exposed to methylmercury postnatally are also exposed prenatally, and the study has been unable to demonstrate any mercury-related deficits in the neurological development of children. However higher postnatal methylmercury exposure had a positive association with test scores. It was suggested that this may be because a higher mercury level indicates a high fish intake and therefore a diet rich in n-3-polyunsaturated fatty acids and vitamin E, which have beneficial effects and may mask any subtle neurological deficits due to chronic low level exposure to methylmercury.

46. The risk is greater for women who are pregnant or likely to become pregnant within the following year because of the effects of methylmercury on the developing central nervous system of the fetus. There is uncertainty with respect to whether infants and young children are at greater risk of methylmercury toxicity whilst the central nervous system is still developing. The limited data available indicate that this is not the case for children but the possibility of increased sensitivity of infants cannot be discounted. Correlation of intakes by the breast-fed infant and the mother (paragraph 35) indicates that the methylmercury intake of the breast-fed infant is within the 2003 PTWI of 1.6 g/kg bw/week if the mother's intake is within the 2000 PTWI of 3.3 μg/kg bw/week.

Assessment of dietary exposure estimates

47. Dietary exposure to mercury was estimated for those fish species for which reliable consumption data were available[35-38] (salmon, prawns and canned tuna) together with exposure from the rest of the diet. Dietary exposures to these fish were also calculated for adult women as this population group contains the most susceptible populations (Table 1). This table is a revised version of that which appears in the FSIS[1] as it incorporates the most up-to-date consumption and occurrence data available for the rest of the diet from the TDS. Of these fish, canned tuna provided the largest contribution to dietary mercury exposure for high level consumers. Total fish consumption by the high level consumer was equivalent to approximately five portions per week (688g).

48. The estimates of average and high level total dietary exposure for almost all age groups, from fish for which consumption data are available, are within the 2003 JECFA PTWI for methylmercury of 1.6 μg/kg bw/week, and not expected to be harmful. The mercury exposure from the whole diet in toddlers and young people aged 4-6 years who are high level consumers exceeds the 2003 PTWI of 1.6 μg/kg bw/week by between 13 and 26% but are well within the 2000 PTWI. The estimated intakes of toddlers who are high level consumers of canned tuna exceeds the 2003 PTWI by 50%, but again are within the 2000 PTWI. Children of this age (1.5-4.5 years) are likely to be less susceptible to neurodevelopmental effects. Therefore this exceedance of the 2003 PTWI is not likely to result in harmful effects.

49. Estimates were also made of the methylmercury intake resulting from consumption of one portion of shark, marlin, swordfish or fresh tuna, for which consumption data are not available (Table 2), using portion sizes as recorded in the NDNS for fish consumption[36-38]. For comparative purposes similar estimates were made for canned tuna.

50. For adults, consumption of one weekly portion of shark, swordfish or marlin could result in a mercury intake in the range of 2.2 to 3.0 μg/kg bw/week, before considering intake from the rest of the diet (upper bound mean 0.28 μg mercury/kg bw/week, not all as methylmercury). Regular intake at this level during pregnancy, or in the year leading up to pregnancy could be associated with a risk of neurodevelopmental effects in the fetus. The methylmercury intake resulting from consumption of either two 140g portions of fresh tuna or four 140g portions of canned tuna would not be expected to result in neurodevelopmental effects.

51. Regular consumption of more than one portion of shark, swordfish or marlin per week could be associated with a risk of neurotoxicity in adults.

52. Dietary exposure of children is higher because their food intake is greater on a body weight basis. Regular consumption of one weekly portion of shark, swordfish or marlin per week by children under the age of 14 could result in a methylmercury intake in the range of 3.0 to 5.2 μg/kg bw/week, before considering intake from the rest of the diet. Consumption of two portions per week of fresh tuna, or 6 portions of canned tuna would not be expected to result in adverse effects in any of the age groups.

Conclusions

53. We *note* that there has been no new information published to indicate that the 2000 PTWI of 3.3 g/kg bw/week is not sufficiently protective of the general population. We therefore *consider* that a methylmercury intake of 3.3 μg/kg bw/week may be used as a guideline to protect against non-developmental adverse effects.

54. We *conclude* that the 2003 JECFA PTWI of 1.6 μg/kg bw/week is sufficient to protect against neurodevelopmental effects in the fetus. This PTWI should be used in assessing the dietary exposure to methylmercury of women who are pregnant, and who may become pregnant within the following year.

55. We *consider* that a guideline of 3.3 μg/kg bw/week is appropriate in considering intakes by breastfeeding mothers as the intake of the breast-fed infant would be within the new PTWI of 1.6 μg/kg bw/week.

56. We *consider* the NDNS blood level data are reassuring with respect to average and high level consumption of fish. The adults surveyed had blood mercury levels indicating that 97.5% of the population had dietary intakes below 1.6 g/kg bw/week.

57. We *conclude* that average and high-level dietary exposure to methylmercury, resulting from the wide range of fish for which consumption data are available, is not likely to be associated with adverse effects in the developing fetus or at other life stages.

58. We *note* that consuming one weekly 140 g portion of either shark, swordfish or marlin would result in a dietary methylmercury exposure close to or above 3.3 μg/kg bw/week in all age groups. We *consider* that this consumption could be harmful to the fetus of women who are pregnant or become pregnant within a year, but would not be expected to result in adverse effects in other adults.

59. We *note* that the mercury content of tuna is lower than that of shark, swordfish or marlin, but higher than that of other commonly consumed fish. We *consider* that consumption of two 140g portions of fresh tuna, or four 140g portions of canned tuna, per week, before or during pregnancy would not be expected to result in adverse effects on the developing fetus.

60. We *recommend* that further research should include development of analytical methodology to allow direct measurement of methylmercury, mechanistic studies to help elucidate population groups more at risk and research integrating the risks with nutritional benefits of fish consumption.

COT Statement 2003/06

December 2003

Table 1: Estimated mean and high level dietary intakes of mercury from salmon, prawns, canned tuna and the whole diet.

Consumer group	Mercury Intake – µg/kg bw/week[a]							
	Salmon[b]		Prawns[b]		Canned Tuna[b]		Whole Diet[c,d]	
	Mean	97.5%	Mean	97.5%	Mean	97.5%	Mean	97.5%
Infants	0.01	0.01	–[e]	–[e]	0.04	0.13	0.04	0.13
Toddlers	0.18	0.53[f]	0.13	0.45[f]	0.84	2.45	0.56	2.17
Young People aged 4 – 6	0.18	0.39[g]	0.09	0.34[f]	0.53	1.61	0.55	1.82
Young People aged 7 – 10	0.11	0.36[f]	0.06	0.15[f]	0.39	1.26	0.41	1.40
Young People aged 11 – 14	0.09	0.23[g]	0.04	0.13[f]	0.32	0.98	0.29	1.05
Young People aged 15 – 18	0.08	0.15[g]	0.04	0.11	0.27	0.68	0.25	0.84
Adults	0.10	0.32	0.04	0.14	0.30	1.05	0.31	1.19
Adults – Women only	0.11	0.32	0.05	0.16	0.34	1.19	0.34	1.19

a. Consumption data for salmon, prawns and tuna are taken from the following sources:
   - 2002 National Diet and Nutritional Survey: adults aged 19 to 64 years[38].
   - Food and Nutrient Intakes of British Infants Aged 6-12 Months[35].
   - National Diet and Nutrition Surveys Children Aged 1.5 – 4.5 years[37].
   - National Diet and Nutrition Survey: young people aged 4-18 years. Volume 1 report of the diet and nutrition survey[36].

b. Mercury intake from eating the named fish only, for the mean and 97.5th percentile consumers.

c. Mercury exposure from the whole diet for individuals of the whole study population, including those that eat the named fish (taken from the 2000 Total Diet Study[39]). The whole diet mercury exposure does not equal the sum of the mercury exposures from the named fish and other foods in the typical UK diet.

d. The measurement of mercury does not distinguish between inorganic and organic mercury. Therefore although methylmercury is the major contributor to mercury intake from fish, the estimate of intake from the whole diet also includes inorganic mercury.

e. No infant consumption data were recorded for prawns in the Infant Survey.

f. Based on consumption data for fewer than 60 recorded consumers, therefore exposures to be regarded with caution.

g. Based on consumption data for fewer than 20 recorded consumers, therefore exposures to be regarded with extreme caution.

These estimates have been revised to incorporate most up-to-date consumption and occurrence data for the rest of the diet from the TDS.

Table 2: Mercury intake from one weekly portion of shark, swordfish, marlin, fresh tuna or canned tuna.

Age group (years)	Body Weight (kg)	Av. Portion Size[a] (g)	Weekly mercury intake assuming one portion of fish per week[b] (μg/kg bw/week)				
			Shark	Swordfish	Marlin	Fresh Tuna	Canned Tuna
1.5 – 4.5	14.5	50	5.24	4.62	3.79	1.38	0.66
4 – 6	20.5	60	4.44	3.90	3.22	1.17	0.56
7 –10	30.9	85	4.17	3.69	3.04	1.10	0.52
11 – 14	48.0	140	4.44	3.92	3.21	1.17	0.55
15 – 18	63.8	105	2.51	2.21	1.82	0.66	0.31
Adults	70.1	140	3.04	2.68	2.20	0.80	0.38

a. The average portion size that each age group of the population would consume at a single meal event for fish consumption, as recorded in the following National Diet and Nutrition Surveys (NDNS):
   - 1995 National Diet and Nutrition Survey: Children aged 1½ to 4½ years[37].
   - 2000 National Diet and Nutrition Survey: young people aged 4 to 18 years[36].
   - 1990 The Dietary and Nutritional Survey of British Adults[38].

b. This intake estimate does not include the intake from the rest of the diet, which is estimated to be 0.04 mg/kg bw/day (0.28 μg/kg bw/week)[39].

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Statement on a survey of metals in infant food

Introduction

1. The Food Standards Agency (FSA) has recently completed a survey of metals in infant food. This survey was carried out to establish the concentrations of 12 metals in a representative range of commercial infant foods and formulae. The Committee was asked to comment on the survey and assess if the levels of each element in the diet posed a risk to human health.

2. This survey follows on from a previous survey of metals in infant foods, which the Committee considered in 1999[1], concluding:

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

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

Current Survey of Metals in Infant Foods

3. This survey was carried out between March 2001 and July 2002 to establish the concentrations of 12 metals in a representative range of commercial infant foods and formulae. It was designed to provide a picture of the elemental concentrations of the main types and brands of infant foods on sale in the UK and to allow an assessment of infants' exposures from these elements in these foods.

4. To assess the levels of each element in infant foods, 189 samples of commercial baby foods (infant formulae, manufactured baby foods, desserts, rusks and infant drinks) were analysed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), which does not determine the individual species of each metal.

5. In the absence of a more recent NDNS survey for 6-12 month olds three different approaches were used to provide consumption data for estimation of the dietary exposure of infants to each metal. Details of these methods, and descriptions of their limitations are available in COT paper TOX 2003-05 at: http://www.food.gov.uk/science/ouradvisors/toxicity/cotmeets/cot_2003/115049/.

6. The first approach used the same source of consumption data as the previous survey of infant foods[1], i.e. the 1986 survey of British Infants[2] for age 6-12 months, thereby allowing direct comparison of the data. Dietary exposures were calculated using the mean concentration of metal in each food category allowing a dilution factor for dried/concentrated foods. This method provides mean and high level dietary exposure of infants consuming a combination of one or more of any of the foods studied (formulae, manufactured baby foods, drinks and rusks), which is not possible from feeding instructions alone.

7. The second approach used a food consumption figure of 48 g/kg body weight/day for a high level infant consumer which was identified by the Scientific Committee on Food (SCF) when deriving maximum residue levels for pesticides in infant foods[3]. The basis for this consumption value is not clear. Estimates for formulae consumption were based on a volume of 500-600 ml which is recommended by the Committee on the Medical Aspects of food and nutrition policy (COMA) for infants up to 12 months old[4]. The average weight of powder in 600 ml of made-up formula was calculated for use in exposure estimates. This approach did not allow for a contribution to dietary exposure from juices or other drinks.

8. The third approach used manufacturers' feeding guidelines, as detailed on each product label, as the source of consumption data for formula. An average consumption level of food and drinks for each age range from weaning at 4 months of age was calculated from three different manufacturers' feeding guidelines[5,6,7]. The mean concentration of each element was calculated from the concentration of that element in every eligible food for a particular age group (using a dilution factor for samples of dried food). Average weights of 5.9, 7.7, 8.9 and 9.9 kg were assumed for infants of 0-3, 4-6, 7-9 and 10-12 months respectively[4]. Because the selection of infant foods surveyed was based on market share, the resulting mean concentration is assumed to reflect greater weight to more frequently consumed foods (such as ready-to-feed jar meals). Drinks, including juices, were taken into account in this approach. Due to the higher levels of some elements in soya based formula, separate exposure estimates were calculated, one based on soya formulae and infant foods (excluding dairy) and the other based on cows' milk-based formulae (from birth and follow on formulae) and foods. However only three samples of soya based formula were taken, so these data may not be representative.

9. The exposure estimates do not include the metal content of water used to reconstitute formula or dried food, or offered as a drink. They also do not include any contribution from foods not manufactured specifically for infants (e.g. normal 'adult' foods or home-prepared baby meals) or from breast milk. Nor do they consider wastage of food.

10. The Committee considered that the first approach using the 1986 NDNS consumption data was probably an under-estimation, but was useful in providing a comparison with the results of the previous survey. The third approach based on manufacturers' feeding guidelines generated the highest intakes, and could be considered a worst case scenario. Using the data derived from these two approaches provided a range in which the actual exposures are likely to be found. The Committee considered that there were a number of uncertainties and assumptions made in approach 2 and noted that the intakes calculated using this approach always fell within the range created by approaches 1 and 3. Therefore approach 2 was considered superfluous.

11. The survey results are reported in a food surveillance information sheet [8] and are summarised below.

Concentrations of elements in the products surveyed.

12. The levels of arsenic, cadmium, lead, mercury and tin were below the relevant regulatory levels for all foods surveyed[9, 10, 11]. Regulatory levels have not been set for the other metals surveyed. Copper and zinc are added to infant formula to ensure that infants receive adequate intakes of these essential elements. The levels of copper and zinc in all formulae surveyed fell within the acceptable range of fortification set by The Infant Formulae and Follow-on Formulae Regulations 1995[12].

13. With the exception of mercury, the mean concentrations of all elements in the products surveyed were in the region of, or lower than in the previous survey. Antimony, arsenic, cadmium and lead concentrations were above the limit of detection (LOD) in most samples. Zinc was detected in all samples and copper in all but one.

14. Mercury was detected at concentrations at or above the LOD in approximately one quarter of the samples, but the majority of those samples exceeding the LOD were very close to it. The mean concentration and the upper end of the range of mercury in infant foods appeared to be twice those seen in the previous survey (3 μg/kg, range <0.5 – 20, compared to 1.4 μg/kg, range <0.3 – 10). About 50% of this increase is likely to be due to the higher LOD for mercury in this survey (due to a decrease in the sensitivity of the equipment used in the analysis). In the current survey there were more foods containing fish than in the previous survey (7 out of 189 compared to 2 out of 97), however the fish containing meals only provided a minor contribution to the overall mean mercury concentration. Overall, it is apparent that the average mercury concentrations in infant foods have increased since the last survey.

15. With the exception of mercury, the average metal concentrations were higher in soya formula than in cows' milk formula, the most notable differences being seen with nickel and aluminium where concentrations were 2 to 3 times higher in soya formula. The concentrations in soya formula were similar to those reported in the previous survey.

Dietary exposure

16. The estimated dietary exposures for each metal are shown in Table 1, together with the comparable results from the previous survey. These were compared with available tolerable intakes, such as Provisional Tolerable Weekly intakes (PTWIs) set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), taking into account previous COT evaluations. The COT evaluation was also informed by a summary of the toxicological data on these metals, which is available at: http://www.food.gov.uk/multimedia/pdfs/TOX-2003-05.PDF.

17. The term Provisional Tolerable Weekly Intake (PTWI) is used by JECFA in identifying tolerable intakes of food contaminants with cumulative properties. Within this statement, the PTWI has been by divided by 7 to provide a tolerable daily intake for comparison with the estimated daily dietary exposures. Like the Acceptable Daily Intake (ADI) set for food additives, the PTWI is considered to be applicable to all age groups above 12 weeks of age. Evaluation of dietary exposure of younger infants requires case-by-case consideration of the toxicological database.

COT evaluation

18. Water used to reconstitute infant formula and dried foods could make an important contribution to the metal concentration in the food as consumed. This is particularly important in the case of arsenic, where water is a significant source of dietary exposure to inorganic arsenic. The Committee recommended that future surveys of this type should allow for water used in reconstituting foods and formula.

19. The methods used to analyse the concentration of each metal have not determined which metal species are present, only the total concentration. Therefore risk assessment must allow for the possibility that where one form of a metal is more toxic (for example organic or inorganic) and where there is no information on the speciation of that metal in food, it is the more toxic form that is present predominantly in the food. However, this is a worst case scenario. Information on the speciation of each metal would allow for a more robust risk assessment.

Aluminium

20. The maximum estimated intake was lower than for the previous survey and approximately 18% of the JECFA PTWI[13] which is equivalent to 1000 μg/kg bw/day. Aluminium intakes resulting from a soya based diet were higher than those from a normal diet, probably due to the higher levels of aluminium in soya formulae with a maximum intake of 24% of the PTWI. There is no information available on whether infants are more susceptible to the effects of aluminium. However taking into account the additional margin of safety compared with the PTWI and that this is likely to be an over estimate of exposure due to the use of upper bound concentrations and worst case scenario consumption data, the intake of aluminium from infant foods and formulae is unlikely to be of concern.

Antimony

21. The maximum estimated intake was lower than for the previous survey and approximately 29% of the WHO TDI[14, 15] of 0.86 μg/kg bw/day. There is no information available on whether infants are more susceptible to the effects of antimony. However taking into account the additional margin of safety compared with the PTWI and that this is likely to be an over estimate of exposure due to the use of upper bound concentrations and worst case scenario consumption data, the intake of antimony from infant foods and formulae is unlikely to be of concern.

Arsenic

22. All dietary exposures were within the JECFA PTWI for inorganic arsenic[16]. However, in its latest consideration of arsenic in the diet, the COT concluded that there are no appropriate safety guidelines for inorganic or organic arsenic and that exposure to inorganic arsenic should be As Low As Reasonably Practicable (ALARP)[17]. Where comparable data are available, the estimated mean dietary exposure resulting from this survey is similar to that derived from the 1999 survey. The highest arsenic levels were found in fish-containing dishes, which are likely to contain predominantly organic arsenic[18]. Overall, these data suggest that dietary exposure of infants to inorganic arsenic have not increased.

23. Based on the current permitted level of inorganic arsenic in drinking water (50 μg/L), the contribution to the daily inorganic arsenic intake from water used to reconstitute formula could potentially be 3 to 5 μg/kg bw/day§. This is approximately twice the contribution from infant foods. The maximum permitted level of inorganic arsenic in water is due to be reduced from 50 to 10 μg/L in December 2003. However the vast majority of water companies are already complying with the lower level at which the potential inorganic arsenic intake from water used to reconstitute infant formulae could be 0.6 to 1 μg /kg bw/day.

Cadmium

24. The maximum estimated intake was lower than for the previous survey and approximately 72% of the JECFA PTWI for cadmium[19] which is equivalent to 1 μg/kg bw/day. There is no information available on whether infants are more susceptible to the effects of cadmium. However taking into account that this is likely to be an over estimate of exposure due to the use of upper bound concentrations and worst case scenario consumption data, the intake of cadmium from infant foods and formulae is unlikely to be of concern.

Chromium*

25. The maximum estimated intake was lower than for the previous survey and approximately 3% of the guidance level for trivalent chromium of 150 μg/kg bw/day recommended by the Expert Group on Vitamins and Minerals (EVM)[20]. Trivalent chromium is considered to be an essential trace element, whereas hexavalent chromium has been classified as carcinogenic[21]. The vast majority of chromium found in food is in the trivalent form[22] and so comparison of the total chromium levels in food with guidance levels for trivalent chromium is appropriate. There is no information available on whether infants are more susceptible to the effects of chromium. However taking into account the additional margin of safety compared with the PTWI and that this is likely to be an over estimate of exposure due to the use of upper bound concentrations and worst case scenario consumption data, the intake of chromium from infant foods and formulae is unlikely to be of concern.

§ Based on a water consumption of 600ml used to reconstitute formulae and a body weight of between 5.9 to 9.8 kg.

* Essential element.

Copper*

26. The maximum estimated intake was lower than for the previous survey and approximately 20% of the JECFA Provisional Maximal Tolerable Daily Intake (PMTDI)[23] of 500 μg/kg bw/day and 61% of the Safe Upper Level (SUL) of 160 μg/kg bw/day recommended by the EVM[20]. Infants may be less able to absorb copper, but may also be less efficient at excreting copper than adults and so it is uncertain if infants would be more susceptible to copper toxicity than adults. However taking into account the additional margin of safety compared with the PMTDI/SUL and the fact that this is likely to be an over estimate of exposure, the intake of copper from infant foods and formulae is unlikely to be of concern.

Lead

27. The maximum estimated intake was lower than for the previous survey and approximately 17% of the PTWI for lead[24] which is equivalent to 3.6 μg/kg bw/day. The COT has previously concluded that it is not possible to establish a threshold for lead[25]. Infants absorb a higher percentage of lead than adults following oral ingestion and are more susceptible to the neurotoxic effects of lead, particularly those leading to deficits in Intelligence Quotient (IQ). However the JECFA PTWI is a level of exposure that is not expected to increase the blood lead concentration of children. The decrease in exposure compared with the previous survey is consistent with the COT view that efforts should continue to reduce lead exposure from all sources.

Mercury

28. In 2002 when the COT considered methylmercury in fish[26] it concluded that the then current JECFA PTWI[27] may not be sufficiently protective for breast-feeding women because of the potential risk to the neonate. The EPA reference dose[28], which was derived from subtle neurobehavioural effects seen in children exposed prenatally, was used in assessing fish consumption by breastfeeding women in order to protect the young infant. However the COT noted inconsistencies in the evidence and agreed to review this conclusion following the JECFA evaluation of methylmercury in June 2003. JECFA has now revised its PTWI to 1.6 μg/kg bw/week[29]. The new lower JECFA PTWI is intended to be protective of both the general population and the high-risk groups, and therefore it can be used in assessing the dietary exposure of infants to mercury.

* Essential element.

29. The estimated intakes of mercury were higher than those from the previous survey. The maximum estimated intake (0.2 μg/kg bw/day for infants of 9-12 months) was approximately 87% of the JECFA PTWI for methylmercury which is equivalent to 0.23 μg/kg bw/day. Estimated intakes for infants aged 0-3 months, who are at greatest risk from methylmercury, was 30% of the PTWI. It is probable that mercury absorption would be lower in older infants due to concomitant intake of food and formula. In addition, these are likely to be overestimates of exposure estimates due to the use of upper bound concentrations and worst case scenario consumption data, and it is likely that not all of the mercury in infant foods is in the organic form. Overall, the Committee concluded that the estimated mercury intakes did not give cause for concern, but concentrations of mercury in infant foods should continue to be monitored.

Nickel

30. The estimated intakes were lower than for the previous survey. The worst case intakes (based on manufacturers' feeding guidelines) for 7-12 month old infants (normal diet) and for 4-12 month old infants (soya diet) exceeded the WHO TDI[14] of 5 μg/kg bw/day by up to 68%. Taking into account that this is likely to be an over estimate of exposure due to the use of upper bound concentrations and worst case scenario consumption, this exceedance of the TDI was considered unlikely to be of significance. Ingestion of nickel may exacerbate contact dermatitis/eczema in pre-sensitised individuals. Infants are less likely than adults to be sensitised to nickel and are therefore not to be considered a susceptible sub group. Overall, the dietary exposures were not considered to be of concern.

Selenium*

31. The maximum estimate intake was similar to the previous survey. The estimated intakes for all ages were within the upper limit of the safe range recommended for adults by the WHO[30] (400 μg/day) and the SUL of 450 μg/day recommended for adults by the EVM[20]. This comparison assumes that it is appropriate to use bodyweight in scaling from the adult safe upper levels to levels applicable to infants since it is not clear whether this would produce an apparent safe upper level below an infant's nutritional requirement for selenium.

Tin

32. The maximum estimated intake was lower than for the previous survey and approximately 1% of the JECFA PTWI[31] which is equivalent to 2000 μg/kg bw/day and 9% of the EVM[20] guidance level of 220 μg/kg bw/day. There is no information available on whether infants are more susceptible to the effects of tin. However taking into account the additional margin of safety compared with the PTWI and that this is likely to be an over estimate of exposure due to the use of upper bound concentrations and worst case scenario consumption data, the intake of tin from infant foods and formulae is unlikely to be of concern.[7]

* Essential element.

Zinc*

33. The maximum estimated intake was similar to the previous survey. Most of the estimated intakes of zinc exceeded the SUL recommended by the EVM[20], and some exceed the JECFA PMTDI[32]. However the SUL and PMTDI may not be applicable to infants due to their high nutritional requirements for zinc; 4 mg/day 0-6 months (690 μg/kg bw/day), and 5 mg/day 7-12 months (510 μg/kg bw/day). For this reason infant foods are often fortified with zinc (0.5-1.5mg/100 kcal for formulae and 2 mg/100 kcal for infant foods).

34. The intakes calculated using the 1986 NDNS data suggest that the average infant diet does not provide enough zinc, despite fortification (an infant of 7-12 months, with an average weight of 9.8 kg would require approximately 510 μg/kg bw day to achieve 5 mg/day). However, this approach may not reflect current intakes due to the age of the consumption data. Whilst the estimated intakes of zinc were higher for those infants consuming a soya based diet, soya is known to inhibit absorption of zinc in the gut[20] and so it is possible that the actual amount absorbed could be lower than those infants on a normal diet.

Essential elements

35. For most of the metals, estimated intakes have decreased since the previous survey. Whilst this is desirable for contaminants such as lead, cadmium, antimony, nickel and tin, decreasing intakes of essential elements (chromium, copper, selenium and zinc) may have the potential to lead to nutritional deficiency. However, consideration of nutritional deficiency is not within the remit of the COT.

Conclusions

36. We *note* that, with the exception of mercury, the concentrations of each metal in infant foods do not appear to have increased since the previous survey in 1999. Whilst some of the apparent increase in the concentration of mercury in infant foods may be attributable to methodological differences between the surveys we *consider* that the levels of mercury should continue to be monitored. Information on the forms of mercury in infant foods would help to demonstrate an adequate margin of safety for methylmercury.

37. We *note* that the estimates of intake by infants rely on survey data that may now be outdated or on assumptions about feeding patterns that may represent an overestimate of food consumption. Whilst these approaches permit an assessment of the results of this survey we would *welcome* new studies to determine the patterns of consumption of foodstuffs in infants.

* Essential element.

38. We *consider* that there are no relevant tolerable intakes or reference doses by which to assess dietary exposure to either inorganic or organic arsenic. Inorganic arsenic is genotoxic and a known human carcinogen. We therefore *conclude* that exposure to inorganic arsenic should be as low as reasonably practicable (ALARP). However we are *reassured* that since the previous survey arsenic intakes do not appear to have increased.

39. We *welcome* the apparent decline in lead exposure since the previous survey. However since it is not possible to identify a threshold for the association between lead exposure and decrements in intelligence quotient, efforts should continue to reduce lead exposure from all sources.

40. We *consider* that the consumption of the infant foods sampled in the survey will not result in the intake of such quantities of any of the analysed elements such as would give concern for the health of infants.

41. We *consider* that future assessments of metals in infant foods would be more robust if information was made available on the actual species of metal present in the food and on the contribution of the metal concentrations in water used to reconstitute formula and dried foods.

COT Statement 2003/02

July 2003

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Table 1: Estimated dietary exposure of infants to metals from infant foods (excluding water)

		Mean intakes calculated using manufacturers' consumption guidelines – µg/kg bw/day				Mean (and 97.5 percentile) intakes calculated using 1986 NDNS – µg/kg bw/day		Safety guideline
		2002 survey				2002 survey	1999 survey	
		0-3[a]	4-6[a]	7-9[a]	10-12[a]	7-12[a,b]	7-12[a,b]	
Aluminium[c]	Normal Diet	14	142	175	177	22 (76)	39 (98)	JECFA PTWI equivalent to 1000 µg/kg bw/day[13]
	Soya Diet	82	242	222	218	–	–	
Antimony[c]	Normal Diet	0.02	0.08	0.15	0.15	0.03 (0.10)	0.092 (0.29)	WHO TDI of 0.86 µg/kg bw/day (New TDI of 6 µg/kg bw/day proposed)[14,15]
	Soya Diet	0.18	0.25	0.21	0.20	–	–	
Arsenic[c]	Normal Diet	0.09	1.3	1.8	1.8	0.25 (0.87)	0.24 (0.61)	JECFA PTWI for inorganic arsenic equivalent to 2.14 µg/kg bw/day[16] COT has concluded there are no appropriate safety guidelines[17]
	Soya Diet	0.18	1.6	2.0	1.9	–	–	
Cadmium[c]	Normal Diet	0.04	0.35	0.61	0.64	0.09 (0.31)	0.16 (0.41)	JECFA PTWI equivalent to 1 µg/kg bw/day[19]
	Soya Diet	0.22	0.57	0.68	0.72	–	–	
Chromium[c]	Normal Diet	1.2	2.9	3.6	3.6	0.65 (1.91)	1.8 (4.2)	EVM Guidance Level = 150 µg/kg bw/day for total dietary intake of trivalent chromium[20]
	Soya Diet	1.5	3.3	3.7	3.7	–	–	
Copper[c]	Normal Diet	41	72	78	76	13 (40)	21 (52)	JECFA PMTDI = 500 µg/kg bw/day[23] EVM Safe Upper Level = 160 µg/kg bw/day for total dietary intake[20]
	Soya Diet	62	98	82	81	–	–	
Lead[c]	Normal Diet	0.08	0.37	0.51	0.52	0.08 (0.22)	0.21 (0.52)	JECFA PTWI equivalent to 3.6 µg/kg bw/day.[24] COT considered it is not possible to establish a threshold for lead[25]
	Soya Diet	0.22	0.56	0.59	0.61	–	–	
Mercury[c]	Normal Diet	0.07	0.18	0.18	0.19	0.04 (0.11)	0.023 (0.046)	JECFA PTWI for methylmercury equivalent to 0.23 µg/kg bw/day[29]
	Soya Diet	0.07	0.19	0.19	0.20	–	–	
Nickel[c]	Normal Diet	0.7	4.2	5.8	5.9	0.96 (3.0)	2.0 (5.1)	WHO TDI = 5 µg/kg bw/day[14]
	Soya Diet	4.2	8.4	7.6	7.9	–	–	

Continued

		Mean intakes calculated using manufacturers' consumption guidelines – µg/kg bw/day				Mean (and 97.5 percentile) intakes calculated using 1986 NDNS – µg/kg bw/day		Safety guideline
		2002 survey				2002 survey	1999 survey	
		0-3[a]	4-6[a]	7-9[a]	10-12[a]	7-12[a,b]	7-12[a,b]	
Selenium[c]	Normal Diet	1.3	2.2	2.2	2.1	0.43 (1.42)	0.54 (1.4)	The upper limit of the safe range proposed by the WHO was 400 mg/day determined for adults only based on epidemiological data[30]
	Soya Diet	2.5	3.5	2.6	2.6	-	-	EVM Safe Upper Level = 450 µg/day for total dietary intake, equivalent to 7.5 mg/kg bw/day for a 60 kg adult[20]
Tin[c]	Normal Diet	0.57	4.6	18.6	18.5	2.6 (8.9)	8.1 (32)	JECFA PTWI is equivalent to 2000 µg/kg bw/day[31]
	Soya Diet	0.62	4.7	19.7	20.1	-	-	EVM Guidance Level = 220 µg/kg bw/day for total dietary intake[20]
Zinc	Normal Diet	756	1262	1089	1062	198 (687)	220 (690)	JECFA PMTDI = 1000 µg/kg bw/day[32]
	Soya Diet	946	1503	1148	1128	-	-	EVM Safe Upper Level = 42 mg/day (equivalent to 700 mg/kg bw/day in a 60 kg adult) for total dietary intake[20]

Notes:

[a] Age range in months

[b] Dietary exposure in brackets are for the 97.5th percentile consumers

[c] For all metals except zinc, data are upper bound means calculated using the limit of detection (LOD) when an element is not detected in a sample. The LOD was defined as 3 times the standard deviation of measured values for reagent blanks after correction for typical sample weight and dilution.

Statement on twelve metals and other elements in the 2000 Total Diet Study

Introduction

1. The Food Standards Agency has completed a survey of aluminium, arsenic, cadmium, chromium, copper, lead, manganese, mercury, nickel, selenium, tin and zinc in the 2000 UK Total Diet Study (TDS). The results provide up to date information on the concentrations of these elements in foods and were used to estimate dietary exposures for UK consumers. The Committee was asked to comment on the survey results and assess if the levels of each element in the diet posed a risk to human health. The COT last evaluated population and consumer exposures to the twelve elements in the TDS in 1995.

The Survey

2. The TDS is an important part of the UK Government's surveillance programme for chemicals in food and has been carried out on a continuous annual basis since 1966. Results from the TDS are used to estimate dietary exposures of the general UK population to chemicals in food, such as nutrients and contaminants, to identify trends in exposure and make assessments on the safety and quality of the food supply. Analysis for metals and other elements in the TDS is carried out every 3 years.

3. The design of the UK TDS has been described in detail elsewhere[1] and involves 119 categories of foods combined into 20 groups of similar foods for analysis. The relative proportion of each food category within a group reflects its importance in the average UK household diet and is largely based on an average of three previous years of consumption data from the National Food Survey. Foods are grouped so that commodities known to be susceptible to contamination (e.g. offal, fish) are kept separate, as are foods which are consumed in large quantities (e.g. bread, potatoes, milk)[1,2].

4. The foods making up the 20 groups of the TDS were obtained from retail outlets in 24 towns throughout the UK. Each food group obtained from each town was analysed for the twelve elements of interest. The mean element concentrations for each food group were used together with data on the consumption of these food groups[3-10] to estimate dietary exposure for the average UK population and mean and high level (97.5th percentile) consumers.

Concentrations of the elements in the foods surveyed

5. The full results of this TDS will be published in a Food Surveillance Information Sheet[11]. The concentrations of each of the elements in the food groups were lower than or similar to those reported in the previous TDS, conducted in 1997[12], with the exception of aluminium and mercury.

6. The aluminium concentrations in the miscellaneous cereals, sugars and preserves and nuts groups were higher than those reported for the 1997 TDS. The largest increase (approximately 3 fold) seen in the miscellaneous cereals group may be due to increases in the use of aluminium containing preservatives in these foods, or the different proportions of products sampled in this group compared to previous total diet studies.

7. Mercury concentrations were similar to or lower than those reported in the 1997 TDS except for the fish group, in which the mean concentration was 0.071 mg/kg compared to 0.043 mg/kg in 1997.

Dietary exposures

8. Estimates of dietary exposure were compared with available tolerable intakes, such as Provisional Tolerable Weekly intakes (PTWIs) set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), taking into account previous COT evaluations. The COT evaluation was also informed by a summary of the toxicological data on these metals[13]. The PTWI is used by JECFA in identifying tolerable intakes of food contaminants with cumulative properties. Within this statement, the PTWI has been divided by 7 to provide a tolerable daily intake for comparison with the estimated daily dietary exposures (Table 1).

9. Population dietary exposures have also been estimated, using the amounts of food consumed (based on consumption data from the National Food Survey from 1996 to 1998)[8-10]. These are shown in Table 2 with the population dietary exposures for each element from the UK TDS from 1976 to 2000.

COT evaluation

10. The estimated mean and high-level dietary exposures to aluminium, cadmium, chromium, copper and selenium for each consumer group were within the relevant safety guidelines and therefore are unlikely to be of any toxicological concern. Population exposures to these elements have generally declined over the course of the TDS programme, with exposures to most of these elements now at the lowest level.

Arsenic

11. The Committee has concluded previously, when considering 1999 TDS of Total and Inorganic Arsenic, that there are no relevant tolerable intakes or reference doses by which to assess safety of either inorganic or organic arsenic in the diet. Inorganic arsenic is genotoxic and a known human carcinogen and therefore exposure should be as low as reasonably practicable (ALARP)[14].

12. The estimates of consumer dietary exposures to total arsenic in the 2000 TDS were similar to those reported in the 1999 TDS of Total and Inorganic Arsenic[14]. The current population exposure to total arsenic was also similar to that reported in the 1999 TDS (0.055 mg/day and 0.05 mg/day, respectively) and lower than previous estimates (0.065 mg/day in 1997). In discussing the 1999 TDS, the Committee noted that fish was the major contributor to dietary exposure to arsenic and the predominant form of arsenic in fish is organic. Inorganic arsenic contributed less than 10% of the total dietary exposure to arsenic. The Committee noted that the data on inorganic arsenic appeared to be consistent with dietary exposure being ALARP, that the dietary exposure to organic arsenic identified in the survey was unlikely to constitute a hazard to health, and that the downward trend for total arsenic was reassuring. Although different forms of arsenic were not measured in the 2000 TDS, it is likely that there was a similar distribution of inorganic to organic arsenic to that reported for the 1999 TDS, and that the previous COT conclusions are still valid.

13. The Committee recommended that future surveys should measure both total and inorganic arsenic and include consideration of other sources of exposure such as water.

Lead

14. The highest estimate of dietary exposure to lead was 0.47 μg/kg bw/day (for toddlers at the 97.5 percentile of consumption). This is approximately 13% of the JECFA PTWI for lead[15] (equivalent to 3.6 μg/kg bw/day) which is a level of exposure from all sources that is not expected to cause an increase in blood lead concentration in young children. Young children are vulnerable to the effects of lead, because they absorb a higher percentage of ingested lead and are more susceptible to the neurotoxicity, which may result in deficits in Intelligence Quotient. A UK study of lead intake in children of 2 years of age showed that dietary exposure to lead contributed approximately 30% of total lead exposure with the remainder coming mainly from sources such as house dust, water and the air[16]. Thus dietary exposure to toddlers that is within 30% of the JECFA PTWI (i.e. less than 1.2 μg/kg bw/day) is not expected to result in an increase in the blood lead concentration above background levels. Therefore the dietary exposures to lead identified from the 2000 TDS are unlikely to represent a toxicological concern. However, the COT confirmed its previous opinion, from when they considered a survey of metals in infant foods, that because it has not been possible to identify a threshold for the effects of lead, efforts should continue to reduce exposure from all sources[17].

15. Table 2 illustrates that population dietary exposures have declined considerably since 1976, with the current population exposure at its lowest level (7.4 μg/day compared to 26 μg/day in 1997), which is in accordance with the COT opinion on reducing lead exposure.

Manganese

16. Manganese is an essential trace element but is neurotoxic at high occupational levels of inhalation exposure and there is limited evidence of neurological effects at lower doses. The dose response relationship in experimental animals has not been adequately clarified and the effects observed in animals may not reflect the subtle neurological effects reported in humans[18]. There is insufficient information to determine whether there are toxicological risks associated with dietary exposure to manganese and no available safety guideline. The population exposures to manganese have remained fairly constant since manganese was first included in a TDS in 1983 (4.6 mg/day) and there is no basis for assuming that the current dietary exposure to manganese (4.9 mg/day) is a concern for health to consumers.

Mercury

17. With the exception of high-level consumption by children aged 1.5-4.5 years, the estimates of dietary exposure to mercury (mean and high-level) for all consumer groups were within the PTWI for methylmercury set by JECFA in 2003 to protect against neurodevelopmental effects[19] (equivalent to 0.23 μg/kg bw/day). The estimate for high-level consumption by children aged 1.5-4.5 years exceeded the JECFA PTWI for methylmercury by 17%. It is unlikely that all the mercury in the diet is in the form of methylmercury. Inorganic mercury is less well-absorbed than methylmercury by the oral route, and therefore comparing dietary exposure to total mercury to the PTWI for methylmercury is a worst case scenario. Furthermore, the COT has previously noted that toddlers are likely to be less sensitive to the neurodevelopmental effects of methylmercury than the fetus or infant[20]. Therefore the dietary exposures to mercury do not give rise to toxicological concerns for consumers. The Committee also noted that the population exposures to mercury have decreased since 1976 (0.005 mg/day), with the current dietary exposure at its lowest level (0.0015 mg/day).

Nickel

18. The estimates of dietary exposures to nickel for high-level consumers aged 1.5-4.5 years and 4-18 years exceeded the WHO TDI (5 μg/kg bw/day)[21] for nickel by 44% and 6%, respectively. The TDI was set as a basis for establishing a WHO guideline for drinking water quality. It was derived from an animal study showing general toxicity in a 2 year dietary study and incorporated an uncertainty factor of 1000 to allow for inadequacies in the data and a higher absorption of nickel from drinking water than from food. The EVM noted that ingested nickel may exacerbate contact dermatitis/eczema in pre-sensitised individuals[18], however toddlers are less likely than adults to be sensitised and would not therefore be considered to be a sensitive group. Population exposures to nickel have decreased since 1976 (0.33 mg/day), with the current dietary exposure at its lowest level (0.13 mg/day). Overall the Committee concluded that the estimated nickel intakes were unlikely to result in any adverse health effects.

Tin

19. The estimates of dietary exposures to tin for high-level consumers aged 1.5 – 4.5 years were lower than the PTWI of 2000 μg/kg bw/day[22], but exceeded the EVM guidance level of 220 μg/kg bw/day by approximately 29%. The PTWI is not directly applicable to long term dietary exposures since it is based on intakes associated with acute toxicity (the threshold concentration for manifestation of gastric irritation). The EVM guidance level was based on a no observed adverse effect level (NOAEL) of 22-33 mg tin/kg bw/day from a sub-chronic study in rats, in which anaemia and changes to liver cells were observed at higher doses[23]. The EVM used the lower NOAEL (22 mg/kg bw) and an uncertainty factor of 100 to derived the guidance level of 0.22 mg/kg bw/day. The small exceedance of this guidance level is therefore within an area of uncertainty, but is not expected to result in adverse effects.

Zinc

20. The estimated dietary exposure for the high level consumers aged 1.5-4.5 years exceeded the EVM safe upper level (700 μg/kg bw/day)[18] by approximately 8%, but did not exceed the JECFA Provisional Maximum Tolerable Daily Intake (PMTDI) of 1000 μg/kg bw/day[24]. Estimated intakes for other consumer groups were within the EVM safe upper level. The EVM safe upper level was derived from studies of zinc supplementation in adults, taking into account adult dietary intake of zinc, and cannot be directly extrapolated for assessing safety of dietary intake by children. Overall, the Committee concluded that the estimated zinc intakes were unlikely to result in any adverse health effects.

Conclusions

21. We *conclude* that current dietary exposures to aluminium, cadmium, chromium, copper, mercury, nickel, selenium, tin and zinc are unlikely to be of any toxicological concern for consumers.

22. We *note* that the current survey measured total arsenic only, but that the data appear consistent with a survey of total and inorganic arsenic in food, which we reviewed recently. We *reaffirm* our previous conclusions that current dietary exposure to organic arsenic is unlikely to constitute a hazard to health, and exposure to inorganic arsenic should be as low as reasonably practicable (ALARP).

23. We *note* that estimates of total exposure to lead, including that from the diet, do not exceed the PTWI. We *conclude* that current dietary intakes are unlikely to result in adverse effects, but that efforts should continue to reduce exposure to lead from all sources.

24. We *note* there is insufficient information to determine whether there are risks associated with dietary exposure to manganese. However dietary exposures to manganese have remained fairly constant since monitoring began in 1983, and there is no basis for assuming any concern for health.

25. We *recommend* that in future surveys of elements in food, priority should be given to those of greatest toxicological concern, such as arsenic, mercury and lead. Speciation of metals such as mercury, arsenic and chromium would be helpful for the risk assessment.

COT statement 2003/07

December 2003

Table 1: Comparison of the estimated dietary intakes of each element for each population group with the relevant safety guidelines

Elements	Estimated Dietary exposure (µgkg bw/day) a, b, c												Safety Guidelines
	Adults		Toddlers (1.5-4.5 years)		Young People (4-18 years)		Elderly (free living)		Elderly (institutional)		"Vegetarians"[d]		
	Mean	High level	Mean	High level	Mean	High level	Mean	High level	Mean	High level	Mean	High level	
Aluminium	67-68	134-135	165	327	120-121	244-245	59	126-127	81-82	162-163	71-72	133-134	JECFA PTWI equivalent to 1000 µg/kg bw/day[25]
Arsenic	1.5-1.6	5.8	2.7	12	1.7	7.0	1.7	5.6	1.6	4.9	1.4	7.4	JECFA PTWI for inorganic arsenic equivalent to 2.14 mg/kg bw/day[26] COT has concluded there are no appropriate safety guidelines[14]
Cadmium	0.12	0.21	0.31-0.32	0.56	0.22	0.42	0.12	0.21	0.14	0.24	0.13	0.23	JECFA PTWI equivalent to 1 µg/kg bw/day[27]
Chromium	0.66-0.67	1.0-1.11	1.7	2.7-2.8	1.14-1.15	2.1	0.60-	0.98-0.99	0.72	1.1	0.55	0.92-0.93	EVM Guidance Level = 150 µg/kg bw/day for total dietary intake of trivalent chromium[18]
Copper	18	33	46	81	30	56	18	40	20	41	16	29	JECFA PMTDI = 500 mg/kg bw/day[28] EVM Safe Upper Level = 160 µg/kg bw/day for total dietary intake[18]
Lead	0.1	0.18	0.25	0.47	0.17	0.32	0.094-0.095	0.17	0.12	0.19	0.1	0.18-0.19	JECFA PTWI equivalent to 3.6 µg/kg bw/day[15] COT considered it is not possible to establish a threshold for lead[17]
Manganese	67	118	132	235	101	195	57	100	67	113	65	123	None available
Mercury	0.03-0.04	0.12-0.13	0.06-0.07	0.26-0.27	0.04-0.05	0.15-0.16	0.04	0.12	0.03-0.04	0.11-0.12	0.03	0.16	2003 JECFA PTWI for methylmercury equivalent to 0.23 µg/kg bw/day to protect against developmental effects[19]

Table 1: Comparison of the estimated dietary intakes of each element for each population group with the relevant safety guidelines *continued*

Elements	Estimated Dietary exposure (µgkg bw/day) a, b, c												Safety Guidelines
	Adults		Toddlers (1.5-4.5 years)		Young People (4-18 years)		Elderly (free living)		Elderly (institutional)		"Vegetarians"[d]		
	Mean	High level	Mean	High level	Mean	High level	Mean	High level	Mean	High level	Mean	High level	
Nickel	1.5	2.9	3.9	7.2	2.6	5.3	1.3	2.5	1.6	2.8	1.5	3.0	WHO TDI = 5 µg/kg bw/day[21]
Selenium	0.63-0.67	1.2-1.3	1.3-1.4	2.6-2.7	0.86-0.92	1.9-2.0	0.57-0.60	1.1	0.57-0.62	1.0-1.1	0.36-0.4	0.94-0.98	WHO upper limit of the safe range for adults only = 400 µg/day [29] EVM Safe Upper Level for total dietary intake equivalent to 7.5 µg/kg bw/day[18]
Tin	20	70	70	283	38	150	17	76	17	61	26	101	JECFA PTWI is equivalent to 2000 µg/kg bw/day[22] EVM Guidance Level = 220 µg/kg bw/day for total dietary intake[18]
Zinc	141	252	386	759	226	453	133	250	156	250	84	149	JECFA PMTDI = 1000 µg/kg bw/day[24] EVM Safe Upper Level for total dietary intake equivalent to 700 µg/kg bw/day (for 60 kg adult)[18]

Notes

a. Exposures have been estimated from the upper and lower bound mean concentrations, which assume non-detectable concentrations were the limit of detection and zero, respectively. Where the difference between the lower bound and upper bound mean concentrations is very small, rounding of the data leads to a single value.

b. The dietary exposure (mean and high level) for all foods combined is not equal to the sum of the exposure from the individual food. It refers to the dietary exposure by a consumer consuming one or any combination of the foods containing the metals. These values are derived from a distribution of the individual consumer's consumption patterns with regards to the individual foods.

c. Consumption data taken from the relevant National Diet and Nutritional Surveys.[3-7]

d. Some of the respondents of the dietary survey of vegetarians were consumers of fish.

Table 2: Comparison of population dietary exposures to aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), selenium (Se), tin (Sn) and zinc (Zn) from UK Total Diet Studies 1976 to 2000

	Population dietary exposure (mg/day) [a,b]											
Year	Al	As	Cd	Cr	Cu	Pb	Mn	Hg	Ni	Se	Sn	Zn
1976	–	0.075	0.02	0.13	1.8	0.11	–	0.005	0.33	–	4.4	10
1977	–	0.1	0.018	0.17	1.8	0.1	–	0.005	0.26	–	4.2	10
1978	–	0.081	0.02	0.1	1.6	0.11	–	0.005	0.27	–	3.6	10
1979	–	–	0.017	–	–	0.09	–	0.004	–	–	3.2	–
1980	–	–	0.026	–	–	0.12	–	0.005	0.27	–	–	–
1981	–	–	0.019	–	–	0.08	–	–	0.23	–	2.4	–
1982	–	0.09	0.018	–	1.3	0.069	–	0.003	0.15	–	3.1	10
1983	–	0.07	0.018	–	1.2	0.067	4.6	–	0.15	–	2.3	10
1984	–	–	0.019	0.073	1.4	0.065	5.3	–	0.16	–	2.7	10
1985	–	–	0.018	–	1.3	0.066	5.0	–	0.14	0.063	1.7	10
1986	–	–	0.017	–	–	0.06	–	–	0.13	–	2.2	–
1987	–	–	0.018	–	–	0.06	–	–	0.15	–	2.0	–
1988	3.9	–	0.019	–	–	0.06	–	–	–	–	–	–
1991	10	0.07	0.018	0.25	1.4	0.028	6.2	0.002	0.17	0.060	5.3	10
1994	11	0.063	0.014	0.34	1.2	0.024	4.9	0.004	0.13	0.043	2.4	8.4
1995	–	–	–	–	–	–	–	–	–	0.039 c	–	–
1997	3.4	0.065	0.012	0.1	1.2	0.026	–	0.003	0.13	0.039	1.8	8.4
2000	4.7	0.055	0.009	0.046	1.3	0.0074	4.9	0.0015	0.13	0.034	1.4	8.4

Notes

" – " = not included in that TDS for metals and other elements.

a. The above population dietary exposures have been estimated using upper bound mean concentrations for each food group and consumption data taken from the National Food Survey 1997, Ministry of Agriculture, Fisheries and Food (1998). The Stationery Office, London.

b. Changes in the organisation of the TDS from 1981 onwards mean that exposures from TDSs before 1981 and from 1981 onwards are not directly comparable[1].

c. Dietary exposure estimates for selenium from the 1995 TDS are not directly comparable with those from other years as they are based on analyses of composite samples of each food from all the towns in the TDS rather than the upper bound mean concentrations of analyses of each food group from each town.

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Statement on physiologically based pharmacokinetic modelling

Introduction

1. In February 2003 the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) hosted a workshop on physiologically based pharmacokinetic (PBPK) modelling. The workshop comprised several presentations; these considered the use of PBPK models in risk assessment, requirements for PBPK models and parameters for incorporation of PBPK methods into risk assessment. The presentations were followed by a general discussion which focussed on the strengths and weaknesses of PBPK modelling, whether PBPK models could be integrated into COT risk assessments and how this could be achieved.

Background

2. Pharmacokinetics describes the relationship between exposure and the concentration-time profile of a chemical within the body. This relationship is usually expressed as an equation based on a representation of the body as one or more compartments. These approaches are limited, since the equation or model used is essentially empirical, and may bear little relation to the physiological processes involved. A more meaningful approach based on physiological principles rather than observed data would provide greater understanding of what actually occurs following exposure to a chemical. This is the concept of PBPK modelling as first described by Thorsten Teorell in 1937. However, at that time the lack of computing power to solve the resulting mathematical equations meant that the approach was impracticable.

3. Because PBPK modelling accounts for the underlying physiological processes and the physico-chemical properties of the chemical administered it facilitates prediction of events in humans from animal data and explains differences across chemicals. PBPK modelling permits prediction of chemical concentrations at specific target sites and can incorporate different exposure scenarios, disease states or changes with age and co-administration of other chemicals.

4. PBPK models are based on three main elements; physiological parameters, chemical specific parameters and design of the model. The physiological parameters are independent of the chemical and define each tissue or organ by its structure, size, blood flow, and functionality. Overlaid onto physiological parameters in the model are the chemical specific parameters: binding within blood (e.g. to proteins, red cells), tissue affinity (binding, partitioning), membrane permeability, and sensitivity to enzymic modification. The complexity of the model can be varied according to the information required. In a simplified model, tissues with similar physiological properties are considered as a single tissue. Variation of the biological parameters of a model (e.g. body weight) allows some PBPK models to simulate population response to exposure to a chemical by producing a distribution of outputs.

5. PBPK models, although complex and initially demanding of data and resources;

   - are highly informative
   - allow ready integration and scaling of in vitro and structural information
   - allow ready exploration of a wide variety of conditions and
   - improve successful modelling and prediction of pharmacokinetic events.

6. The use of PBPK modelling has become increasingly common in the development and selection of pharmaceutical candidates. The majority of work on development and validation of PBPK models has occurred in this context, although there have been some detailed studies of specific environmental chemicals.

7. Interest in PBPK modelling as a tool in risk assessment is increasing in North America and the EU. Regulators will need to be in a position to respond intelligently to use of PBPK by industry. The information generated will be useful in risk assessment and might provide insights when developing positions on generic risk assessment issues.

Validation of PBPK Models

8. A PBPK model requires validation to establish that the model accurately predicts what happens following exposure to a chemical. Validation contrasts the predictions generated by a model to data observed experimentally. However, little consensus exists on the nature and extent of experimental data required for validation of a model.

9. Where there are considerable data on the concentration of a chemical in human and animal systems, validation is relatively straightforward. When a model appears to predict the empirical fate of a chemical in the body with accuracy over a range of inputs there can be a high level of confidence in the model. This is relatively easy for a pharmaceutical compound where there are generally human data available to compare with the model output. There might occasionally be sufficient data available to validate a model in occupational settings.

10. In the case of contaminants and other non-pharmaceutical chemicals of toxicological concern human data is often scarce. The validation process may be limited to contrasting model predictions with observed data in animals. However, in contrast to the situation in humans, in animal studies it is also possible to undertake mechanistic validation, e.g. by manipulating the activity of a specific process. Nevertheless, generally there will be greater uncertainty about the accuracy of models generated for non-pharmaceutical chemicals and their ability to predict the behaviour of the chemical in humans.

Utility of PBPK modelling in the risk assessment/management process

11. Validated models can be very useful for informing the risk assessment and risk management of chemicals. An example is the use of PBPK to determine tissue levels following different exposure scenarios based on occupational exposure limits for carbon monoxide in order to examine options for setting new limits. However, generation and validation of a PBPK model is resource and time intensive, and it would be neither possible nor practicable to generate PBPK models for all chemicals. The emphasis should be that where models are available they have the potential to decrease the reliance on default assumptions about interspecies extrapolation and animal experiments.

The applicability of PBPK to risk assessments as carried out by the Committee

12. The majority of the risk assessments undertaken by the Committee are reactive. The effort and time required to produce and validate a model mean that it would not be feasible to generate a model in the usual time-scale for preparing papers for the Committee. However, where PBPK models already exist for a chemical these should be included in the information considered by the Committee in undertaking the risk assessment. The Committee noted that for many chemicals there were limited toxicological databases and little or no pharmacokinetic data. In addition, for most contaminants it was unlikely to be ethically possible to generate human pharmacokinetic data to fully validate the model. The adequacy and predictability of the model would then be a greater source of uncertainty.

13. There may, however, be the possibility to generate models that inform the risk assessment process for a chemical in more proactive risk assessments. This would require identifying and defining specific questions that the model would need to answer but could be valuable in an integrated risk assessment examining the relative contributions of different routes when exposure occurs via several routes. Considerable resource investment would be necessary in order to generate the model and, if necessary, data for validation.

14. The development of a small range of generic models with consensus biological parameters might be of value in comparing chemicals with different but limited data.

Conclusions

15. We *conclude* that PBPK modelling is an established technique, capable of predicting the behaviour of chemicals in the body, which is widely used in the development and assessment of pharmaceuticals. We *consider* that PBPK models can be used as part of the risk assessment process. We *note* that PBPK modelling may also be helpful in evaluating risk management options. PBPK models can also be valuable in identifying those parameters exerting most influence on the behaviour of the compound (sensitivity analysis) and as a means of exploring inter-individual variability.

16. We *conclude* that the generation and development of PBPK models is an iterative process and that each model requires validation. We *note* that the concept of validation is often based on empirical verification but currently there appears to be little consensus amongst practitioners on criteria for the adequacy of validation.

17. We *recognise* that full validation usually requires verification of the model predictions with human data. We *recognise* that for many chemicals it may not be possible to generate human data. We *note* that animal data can go a long way towards validation if it can be assumed, or there is evidence, that the chemical behaves similarly in animals and humans. We *conclude* that there would be limited confidence in the predictions of such models; this would need to be expressed as a source of greater uncertainty in the risk assessment.

18. Whilst validation requires data on the pharmacokinetics of a chemical in human and/or animal systems, we *note* that for many chemicals evaluated by this Committee there are very limited data on pharmacokinetics. We *conclude* that for many of these chemicals full validation of a specific PBPK model would not yet be possible. Validation could be enhanced by mechanistic studies in experimental animals

19. We *note* that the generation and validation of a PBPK model is resource and time intensive. We *conclude* that it would not be feasible to undertake PBPK modelling routinely for our risk assessments but that we should incorporate existing published PBPK models into our assessment when available.

COT Statement 2003/05

December 2003

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Urgent opinions requested of the COT

Fumonisins in maize meal: risk assessment

Possible health risks from consumption of contaminated maize meal

Issue

1. As part of a wider FSA survey of maize products analysis of two maize meal products (the only maize meal samples taken), 'Fresh and Wild' and 'Infinity Foods' organic maize-meal were found to contain high levels of fumonisins. The original laboratory found levels of 3605 and 8358 mg total fumonisins/kg, respectively. These samples were reanalysed and found to contain significantly higher levels 4712 and 20435 mg total fumonisins/kg, respectively. A further 30 samples have now been taken and are currently being analysed. Levels of fumonisins were low in other maize products analysed, such as maize flour.

Background

2. Fumonisins are mycotoxins, which are considered to be possibly carcinogenic in humans although evidence for their genotoxicity and carcinogenicity was considered inadequate by the Scientific Committee on Food (SCF). Information on the mechanism of action justified a threshold approach. Therefore, in 2003 the SCF set a tolerable daily intake (TDI) of 2 μg/kg bw/day for fumonisin B1, B2, and B3, alone or in combination (the committee considered a group TDI was appropriate). This was based on kidney and liver damage in sub-chronic and long-term studies in rodents (immunotoxicity was also observed at higher dose levels) and incorporates a 100-uncertainty factor. The TDI also took account of neurotoxicity in a short-term study in horses, as this was a severe effect that did not need long-term exposure. The committee considered that this effect, if induced in humans, would also be observed after short-term exposure. Fumonisin B1 has a low acute toxicity in several animal species and there are no reports of acute effects in humans (despite levels of about 120 mg/kg in some home grown maize).

Risk assessment

3. One-off consumption of a product containing fumonisin levels resulting in exposures exceeding the TDI is unlikely to have an appreciable effect on health. However, the risks of consumption of products containing high levels of fumonisins sufficient to exceed the TDI for longer periods of time is uncertain.

4. Assessment of consumption of maize meal and consumer exposure to fumonisins is difficult. There are too few consumers of maize meal in the National Diet and Nutrition Survey (n=6) to derive reliable intake data.

5. The TDI set by the SCF earlier this year is 2μg/kg bw/day for total fumonisins. Using the data from the second analysis of the maize meal, which gave levels of total fumonisins of 4712 and 20435μg/kg the TDI would equate to an intake of 29 or 7g of these maize meal samples for adults and 17 or 3g for young persons (4-18yrs) for the 2 samples analysed. The SCF TDI used an uncertainty factor of 100 and was based on kidney and liver damage in rats from sub-chronic and long-term studies.

6. As the intake data are unreliable due to the small sample sizes, data on possible intakes can only be estimated from individual recipes (see Annex A). Maize meal is used as a thickening agent or an alternative to bleached cornflour, or in gluten-free foods, and it can also be used as part of some ethnic diets. Generally the recipes considered indicate intakes of maize meal of 5-20g/serving from foods such as tortillas, corn bread, muffins, scones etc. Intakes from dishes including polenta could be as high as 80-125g maize meal/serving but data from the survey have not found high levels of fumonisins in retail polenta products. We do not have any information on possible intakes of maize meal from ethnic diets, but it is known to be used to make a weaning porridge in some parts of Africa and could be used in the same way by some ethnic groups in the UK.

7. If it is assumed that one serving of breads, cakes etc might be consumed daily, this could lead to an intake of maize meal of up to 20g/day. This would equate to a fumonisins intake of 3xTDI for adults and 7xTDI for young persons consuming the most highly contaminated sample. However, it is unlikely that such consumption would be repeated on a daily basis.

Annex A

Maize Meal Consumption

1. There were too few consumers of maize meal (N=6) in the NDNS from which to derive reliable intake data and no suitable food substitutes were available in the programme, hence it was decided that the best way forward was to use consumption data from typical recipes.

2. The following information on the typical uses of the products has been sourced from the retailers:

   - Uses described over the phone: It is used mainly as a thickening agent, as an alternative to bleached cornflour, by consumers who require a gluten-free diet. It is used in desserts, soups and in Mexican foods such as tortillas.

   - Fresh and Wild Organic Maize Meal, uses described on the label: Muffins, tacos, breads, cakes and polenta. Use as a coating for fried foods. Do not confuse with cornflour/starch, which should only be used as a thickening agent.

3. Below we have provided a table of typical recipes that use maize meal. Most of these recipes were sourced from 'gluten-free' cookbooks designed for sufferers of Coeliac Disease. Those recipes sourced from the Sainsbury's recipe database are designed for individuals without specific dietary requirements, but may be consumed by Coeliacs if they are gluten-free.

4. Recipes quoted in the table below assume that corn meal and maize meal are synonymous. Recipes using just corn/maize flour (UK) or corn/maize starch (US) were not included (typically this is used as a thickening agent, a common use is in custard). An exception to this is the tortilla recipe, which contains masa harina (maize flour). This was included as the retailer suggested that the product might be used for tortillas. No other information on ethnic foods, such as maize meal used as a weaning porridge (typically eaten in certain parts of Africa), have been included.

5. The maize meal in a typical adult serving has been given for each recipe where that recipe specified the number of people it would serve. Where this information was not available an amount of maize meal per individual unit is given e.g. per muffin or slice. The recipes in the table below directly indicate the use of maize/corn meal. Maize meal may, however, be used as part of a gluten-free flour mix that can substitute for flour in any cake, bread or biscuit (resulting in a heavy texture). Maize flour or dry polenta typically makes up 38% of the mix. Many more polenta recipes are available on the Sainsbury's recipe database; here we have just given a couple of examples (including the recipe that used the highest serving of polenta in the database). Any other assumptions made are detailed in the notes section of the table (see below).

Table of typical gluten free recipes using maize/corn meal[1,2,3,4,5]

Recipe	Amount of cornmeal per single serving (g)	Notes
Hard polenta and grilled courgettes[1]	125	Per adult serving Highest single adult serving from Sainsbury's database polenta recipes
Polenta sandwich[1]	83	Per adult serving
Grilled vegetable pizza[2]	19	Per adult serving
Corn Tortillas[1*]	17	Per tortilla
Cheese and onion corn bread[2]	16	Per 1 slice, assuming each slice is 85g (approximated to banana loaf slice – MAFF portion sizes)[4] Recipe specifies 175g maize meal per 900g loaf
Apricot and orange muffins[2]	14	Per muffin
Scones made with cornmeal[5]	10	Approximate cornmeal per 50g scone (approximated to wholemeal scone – MAFF portion sizes)[4]
Wild mushroom and broccoli flan[2]	9	Per adult serving
Corn crisp bread[3]	8	Per crisp bread
Corn, Buckwheat and Rice Bread[2]	5	Per 1 slice, assuming each slice is 85g (approximated to banana loaf slice – MAFF portion sizes)[4] Recipe specifies 50g maize meal per 900g loaf

1 Sainsbury's recipe database: http://recipe.sainsburys.co.uk/recipe/search

2 Anne Sheasby 'Gluten Free Cooking' 2000, Annes Publishing Ltd.

3 Peter Thompson 'Gluten-free cookery' 2001, Hodder and Stoughton.

4 MAFF 'Food Portion Sizes' 1998, 2nd Ed. The Stationery Office.

5 Marilyn Le Breton 'The AiA Gluten and Dairy Free Cookbook' 2002, Jessica Kingsley Publishers, London and Philadelphia.

* This recipe is not gluten free.

Nickel leaching from kettle elements into boiled water

Background

1. The Committee was asked for urgent advice on the health implications of nickel leaching from kettle elements into boiled water, based upon results of a study commissioned by the Drinking Water Inspectorate (DWI). Members were provided with the full DWI study report, a summary of the data drafted by the Secretariat, the draft risk assessment of nickel from the Expert Group on Vitamins and Minerals (EVM) and an opinion previously provided by a COT expert to support the EVM evaluation.

2. Seven Members were able to provide written comments in the time available. The following summary of the comments was drafted by the Secretariat and approved by the Chairman.

Nickel in boiled water

3. The new data show that boiling of water in some types of exposed element kettle results in an increase in the nickel content. This is consistent with the results of previous studies reviewed in the DWI report.

4. There is no statistical analysis of the data in the DWI report and it is therefore not possible to draw conclusions with respect to possible effect of water filtration. The study design involved 3 types of filter, 8 models of kettle, 4 kettles per model, 2 samples of water, which were sometimes but not always pooled for analysis, with 8 sampling days. Specialist statistical expertise is required to analyse these data.

5. The data indicate that there might be a difference between filtered and unfiltered water. However this depends on a number of factors such as the age of both the kettle and the filter, type of kettle, whether the water is hard or soft, and how long the water is allowed to stand in the kettle after boiling. In practice, it is likely that kettles are often boiled containing appreciable amounts of water from previous use. The results of repeated boiling studies are odd since the nickel content of the water appears to decrease. The limited study of water boiled under "domestic conditions" indicates that if there is a difference it is much less marked when the kettle and filter are older.

6. The data only address water from two specific locations and it is not possible to determine whether these are representative of all water supplies, or how variable the results would be using water from other areas. Potential variables include the presence of organic compounds (e.g. in peaty water), differences in reservoir conditions, distance water has been piped and types of piping used.

7. In considering the nickel levels that should be used in a risk assessment, Members concluded that it would be preferable to have more complete data on boiled water corresponding to normal usage of kettles. Members considered that if it is necessary to conduct a risk assessment on the currently available data, then since the leaching declines rapidly during the first seven uses, it would be more relevant to use the data from the later sampling points.

Health risks associated with ingestion of nickel

8. As noted by the EVM, ingestion of nickel may result in an elicitation or exacerbation of allergic reactions in individuals who already have allergic sensitisation to nickel. Nickel-sensitised individuals should therefore be considered to be at greater risk; nickel sensitisation is more prevalent in women than in men. There is limited evidence that atopic dermatitis sufferers may have a higher rate of nickel sensitisation (Cronin *et al*., 1993). An allergic skin reaction may result from single exposure to pre-sensitised individuals, but is not considered to be a serious health risk.

9. Oral exposure is not considered to cause skin sensitisation in the absence of prior exposure via jewellery or other skin contact. Oral exposure to nickel at an early age (prior to ear piercing) may inhibit subsequent development of allergic hypersensitivity to nickel (Todd *et al*., 1989; Van Hoogstraten *et al*., 1991; Vreeburg *et al*., 1984). Therefore infants are not considered to be at greater risk that adults. In addition, there is some evidence that oral exposure to nickel may reduce sensitisation in those who already have contact hypersensitivity (e.g. Jovall *et al*., 1987; Panzani et al., 1995).

Information needs for risk assessment

10. Since absorption of nickel from beverages such as tea or ingested with food is greatly reduced compared with absorption from water alone, information is required on the ways in which boiled water may be consumed, and the amounts consumed.

11. More information is required on the factors influencing leaching, using water samples that are representative of different supplies. A study with distilled water would be useful for comparison. More information is needed on different types of filter and kettle, on the time-course of changes in nickel leaching with the age of the kettle used, frequency and effects of descaling and how frequently consumers replace their kettles. Exposure data to be used in a risk assessment should be based on water boiled under conditions similar to those used in homes, the work-place and catering establishments.

Conclusions

12. The Committee concluded that the results of the DWI report support previous observations that boiling water in some types of kettle may result in elevated levels of nickel in the water. No other conclusions could be reached in the absence of statistical analysis of the data.

13. Individuals with prior allergic sensitisation to nickel are at greatest risk of adverse effects arising from nickel ingestion. A single exposure to high levels of nickel in food or water may result in exacerbation or elicitation of an allergic skin reaction in these individuals. This is not considered to be a serious health risk. Infants are not considered to be at greater risk than adults.

14. In order to assess the risks associated with nickel in boiled water, more information is needed on the possible exposure resulting from use of different types of filter and kettle under normal conditions of use.

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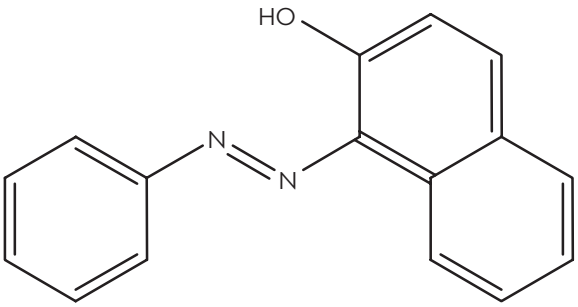
Sudan 1 found in chilli powder

Risk Assessment of Sudan I



1-phenylazo-2-naphthol
Major synonyms: Sudan I, C.I. Solvent Yellow 14

1. In 1975 the International Agency for Research on Cancer (IARC) reviewed the available data on Sudan I. It placed Sudan I in Group 3[§] based on the limited data available at that time (IARC 1975). IARC also cited an evaluation carried out by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which concluded that on the basis of toxicological evidence, Sudan I is considered to be unsafe for use in food. Subsequent to the IARC evaluation further data have become available on Sudan I.

In vitro genotoxicity data

2. Garner and Nutman (1977) reported a negative result in the Ames test with concentrations up to 100mg/plate. Cameron *et al.* (1987) reported that Sudan I induced revertants in *S. typhimurium* TA1538 in the presence of hamster liver S9, with a concentration related response from 3333mg/plate. There was also an apparent concentration-related response with rat liver S9, but it did not reach the level of twice the spontaneous revertants, which was a specified criterion for a positive response at that time. No increase in revertants was seen in the absence of S9. Tennant *et al.* (1987) reported Sudan I as being positive with metabolic activation in the Ames test with a lowest positive concentration 0.3 μg/plate, but insufficient detail is available to judge the validity of this result.

3. In Chinese Hamster Ovary (CHO) cells, Sudan I induced sister chromatid exchange in the presence and absence of metabolic activation, but did not induce chromosome aberrations (Tennant *et al.,* 1987); Ivett *et al.,* 1989). It has also given positive results in the mouse lymphoma assay (Tennant *et al.,* 1987; Cameron *et al.*, 1987; McGregor *et al.,* 1991).

[§] Group 3: The agent (the mixture, the circumstances surrounding exposure) may not be classed in terms of its carcinogenicity for man. This category groups those agents for which proof of carcinogenicity is insufficient for man and insufficient or too limited for laboratory animals.

4. Stibovora *et al.* (2002) reported that metabolism of Sudan I by CYP1A1 (and possibly CYP3A4) in human microsomes produces a product that can form DNA adducts. This compound exhibited the same chromatographic properties as the 8-(phenylazo)deoxy-guanosine DNA adduct produced following metabolism of Sudan I by rat microsomal enzymes.

In vivo genotoxicity data

5. Oral administration of Sudan I has been reported to induce micronuclei in rat and mouse bone marrow (Elliot *et al.,* 1997). In both rat studies, there was a significant increase in micronuclei at 24 and 48h harvest times at the only dose tested, 5000 mg/kg, which was reported as clear positive result. In mice, there were some inconsistencies between the two experiments with respect to results at the different harvest times and depending on the number of cells scored. Positive results were obtained at a dose of 5000 mg/kg at both harvest times, and also at 2000 mg/kg when 6000 cells at the 24 h harvest. The authors reported this as a weak effect because the fold increases above control were less than for rat. There were no signs of toxicity in either rats or mice, but an orange coloration of urine and faeces indicated that Sudan I was absorbed.

6. Westmoreland and Gatehouse (1991) reported a dose-related increase in bone marrow cell micronuclei in male PVG rats 24 hours after a single oral dose of 250, 500 or 1000 mg/kg. Administration of 1000 mg/kg Sudan I induced micronucleus formation in bone marrow cells and peripheral blood reticulocytes of F344 rats (Wakata *et al.* (1998). In contrast, Westmoreland and Gatehouse reported a negative response in a bone marrow micronucleus assay in male CRH mice at single doses of either 500, 1000 or 2000 mg/kg.

7. Negative results have been reported for induction of rat liver unscheduled DNA synthesis assay (UDS) at doses of up to 2000 mg/kg (Mirsalis *et al*., 1989; Elliot *et al.,* 1997; Westmoreland and Gatehouse, 1991). Mirsalis *et al*. (1989) and Westmoreland and Gatehouse (1991) reported negative or equivocal responses in the induction of S-phase DNA synthesis.

8. Tsuda *et al.* (1999) reported that oral administration of 1000 mg/kg Sudan I to male ddY mice produced a positive result in a Comet assay in stomach and colon 8, but not 3 or 25 hours after dosing. No evidence of genotoxicity was seen in kidney, liver, bladder, lung, brain or bone marrow.

Carcinogenicity data

9. Since the IARC evaluation, the carcinogenicity of Sudan I has been investigated as part of the National Toxicology Programme (NTP, 1982). Groups of 50 male and 50 female F344/N rats were fed diets containing 0, 250 or 500ppm Sudan for 103 weeks. These diets provided doses of 0, 13-36 and 26-73 mg Sudan I/kg bw/day to the males and 0, 12-32 and 25-68 mg/kg bw/day to the females. Groups of 50 male and 50 female B6C3F1 mice were fed diets containing 0, 500 or 1000ppm Sudan I for 103 weeks, providing doses of 0, 115-198 and 243-405 mg Sudan I/kg bw/day to the males and 0, 116-208 and 226-416 mg/kg bw/day in the females.

10. No compound-related clinical signs or effects on survival were observed. Neoplastic nodules of the liver occurred in rats of both sexes with a significant dose-related trend (male, $P<0.001$; female, $P=0.005$), which was not associated with non-neoplastic pathology in the liver. The authors concluded that the study provided unequivocal evidence of carcinogenicity following dietary administration in both sexes of F344 rats.

11. Lymphomas or leukemias occurred in the low-dose female mice at an incidence significantly ($P<0.05$) higher than that in the controls (control, 12/50; 500ppm, 23/50; 1000ppm, 17/50). However, because of the lack of a dose-related trend and because the incidence in the 1000ppm group was not significantly increased, an association between the incidence of hematopoietic tumors and the administration of Sudan I was not clearly established. The incidence of lymphomas or leukemias in male mice was not significantly increased (control, 5/49; 500ppm, 10/50; 1000ppm, 10/50). The authors concluded that Sudan I was not considered to be carcinogenic for B6C3F1 mice of either sex.

12. Hepatomas have been reported in mice following subcutaneous administration (IARC, 1975).

Additional data

13. There is evidence that Sudan I can cause allergenicity in the form of contact dermatitis following its use as a red dye in "kumkum" (an Indian cosmetic) at concentrations ranging from 2.8 mg/g to 8.7 mg/g (Kozuka *et al*., 1988). Additional evidence of allergenicity is reported by Kato *et al* (1986) who demonstrated that a metabolite of Sudan I (4'-hydroxy-1-phenylazo-2-napthol) elicited a positive allergenic response in guinea pigs sensitised to Sudan I. The authors concluded that the *para*-hydroxylation of the phenyl group of Sudan I may play an important role in its allergenicity

Conclusions

14. The Chairman of COT, COC and COM noted that, although there are as always some incomplete and possibly equivocal results, overall the azo structure, the genotoxicity data and the carcinogenicity data lead to the conclusion that it is prudent to assume that Sudan I is a genotoxic carcinogen. Dietary exposure should therefore be as low as reasonably practicable (ALARP).

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Declaration of COT members' interests during the period of this report

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Professor I Hughes (Chairman)	Pfizer	Education Adviser	Archives of Disease in Childhood	Commentary Editor
	BP Amoco	Shares	Academy of Med Sciences	Fellow
	BP Amoco	Daughter is an employee of this company	Soc for Endocrinology Royal College of Paediatrics and child Health	Member
	Topical Endocrinology	Editorial Board Member	Medical Res Council	Fellow, Senior Examiner, Regional Academic Adviser Member of Advisory Board
			Pfizer Aventis NovoNordisk Diabetes UK Wellcome Trust Juvenile Diabetes Fund	Funds received from all these sources for Departmental research and education in medicine and health related topics
Professor Alan Boobis	NONE	NONE	GlaxoSmithKline	Support by industry
			Servier Pharmaceuticals	Support by industry
Dr P Carthew	Unilever	Employee	NONE	NONE
	Provalis	Share Holder		
Professor J K Chipman	Sequani	Training	AstraZeneca	Research Support
		Consultancy	Glaxo Smith Kline	Research Support
	AstraZeneca	Consultancy	ICI	Research Support
	Inamed	Consultancy	HSE	Research Support
	Unilever	Consultancy	CEFIC-LRI	Research Support
	Syngenta	Lecture fee	Dept of Health	Research Support
			Inamed	Research Support

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Dr J Hinson	GlaxoSmithKline	Shareholder	NONE	NONE
Dr P Jackson	Bristol Myers Squibb	Lecture fees	Novartis	Research grant
	Merck Sharp Dohme	Lecture fees	Merck Sharp Dohme	Research grant
	British Heart Foundation	Lecture fees	Servier	Research grant
			Bristol Myers Squibb	Research grant
			Pfzier	Research grant
			AstraZeneca	Research grant
			Bohringer Ingelheim	Research grant
			Medtronic AVE	Research grant
			Department of Health	Research grant
Dr M Joffe	NONE	NONE	NONE	NONE
Professor J Lunec	NONE	NONE	Scilucent LLC. USA	Funding research group to investigate toxicology of soya-bean oil implants
Dr A Piersma	NONE	NONE	NONE	NONE
Professor D Ray	CEFIC (European chemical industry body)	Advisory panel fee	NONE	NONE
Professor I R Rowland	Colloids Naturels International Rouen (CNI)	Consultancy	Various	Departmental teaching and research funded by various food companies
	Cerestar	Consultancy		
	Halifax	Shares		
	Woolwich	Shares		
Dr L Rushton	Institute of Petroleum	Consultancy, Contracts and Grants – Completed	Concawe	Contracts to Institute for Environment and Health (IEH) Now Completed
	Transport and Geneal Workers Union	consulancy – Completed	European Silica Industry	Ongoing Cohort Study, Contract to IEH
	Friends Provident	Shares	International Manganese Institute	Contract to IEH to prepare criteria document
	Northern Rock	Shares	American Chemistry Council	Contract to IEH for systematic review and meta-analysis
	Unilever	Consultancy – advice on design of an epidemiological survey relating to dermatitis		

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Ms J Salfield	NONE	NONE	NONE	NONE
Dr A Smith	Abbey Natiional	Share Holder	Rhone Poulenc	Past Research
	British telecom	Share Holder	Glaxo Wellcome	Support
	MMO2	Share Holder	CEFIC – LRI	Past Research
	HBOS	Share Holder		Support
	Latham & Watkins	Consultancy		Research Support
Dr L Stanley	CXR Biosciences	Salary	Cyclacel	Company contract
			Euro Chlor	Company contract
			Halogenated Solvents Industry Alliance	Company contract
			Association of Plastics Manufacturers, Europe	Company contract
			Astra Zeneca	Company contract
Professor S Strobel	NONE	NONE	Syngenta	Research Grannt
			FSA	Contract Research
Professor S Strobel	NONE	NONE	Syngenta	Research Grant
			FSA	Contract Research
Dr M Tucker	Zeneca	Pension	NONE	NONE
Miss A Ward	NONE	NONE	NONE	NONE

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

Preface

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 independent authoritative advice on potential mutagenic hazards and risks is required. Frequently recommendations for further studies are made.

During 2003, the Committee provided advice on a wide range of chemicals which included DEET (an insect repellent), malathion (an insecticide), Flunixin (in the form of its meglumine salt) and malachite green (both used as veterinary medicines), 2-phenylphenol (a biocide) and a number of contaminants such as 1,3-dichloropropan-2-ol and polycyclic aromatic hydrocarbons (PAHs).

The Committee has an ongoing responsibility to provide Government Department's and Regulatory Authorities with advice on developments in procedures for the evaluation and risk assessment of mutagens. In this regard the Committee provided advice on the conduct of *in-vitro* mammalian cell mutation assays, the investigation of germ cell mutagens and a strategy for the assessment of the significance of in-vivo mutagenicity seen at high doses in bone-marrow tests. This latter statement is particularly recommended for regulatory agencies who may have to assess such data.

During 2003, the Committee also said farewell to three highly respected members. Professors Ashby, Cooper and Tweats have provided valuable advice to the Committee over many years. I wish to record my thanks for their scientifically excellent contribution and their commitment to public health during their terms of office with COM.

Professor P B Farmer Chair
MA DPhil CChem FRSC

1,3-Dichloropropan-2-ol (1,3-DCP) : new *in vivo* mutagencity studies

2.1 1,3-Dichloropropan-2-ol (1,3-DCP) is a member of a group of chemicals called chloropropanols, which also includes 3-chloro-1,2-propanediol (3-MCPD) 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. The COM considered the mutagenicity of 1,3-DCP in 2001 and noted that the *in-vitro* data indicated that it had mutagenic potential. In the absence of any *in-vivo* data in mammals, it was concluded that it would be prudent to assume that 1,3-DCP was potentially genotoxic *in-vivo* and agreed it should be tested *in-vivo* using the approach set out in the COM guidelines.

2.2 Two studies, a bone marrow micronucleus assay and an unscheduled DNA synthesis (UDS) assay had been carried out to investigate the *in-vivo* genotoxicity of 1,3-DCP. Members discussed the results of the two new studies and considered that both assays had been conducted to relevant guidelines and that the results were clearly negative. In the micronucleus assay, Members noted that the PCE:NCE ratio was very variable amongst controls and that the top and bottom ends of the range was outside the range for historical controls. These findings were not considered to invalidate the study but Members requested that the contract laboratory be asked to comment on the reason for the variability.

2.3 Members considered that it would be appropriate to consider these studies provided evidence that 1,3-DCP was not an *in-vivo* mutagen.

2.4 A statement is appended at the end of this report.

DEET (Diethyl-m-toluamide)

2.5 The COM considered the available mutagenicity data on DEET in April 2002 and concluded that whilst there were apparently no concerns regarding mutagenicity, there was a need to consider the full reports of the two unpublished studies (carried out for the DEET Joint Venture Group), namely a metaphase assay for clastogenicity in CHO cells and an assay for UDS in rat hepatocytes. The COM considered these additional reports.

2.6 Members noted that DEET appeared to be highly toxic *in-vitro* to mammalian cells in both CHO cells and rat hepatocytes and hence adequate results were only available from a relatively narrow concentration test range in both assays.

2.7 The COM concluded that there were limitations in all of the submitted studies. In particular, the COM considered that the exogenous metabolising fraction used in the *in-vitro* chromosome aberration assay had not produced satisfactory results with cyclophosphamide. However, overall the results of the *in-vitro* cytogenetics assay in CHO cells and an *in-vitro* UDS assay in hepatocytes were negative. This information together with the information previously reviewed by the Committee (lack of structural alerts with DEET, negative Ames tests and negative carcinogenicity studies) suggest there is no concern with regard to the mutagenicity of DEET.

2.8 An amendment to the published statement to include this conclusion was made (http://www.doh.gov.uk/pdfs/deetstatement.pdf.)

Flunixin, meglumine and Flunixin-megulimine

2.9 Flunixin in the form of the meglumine salt is a non-steroidal anti-inflammatory (NSAID) drug and a non-narcotic analgesic drug with antipyretic activities. It is used in veterinary medicine (including food-producing animals) but it is not used in human medicine. Flunixin-meglumine dissociates *in-vivo* to Flunixin and meglumine. In 1997, the European Medicine Evaluation Agency's (EMEA) Committee on Veterinary Medicinal Products (CVMP) considered the safety of Flunixin-meglumine as part of its review of old veterinary medicinal products, operating under Council Regulation 2377/90, which covers the marketing authorisation of pharmacologically active substances used in veterinary medicines. The Food Standards Agency was concerned about the possible mutagenicity of Flunixin, meglumine and Flunixin-meglumine and asked for an opinion from the COM.

2.10 The COM considered the mutagenicity data on these compounds at meetings in 2001/2 and heard a presentation from the data holder (Schering-Plough) at the February 2003 meeting.

2.11 Most of the mutagenicity data were relatively old and had limitations. Members agreed there was no evidence to suggest that flunixin itself had mutagenic potential *in-vivo* but the *in-vitro* data were inadequate. There was limited evidence to conclude that flunixin meglumine was an *in-vitro* mutagen, which suggested that it was the meglumine component that was responsible for the mutagenic activity. There was a negative *in-vivo* bone marrow micronucleus test with flunixin meglumine, but this study was considered too limited to allow conclusions to be drawn. Meglumine was not considered to have any structural alerts. A positive result had been reported with meglumine itself in a bone marrow micronucleus test. In this study involving administration of 2 doses 24 hours apart with harvest at 6 and 24 hours later, a positive result was produced only at the 6 hour sampling point. Negative results were also obtained in a second assay using a similar dosing schedule, but with harvest only after 24 and 48 hours.

2.12 New *in-vivo* micronucleus data was considered at a further meeting and agreed to be inconclusive. One study did indicate activity 6 hours after the 2 doses spaced 24 hours apart. No activity was seen at 24 hours after the second dose. The Committee noted that there was much variability in the results with individual animals, and the effects may possibly be due to toxicity. Members agreed that on the basis of the available data, the situation regarding the *in-vivo* activity of meglumine was not completely resolved.

2.13 The COM considered that further studies were needed before it could be concluded that the mutagenic activity seen *in-vitro* with flunixin meglumine (believed to be due to the meglumine component) could be discounted.

2.14 The presentation from the data holder and subsequent COM discussion focused on the most appropriate testing strategy given the complex set of mutagenicity data on these compounds. The data holder accepted the proposed strategy which is outlined in para 2.15 below.

2.15 The COM concluded:

i. The mutagenicity data on Flunixin, Flunixin-meglumine and meglumine are relatively old and the studies have not been conducted to contemporary standards.

ii. It is therefore difficult to draw any definite conclusions on the mutagenicity of these chemicals. A number of prudent conclusions were agreed:

a. For Flunixin (a non-steroidal anti inflammatory veterinary medicine) there was limited evidence that Flunixin was mutagenic *in-vitro* but there was no evidence to suggest that flunixin had mutagenic potential *in-vivo*. Members felt the *in-vitro* mutagenicity data on this compound were inadequate and considered this should be raised with the CVMP.

b. For Flunixin-meglumine (Flunixin in the form of the meglumine salt), there is limited evidence for a mutagenic effect *in-vitro*. In addition the inconclusive *in-vivo* data on meglumine suggest that a definite conclusion regarding flunixin-meglumine cannot be reached.

c. For meglumine, the available *in-vivo* mutagenicity data are inconsistent with some positive and negative results. It is recommended that additional *in-vitro* mutagenicity tests conducted to modern standards are undertaken with meglumine to assess whether meglumine has any mutagenic potential using the approach recommended in the COM guidance. Negative results are available for a gene mutation assay in *Salmonella*. Therefore these additional tests should comprise:

i. An *in-vitro* chromosomal aberration test in mammalian cells.

ii. An *in-vitro* mouse lymphoma assay.

2.16 A statement is appended at the end of this report.

Malachite Green

2.17 Malachite green is a cationic triphenylmethane dyestuff used in a number of industries, including fish farming. It is used in freshwater fisheries for the treatment of external fungal and other infections, its main use being to stop fungal growth on the eggs. The COT has asked for advice on the mutagenicity of malachite green and the lipophilic metabolite leucomalachite green.

2.18 The COM last considered the mutagenicity of malachite green and leucomalachite green in 1999. A statement had been forwarded to the Committee on Toxicity (http://www.doh.gov.uk/com/malachit.htm). The COM had concluded that, on the basis of the limited data available, and specifically a ^{32}P-post-labelling study, both compounds should be considered as potential *in-vivo* mutagens. Further results were now available from the NTP studies on the mutagenicity and carcinogenicity of leucomalachite green. These new data included results of *lacI* mutations in the livers of BigBlue® rats fed leucomalachite green at levels of up to 543 ppm in the diet for 4, 16 and 32 weeks and results of DNA adducts measurements in the livers of rats fed leucomalachite green for 4 weeks using ^{32}P-postlabelling assays. (Culp SJ *et al, Mutation Research* vol 506-507, 55-63, 2002).

2.19 The Food Standards Agency had asked the COM for advice on these new data. The mutagenicity data were from studies on leucomalachite green fed to BigBlue® rats. An approximate 3-fold increase in *lacI* mutations was reported in the liver only at the top dose level at the 16-week time interval. An apparent dose-related increase in DNA adducts using the ^{32}P-post-labelling assay was reported.

2.20 Members agreed that the results for *lacI* mutations were unusual in that the claimed positive response seen at 16 weeks feeding had disappeared after 32 weeks of feeding leucomalachite green. It was noted that the inter-animal variation in mutation frequency was high and the apparent positive result might relate to one only out of the five animals fed leucomalachite green for 16 weeks. The Committee was not convinced that a clear positive response had been obtained in this mutagenicity study. A reported increase in DNA adducts in the liver of female BigBlue® rats did correlate with the increasing dose of leucomalachite green. However, it was noted that adduct levels produced were very low; the highest level of DNA adducts found equated to approximately 1 adduct per 10^8 nucleotides (this was considered to be a difficult adduct formation frequency to detect). The author's explanation was that the reported increase in mutation frequency at 16 weeks may have been due to a proliferative response of background mutations rather than induced by leucomalachite green. The Committee did not find this particular argument convincing and agreed that overall no conclusions could be reached from the investigations reported in this published paper.

2.21 Members heard that additional information from an NTP bioassay in rats with leucomalachite green would be forthcoming and that it was possible that additional in-vivo mutagenicity studies using BigBlue mice would be reported.

2.22 In answer to the questions posed by the FSA, members agreed that no conclusions could be reached from the new mutagenicity data on leucomalachite green summarised in the paper by Culp SJ *et al (Mutation Research* vol 506-507, 55-63, 2002).

2.23 The Committee agreed there was no need to alter its previous advice on malachite green and leucomalachite green, but that the situation should be reviewed when full reports of the carcinogenicity bioassay became available.

Malathion

2.24 Malathion is an organophosphorus insecticide. It has been marketed in the UK for use in agriculture and horticulture since 1956. There were three products with approvals for use in agriculture and horticulture, home garden and use in pigeon lofts at the time when this review was initiated in January 2002. A number of products containing malathion are also licensed as human medicines for use in the control of head lice.

2.25 The Advisory Committee on Pesticides reviewed the available toxicological information on malathion as part of its ongoing review of organophosphorus compounds. The ACP asked for advice from COM and COC on mutagenicity and carcinogenicity at its 289th meeting on 17 January 2002. The Chairs of COM and COC agreed that a joint statement was required in view of the need for a full review of all mutagenicity and carcinogenicity data.

2.26 The COM undertook an initial consideration of the in-confidence mutagenicity data provided by the pesticide data holder and the available published information provided by the data holder at its 25 April 2002 meeting. A number of additional published papers on malathion and impurities present in technical grade malathion were also considered at this meeting. At its meeting of 10 October 2002 the COM considered some additional information provided in-confidence by the pesticide data holder (a report of one additional *in-vivo* study and information on the potential for variation in impurities between different sources of malathion) together with a number of published studies not previously reviewed.

2.27 The Committee agreed a number of conclusions, which were forwarded to the pesticide data holder. Additional data on the conduct and results of the *in-vivo* oral rat liver UDS assay were submitted by the pesticide data holder and were considered at the February 2003 meeting of COM. The Committee was informed that the data holder had responded to the 1st draft statement and there had been an exchange of views between the COM and the data holder particularly with regard to the adequacy of the in-vivo rat liver UDS assay. A revised draft COM statement containing additional comments in response to the data holder's submission was agreed.

2.28 Malathion was also considered at the COC meeting of 27 June 2002. A full statement from COC and COM is appended at the end of this report.

Phenol

2.29 The COM had previously considered phenol on a number of occasions (1994, 1995 & 2000) (http://www.doh.gov.uk/hydphen.htm). The *in-vitro* data on this compound were poor. The committee had decided that phenol should be regarded as an *in-vivo* somatic cell mutagen based on positive results at high doses in the bone marrow assays for clastogenicity. Negative results were obtained in carcinogenicity bioassays in rats and mice. The committee agreed that a threshold for mutagenicity could be assumed for the oral route because there was evidence to show that any phenol active metabolites formed *in-vivo* were rapidly detoxified by multiple pathways following ingestion. But a threshold for mutagenicity for exposure by other routes, such as inhalation or dermal, could not be assumed.

2.30 Since then, data have become available to provide a plausible mechanism to support the view that positive results in the bone marrow assays were not due to a direct mutagenic effect of phenol, but were due to a secondary threshold toxic effect, namely hypothermia occurring at dose levels associated with positive results in the micronucleus assays.

2.31 The Committee was asked to consider whether these new unpublished data together with the absence of any other positive *in-vivo* mutagenicity data and the negative results in the carcinogenicity bioassays indicated that phenol did not produce clastogenic effects at doses below those that produced hypothermia. These new data indicate that phenol can produce hypothermia in mice at doses that result in micronuclei formation. An intraperitoneal dose of 300 mg/kg phenol produced significant and prolonged hypothermia with a drop in body temperature of up to 7 degrees centigrade. No effects on body temperature were seen at doses below 300 mg/kg, and single doses of 400 and 500 mg/kg reduced body temperature and cause marked lethality. To investigate micronucleus induction single intraperitoneal doses of phenol were administered at 0, 30, 100 or 300 mg/kg and bone marrow harvested at 24 and 48 hours post dose (cyclophosphamide was used as a positive control and produced micronuclei). An increase in micronuclei was seen only at 300 mg/kg phenol, which was associated with significant and prolonged hypothermia. The investigators argued that the induction of micronuclei by phenol at the maximum tolerated dose is threshold related and may be causally related to hypothermia.

2.32 The COM considered that the new data provided a plausible mechanism. Members agreed the function of spindle fibres could be inhibited at low body temperatures, which could result in adverse chromosome effects, such as aneuploidy. The Committee was also aware of other data that indicated that hyperthermia can also induce chromosome damage both *in-vitro* and *in-vivo*, and that high body temperature induces micronuclei in mouse bone marrow. Members agreed that inhibition of the spindle function and disturbance of the mitotic apparatus was also a possible mechanism for this effect.

2.33 Members agreed that before definite conclusions could be drawn on the significance of these new data they would like to see a peer reviewed published report of this study. Members requested further data on the dose-response of hypothermia induced by phenol. It was also agreed that strong evidence to support this hypothesis would be provided if micronuclei were not induced by phenol in a separate group of animals maintained at normal body temperature (e.g. by the use of heated plates and warm beds). If such information could be provided members agreed that phenol could be regarded as having no significant *in-vivo* mutagenic potential at dose levels that do not produce any significant toxic effects (hypothermia).

2-Phenylphenol

2.34 2-Phenylphenol and its sodium salt are broad spectrum fungicides that are approved in the UK under the Control of Pesticides Regulations (COPR 1986 amended) for use as wood preservatives. They are also used as surface biocides in a number of areas. The COM have advised on the mutagenicity of these compounds, specifically in the context of the mechanism of the bladder tumours seen in male

rats fed high doses of these compounds, on a number of occasions, the most recent being in 1997. Significant new relevant data are now available. Furthermore an EU review of the use of 2-phenylphenol and its sodium salt in wood preservation is shortly to be initiated under the Biocidal Products Directive (98/8/EC). It would thus be timely to update the COM view on the mutagenicity of these compounds. This information would be helpful in developing the UK position with regard to the EU in the context of the Biocidal Products Directive.

2.35 At high dose levels 2-phenylphenol induces bladder tumours in the rat and it is particularly important to be able to exclude a genotoxic mechanism being involved. In 1992 the COM noted that although negative results were obtained in bone marrow assays and germ cell assays for mutagenicity *in-vivo*, some conflicting results were obtained in in-vivo assays for effects on the DNA in bladder epithelium. The COM recommended that data from an *in-vivo* study to investigate DNA adduct formation in bladder epithelium should be carried out using more sensitive methods, to provide definitive information regarding the absence of a genotoxic mechanism. At that time the Committee felt that there was insufficient concern to recommend a departure from a risk assessment approach based on the use of uncertainty factors to estimate safe levels of exposure (i.e. a threshold approach was adopted). In 1997 the Committee considered data from an *in-vivo* ^{32}P- post-labelling study on DNA adducts in the bladder but they had concerns at the limitations of the method used and recommended that this be further investigated. This recommendation was not made into a regulatory request because of other data on carcinogenicity provided to the ACP who felt that the post review regulatory requirements had been provided, and that a threshold based risk assessment was appropriate.

2.36 Considerable additional data has now been published relating to the metabolism and mutagenicity of 2-phenylphenol, including further *in-vivo* DNA adduct work. The Committee agreed that the new Accelerator Mass Spectrometry study (AMS) study provided good evidence for the lack of covalent DNA binding in the male rat bladder. The method was a particularly sensitive technique and the study had been well performed. Protein but not DNA binding had been clearly shown. Members agreed that the weight of evidence with regard to the DNA binding studies in the rat bladder was now sufficient to conclude that significant DNA binding was unlikely to occur.

2.37 The Committee agreed the following overall conclusions regarding the mutagenicity of 2-phenylphenol and its sodium salt, after consideration of all the new data:

i. Data from several assays to investigate the ability of 2-phenylphenol or its sodium salt to produce gene mutation in Salmonella typhimurium were consistently negative.

ii. Positive results were obtained in *in-vitro* metaphase analysis studies in CHO cells and also in mouse lymphoma assays in both cases in the presence of an exogenous metabolic activation system. The induction of small colonies in the latter assay is consistent with the compound having clastogenic potential.

iii. Phenylhydroquinone (PHQ) and phenylbenzoquinone (PBQ), which are metabolites of 2-phenylphenol, (PHQ usually being present as conjugates), have been shown to produce oxidative DNA damage and single strand DNA breaks *in-vitro* using HL60 and V-79 cells.

iv. Negative results were obtained in bone marrow assays for clastogenicity *in-vivo* and also in germ cells (dominant lethal assay), suggesting that any possible clastogenic potential was not expressed in the whole mammal.

v. The weight of evidence from *in-vivo* studies to investigate 2-phenylphenol binding to DNA in the male rat bladder was negative: a recent study using a highly sensitive AMS techniques was particularly important in this regard. However the possibility of prolonged high level exposure producing DNA adducts cannot be entirely discounted.

vi. Although a contributory role of oxidative DNA damage cannot be excluded when considering the mechanisms of bladder tumour induction in the male rat, this would not be expected to occur at low dose levels.

vii. The Committee concluded that it would be reasonable to adopt a threshold based risk assessment for 2-phenylphenol and its sodium salt.

2.38 A statement is appended at the end of this report.

Polycyclic Aromatic Hydrocarbons in air pollution: A discussion of approaches to risk assessment

2.39 The COC has recently evaluated published carcinogenicity data on dibenzo(a,l)pyrene (DB(a,l)P) and had agreed that this compound was between 10-100 times more potent than benzo(a)pyrene depending on the test system used (see section 3.1 of the COC Annual Report). Thus a paper had been drafted which considered the potential impact of DB(a,l)P and other high carcinogenic potency PAHs on the existing approaches to risk assessment of PAHs in air pollution.

2.40 Three approaches to carcinogenic risk assessment have been advocated: the use of Potency Equivalency Factors (PEFs) (equivalent to Toxicity Equivalent Factors, TEFs, for general toxicity); the complete mixture method; and use of B(a)P as a surrogate carcinogen for all PAHs. It was possible that the B(a)P surrogate approach which is currently used to monitor for compliance with UK air pollution standard, might not be appropriate if high potency PAHs were present in air pollution and if the concentrations of these compounds varied significantly when compared to B(a)P. A review of the possible approaches to risk assessment has been undertaken, taking into account available data on carcinogenicity, including a discussion of kinetics of PAH uptake via the lung and self-induction of PAH metabolism. COM advice was particularly sought on the proposals for use of DNA adducts or mutations as surrogate end-points for carcinogenic potency.

2.41 Members agreed that the majority of these compounds were activated *in-vivo* to diol-epoxides. It was possible to make informed predictions regarding the pathways of metabolism and reaction of diol-epoxides with DNA from the structure of PAHs. Members agreed that PAHs could be considered as members of a single group of genotoxic carcinogens whose mechanism of activation, formation of DNA adducts and metabolism were all broadly similar. There were some exceptions where PAHs were also metabolised by another minor route also leading to activation and DNA binding, for example the bis-diol-epoxide formed by dibenz(a,h)anthracene (DB(a,h)A). It was agreed that PAHs activated by routes other than via a diol-epoxide would need to be considered carefully. Members noted the carcinogenic process leading to PAH induced tumourigenicity was complex and included factors such as tumour promotion and cell proliferation which would also affect carcinogenic potency. PAHs also had other molecular targets (e.g. oestrogen receptors) which might potentially impact on the carcinogenic process in target tissues.

2.42 The Committee reviewed the available data regarding use of DNA adducts as a surrogate marker for PAH carcinogenic potency. There was evidence that DNA adducts may be an adequate endpoint for this group of genotoxic carcinogens from two research groups using different experimental approaches. Dermal application of PAHs to the skin of mice showed a good correlation for DNA adducts in skin and lung with applied skin dose. Studies using intraperitoneal administration of PAHs to A/J mice showed a good correlation between total DNA adducts (Time Integrated DNA Adduct Levels; TIDAL) in lung and administered PAH dose. Both research groups had documented a good correlation between DNA adducts and tumourigenicity. It was suggested that DNA adducts could serve as a pragmatic marker (it is argued for DNA adduct formation from reactive diol-epoxides of PAHs) that correlates with tumourigenicity. It was noted that there was also data from studies in A/J mice using administration of mixtures of PAHs that suggested that any departure from additivity was limited.

2.43 Members noted the evidence for a lack of correlation between PAH induced mutation frequency in transgenic mice given oral doses of PAHs and target organ for carcinogenicity. Members felt that it would be important to evaluate dose-response for PAH induced mutation frequency in cancer target tissues in a similar way to the DNA adduct data before conclusions regarding the utility of mutations as a surrogate marker for PAH induced carcinogenicity could be drawn.

2.44 The Committee considered the proposals for experiments to rank carcinogenic potency of PAHs. It was agreed that intratracheal instillation of small doses in rats, with measurement of total DNA adducts in lung, would be appropriate for inhalation of PAHs. Members also considered that measurement of DNA adducts in skin following topical treatment would also yield a similar ranking of PAHs. It was suggested that it might be possible to undertake DNA adduct measurements and determination of PAH induced mutation frequency in the same animals, although it was acknowledged this would represent a very large research project and that it was not a practical approach for ranking PAHs. Members briefly considered whether *in-vitro* approaches could yield relevant data but agreed that differences in metabolism between *in-vitro* cultures and whole animals would limit the value of *in-vitro* studies.

2.45 The Committee agreed the proposals in the draft paper. It was noted that the paper would be provided to COC for comment and then submitted to a peer review journal for publication. Members asked for detailed information regarding air levels of highly potent carcinogenic PAHs when available.

Review of Committee Procedures

Horizon Scanning

2.46 The Code of practice published by the Office for Science and Technology (OST) on Guidance for Scientific Advisory Committees (http://www.ost.gov.uk/policy/advice/copsac/) encouraged Committees to develop strategies for the early identification of issues. This included i) "new issues", eg previously unidentified potential chemical mutagens/carcinogens which may represent a risk to public health and where advice is required, and ii) "new or unexpected developments in science.."

2.47 The COM considered a number of topics for potential future consideration. Members considered that future consideration of the potential for formation of mutagens during food processing/cooking (particularly frying) would be valuable. On a separate subject it was suggested that the significance of mitochondrial mutation and the possible genotoxicity of phytoestrogens could be possible topics for future COM consideration.

2.48 Regarding new methods of evaluation, members agreed there could be a review of changes in gene expression induced by chemical mutagens. Also further consideration could be given to the appropriate weight of evidence provided by negative *in-vivo* results in two tissues (as suggested by the COM guidance) when discounting positive results *in-vitro*.

2.49 It was agreed that members could raise new issues or developments in chemical induced mutagenicity at any time and that the committee should formally discuss issues relating to horizon scanning at least once per year.

Test Strategies and Evaluation

In-vitro Mammalian cell mutation assays

2.50 The COM guidance on a strategy for testing chemicals for mutagenicity (http://www.doh.gov.uk/com/guidance.pdf) published in 2000 includes the use of a mammalian cell mutation assay as the third of three *in-vitro* screening tests recommended in Stage 1 of the testing strategy. The COM concluded that the mouse lymphoma assay using L5178Y cells was the preferred test. The Committee was asked to review this conclusion in the light of an expert opinion submitted by one pesticide applicant which focused on the use of mammalian cell mutation assays to screen for gene mutagens.

2.51 The Committee agreed that that there was no evidence that the mouse lymphoma assay had any higher inherent sensitivity for the detection of point mutations than other assays in mammalian cell such as the *hprt* assay in CHO cells, provided that adequate number of cells had been used in each assay. This presented, on statistical grounds, some practical difficulty with regard to the use of surface cultures of CHO cells, rather than with suspension cultures of mouse lymphoma cells. The number of cells needed is governed by the spontaneous mutation frequency of the cell system used. This is covered in some detail in the OECD test guideline in which it is stated that, as a general guide, the cell number should be at least 10 times the inverse of the spontaneous mutation frequency. The Committee endorsed this and indicated that, allowing for cytotoxicity, it would usually be necessary to use 10^7 cells or more in the *hprt* CHO cell assay.

2.52 Members also confirmed that another reason why the mouse lymphoma assay was preferred as part of Stage 1 in the COM strategy (*in-vitro* screening for potential mutagenicity) was that it detected a wider range of genetic endpoints, for example, chromosome deletions as well as gene mutations. This gives the mouse lymphoma assay added value as the second *in-vitro* mammalian cell assay in addition to cytogenetics in mammalian cells. Members accepted that the *hprt* CHO cell assay might have a role in mechanistic studies where it was an advantage to specifically measure gene mutation.

Strategy for investigating germ cell mutagens

2.53 The COM guidance on a strategy for testing chemicals for mutagenicity (http://www.doh.gov.uk/com/guidance.pdf) published in 2000 recommended that in the initial stages of investigating whether a chemical has in-vivo activity there was no need to screen for germ cell mutagens. This was because all germ cell mutagens had been shown to produce positive results in bone marrow assays, and there was no evidence for any germ cell specific mutagens. It was recognised that the reverse was not true, and not all somatic cell mutagens were germ cell mutagens.

2.54 The Committee was made aware of a recent published paper (Witt Kl et al, Environmental and Molecular Mutagenesis, volume 41, 111-120, 2003) where evidence had been reported that N-hydroxymethylacrylamide produced positive results in the mouse dominant lethal assay for germ cell mutagenicity, but gave negative results in the micronucleus test in both bone marrow and peripheral erythrocytes. This chemical was negative in a range of *in vivo* bone marrow assays (using acute, sub-acute and subchronic dosing regimes). Positive results, however, were obtained when an especially sensitive method involving flow cytometry was used. Positive results were obtained in the dominant lethal assay in mice after one week and 8 weeks dosing. At the latter time point negative results were obtained in a micronucleus test using peripheral blood. A single dose dominant lethal assay also gave negative results.

2.55 The Committee was asked whether the data warranted any modification to the COM strategy of testing as outlined in the 2000 guidelines.

2.56 Members agreed that there were a number of possible reasons why this new study on N-hydroxymethylacrylamide detected a positive mutagenic result in germ cells but not in the bone marrow. In relation to the analysis for micronuclei in peripheral erythrocytes, it was noted that the spleen could have removed damaged cells. Members felt that accumulation in the germ cells (positive results had only been detected after repeated administration) could also be significant in producing positive effects on germ cell DNA. Additionally, a certain stage of a developing germ cell could be more susceptible to N-hydroxymethylacrylamide genetic damage, and germ cells might stay in a susceptible stage for a longer period of time than the faster dividing bone marrow cells. Members also felt that some of the bone marrow micronucleus tests involving repeated administration were equivocal, rather than negative.

2.57 The Committee noted that if the COM guidance was followed, a second *in vivo* assay in a different somatic tissue was needed. After consideration of all the available information, members agreed that N-hydroxymethylacrylamide was not a germ cell specific mutagen.

2.58 Members considered that this one study by itself did not warrant any change to the COM strategy as outlined in the 2000 guidelines, but that this issue should be kept under review in case other similar examples were found in the future.

Significance of *in-vivo* mutagenicity at high doses

2.59 The COM has previously agreed that it is prudent to assume that there is no threshold for mutagenicity unless appropriate mechanistic data can be provided to identify a threshold related mechanism. *In-vivo* studies in the bone marrow provide key data in identifying compounds as *in-vivo* mutagens. In some cases the only data available to indicate such *in-vivo* activity is from mutagenicity studies using excessively high doses (by current guidelines) associated with severe toxicity/lethality, and there is no evidence from carcinogenicity bioassays to suggest that a compound is a genotoxic carcinogen. Due to the importance of these data in risk assessment, where positive results normally lead to a 'non-threshold' approach being adopted by regulatory agencies, it is important that the observed effects are not secondary to toxicity. The Committee considered a paper drafted by the Secretariat at its October 2002 meeting which presented "in-confidence" data from submissions received by regulatory authorities. These were to be used as examples in order to draft generic guidance which could be published.

2.60 The COM undertook further consideration of this subject at its February and May meetings in 2003. A number of potential mechanisms with a threshold of action (eg hypothermia and erythropoiesis) had been identified in the discussion papers as well as severe toxic effects. The COM placed considerable emphasis on considering all the available data on a case-by-case basis, including results from adequately conducted carcinogenicity bioassays. Due to the close association between mutagenicity and carcinogenicity, two members of the COC attended the February 2003 meeting for the discussion of this item to provide advice on the weight of evidence to be given to carcinogenicity studies.

2.61 Members of COM and COC agreed that consideration needed to be given to the whole data package, including chemical reactivity, toxicokinetics and whether the carcinogenicity data of a compound is consistent with the profile of a genotoxic carcinogen. Chemicals needed to be considered on a case-by-case basis. A negative carcinogenicity bioassay on its own could not discount a 'high dose' positive *in-vivo* mutagenicity result. Conversely a positive result in a carcinogenicity bioassay may not necessarily mean that a chemical was a mutagen, as there were 'non-genotoxic' mechanisms that could induce cancer.

2.62 The committee had previously agreed that *in-vivo* mutagenicity data from dose levels associated with lethality (ie clearly above the top dose recommended by OECD guidelines) could not be interpreted with any certainty due to the confounding effects of toxicity. If adequate information were not available from lower doses then further testing would be required with more appropriate lower doses. Members considered that mechanistic data were valuable in discounting positive mutagenicity results seen at toxic doses i.e. to demonstrate the response was secondary to toxicity.

2.63 It was agreed that adequately conducted carcinogenicity bioassays, if available, provided important information to help evaluate the significance of positive results from mutagenicity studies at 'high' doses. Adequate negative carcinogenicity bioassays, preferably in two species, could be used to help discount evidence of mutagenicity at lethal doses, and where there was some concern at the relevance of the *in-vivo* mutagenicity data. Members discussed the need to take into account the differences in the maximum dose levels in these studies. It was noted that consideration of the differences in maximum dose levels would be of limited value in view of the differences in duration of dosing (usually 1-2 days compared to 2 years). However consideration should always be given to available toxicokinetic data.

2.64 Overall the COM considered that a weight of evidence approach using the whole data package on a case-by-case basis had to be used when considering whether a 'high' dose positive *in-vivo* result could be discounted in somatic cells due to a biologically plausible mechanism.

2.65 The Committee agreed that the following points need to be considered when deciding whether positive *in-vivo* bone marrow clastogenicity data can be discounted with regard to considering a compound as an *in-vivo* mutagen.

i. The totality of the relevant data relating to the specific compound in question needs to be considered, including any information on its chemical reactivity, toxicokinetics, its mutagenic profile (*in-vitro* and *in-vivo*), and also any available data from carcinogenicity bioassays to enable an assessment to be made as to whether the compound appears to be a genotoxic carcinogen.

ii. Positive bone marrow *in-vivo* mutagenicity data from dose levels that are associated with high levels of toxicity or lethality (ie above the maximum dose level recommended in the current OECD guidelines) cannot be interpreted with any certainty because of the confounding effects of toxicity. If adequate information is not available at lower, non-lethal dose levels then retesting is necessary.

iii. It is also important to consider whether there is any evidence for a plausible mechanism to support the contention that the observed positive results in the bone marrow assays at high dose levels may be secondary to other non-genotoxic effects rather than being a mutagenic effect of the compound (or its metabolites). Examples of such mechanisms include (but are not limited to) hypothermia, hyperthermia, and erythropoiesis.

iv. Data from adequately conducted carcinogenicity bioassays, if available, provide important information to help in the assessment of the significance of such high dose bone marrow mutagenicity results. Such data may indicate that the carcinogenic profile of the chemical is consistent with either a genotoxic or a non-genotoxic mechanism, or that the compound is not carcinogenic.

v. Only generic advice can be given in this area and it should be emphasised that each compound needs to be considered in a case-by-case basis. However consideration of the above factors, with expert judgement, may provide sufficient evidence to conclude that the positive *in-vivo* bone marrow data at high dose levels was due to a non-genotoxic effect. A threshold based risk assessment may thus be appropriate.

2.66 A statement is appended at the end of this report.

Ongoing reviews

Chromium picolinate

2.67 Chromium picolinate is a widely available food supplement. The adverse effects of chromium were recently reviewed by the Expert Group on Vitamins and Minerals (EVM). The reports of genotoxicity associated with chromium picolinate were noted and chromium picolinate was excluded from their recommendations for an upper safe level. Following the publication of the EVM report, the Food Standards Agency advised that consumers should use other forms of trivalent chromium supplements until more detailed advice is available. The COM undertook a preliminary discussion of available mutagenicity data at its October 2003 meeting. A request for additional information has been forwarded to the manufacturers who supply chromium picolinate to the food supplement industry.

Statements agreed by COM during 2003

1,3-Dichloropropanol

Flunixin, meglumine and Flunixin-meglumine

Joint statement on review of malathion

2-Phenylphenol

Significance of in-vivo mutagenicity at high doses

Statement on the mutagenicity of 1,3-dichloropropan-2-ol

Introduction

1. 1,3-dichloropropan-2-ol (1,3-DCP) is a member of a group of chemicals called chloropropanols, which includes 3-monochloropropane-1,2-diol (3-MCPD) and 2,3-dichloropropan-1-ol (2,3-DCP). 1,3-DCP and 3-MCPD can be present as process contaminants in some foodstuffs and as contaminants of polyamine flocculants in drinking water. 1,3-DCP was last considered by the COM and the COC in 2001. COM concluded that it would be prudent to consider 1,3-DCP as potentially genotoxic *in vivo* but agreed that it should be tested for genotoxicity *in vivo* using the approach set out in the COM guidelines.

2. 1,3-DCP has been considered by the COC, originally in 1991 and more recently in 2001. The COC noted that in a carcinogenicity study undertaken by Hercules Inc[1]. 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 concluded that "it was prudent to assume 1,3-DCP is a genotoxic carcinogen and that exposure to 1,3-DCP should be reduced to as low as technologically feasible"[2].

COM evaluation: 2001

3. 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 1,3-DCP had been found to be mutagenic to *Salmonella typhimurium* strains TA1535 and or TA 100[3-10]. Studies with mammalian cells have produced increased frequencies of sister chromatid exchanges and chromosome aberrations[11,12]. A positive result has been obtained in a mouse lymphoma assay[13,14]. 1,3-DCP was negative in the wing spot test in *Drosophila melanogaster* (a somatic mutation and recombination test)[15]. No *in-vivo* mammalian studies had been carried out.

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

COM evaluation: 2003

5. The Committee considered two new *in-vivo* genotoxicity studies at its May meeting. These comprised a rat bone-marrow micronucleus test and a rat liver unscheduled DNA synthesis (UDS) assay, both of which are widely used to assess genotoxicity *in vivo*.

Rat in-vivo bone-marrow micronucleus test [16]

6. The assay followed the current OECD guideline (No. 474). The highest dose used in the study was selected so that it would produce some signs of toxicity, but not severe effects, based on the results of a range-finding study. In the main study, 1,3-DCP was administered once daily for two consecutive days to groups of six male Han Wistar rats at doses of 25, 50 and 100 mg/kg. Bone marrow was harvested 24 hours after the final dose. A single sex study was considered adequate because no substantial difference in toxicity was observed between males and females in the range-finder.

7. Weight loss (25 mg/kg), piloerection and weight loss (50 mg/kg) and piloerection, weight loss and lethargy (100 mg/kg) were observed in treated animals, indicating that the test was conducted at adequate doses. The Committee noted that the ratios of polychromatic to normochromatic erythrocytes (PCE/NCE) were variable amongst individual animals of the control group. However, the mean value was within the range of historical control group mean data. Rats treated with 1,3-DCP at all doses exhibited group mean PCE/NCE ratios that were similar to those of the control group and within the normal range. Therefore toxicity to the bone marrow was not demonstrated, although 1,3-DCP clearly caused systemic toxicity.

8. There were no statistically significant increases in micronucleus frequency at any dose of 1,3-DCP. The positive control agent, cyclophosphamide, produced a clear increase in micronuclei.

Rat liver in-vivo UDS assay[17]

9. The UDS assay protocol conformed to the current OECD guideline (No. 486). Based on the results of the range-finder for the micronucleus study, the study was conducted in male rats and the highest dose was 100 mg/kg. Single doses of 40 and 100 mg/kg were administered to groups of four male Han Wistar rats. Hepatocytes were isolated for analysis for UDS by the autoradiographic technique after 12-14 hours in the first study (3 rats per dose group) and at 2-4 hours in the second study (3 rats per dose group).

10. In the 2-4 hour experiment, piloerection was observed in all 1,3-DCP treated rats and lethargy was observed at 100 mg/kg. There were no clinical signs following dosing in the 12-14 hour experiment. There was no evidence for any increase in UDS at either dose level or time point. The positive control compounds 2-AAF and DMN both gave clear positive results.

COM discussion

11. Members agreed that the two new studies met the previously stated requirement that 1,3-DCP should be tested for genotoxicity *in-vivo* using the approach set out in the COM guidelines. The studies were adequately conducted and gave clear negative results, and therefore Members considered that these studies provided evidence that 1,3-DCP was not an *in-vivo* mutagen. Members then gave consideration as to possible mechanisms whereby mutagenic activity observed *in vitro* was not expressed *in vivo*.

12. The role of metabolism in the *in-vitro* mutagenicity is unclear. Most bacterial studies have shown mutagenicity both in the presence and absence of metabolic activation. Two studies indicated that metabolism increased the mutagenicity in TA100 and/or TA1535[7,9], whereas one reported a decrease in TA100 revertants in the presence of metabolic activation[8]. Activation was found to result in reduced frequency of sister chromatid exchanges in V79 cells[11].

13. It has been postulated that metabolism to epichlorhydrin could be responsible for the mutagenicity of 1,3-DCP[18]. There is some evidence that bacteria can convert 1,3-DCP to epichlorhydrin[19], which could account for the direct activity seen in the bacterial mutagenicity tests, although no data are available with respect to *Salmonella typhimurium*. It has been suggested that epichlorhydrin may be formed non-enzymically during the pre-incubation stage of the SOS chromotest assay with *Escherichia coli* stain GC4798[6].

14. An alternative active metabolite is 1,3-dichloroacetone. It has been postulated that this may be formed from 1,3-DCP by action of alcohol dehydrogenase[20] or CYP2E1[21]. Glutathione conjugation is believed to be a detoxification pathway since 1,3-DCP depletes glutathione both *in vitro*22 and *in vivo*[23], and glutathione depletion has been shown to potentiate the toxicity of 1,3-DCP to rat hepatocytes[22].

15. One known route of 1,3-DCP metabolism in rats involves hydroxylation to 3-monochloropropane-1,2-diol (3-MCPD), accounting for 1 to 14% of a 10 mg subcutaneous dose in rats[24]. The COM has concluded that 3-MCPD can be regarded as having no significant genotoxic potential *in vivo*[25].

16. The COM considered that the metabolism of 1,3-DCP had not been fully elucidated. Metabolic activation *in vivo* to two active metabolites had been postulated. In both cases the compound formed would be expected to be rapidly de-activated *in vivo* by glutathione. In one case deactivation would also occur by the action of epoxide hydrolase. Thus once formed, the active metabolite is rapidly detoxified and hence 1,3-DCP would be unlikely to have significant activity *in vivo*. This is supported by the negative results obtained in the two new *in-vivo* mutagenicity assays.

Conclusions

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

18. The Committee noted the uncertainties with regard to routes of metabolic activation of 1,3-DCP and agreed that the two new mutagenicity studies supported the view that reactive metabolites, if formed, did not produce genotoxicity *in vivo* in the tissues assessed.

19. The Committee concluded that 1,3-DCP can be regarded as having no significant genotoxic potential *in vivo*.

COM/03/S4 October 2003

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Statement on flunixin, meglumine and flunixin meglumine

Background to COM review

1. Flunixin in the form of the meglumine salt is a non-steroidal anti-inflammatory (NSAID) drug and a non-narcotic analgesic drug with antipyretic activities. It is used in veterinary medicine (including food-producing animals) but it is not used in human medicine. Flunixin-meglumine dissociates *in-vivo* to Flunixin and meglumine. In 1997, the European Medicine Evaluation Agency's (EMEA) Committee on Veterinary Medicinal Products (CVMP) considered the safety of Flunixin-meglumine as part of its review of old veterinary medicinal products, operating under Council Regulation 2377/90, which covers the marketing authorisation of pharmacologically active substances used in veterinary medicines.

2. With regard to meglumine, the CVMP considered that it could be regarded as an excipient that had the purpose of increasing the solubility of flunixin. The CVMP concluded that "meglumine used as an excipient at up to 1.5 mg/kg bw does not fall within the scope of Council Regulation (EEC) number 2377/90"[1].

3. The Food Standards Agency is concerned about the possible mutagenicity of Flunixin-meglumine, meglumine and Flunixin. The Food Standards Agency considered that it would be useful to seek the opinion of the COM.

Assessment of Mutagenicity data

4. As a general comment Members noted that relatively high levels of micronuclei had been documented in all of the *in-vivo* bone-marrow micronuclei studies submitted on these chemicals which had all been undertaken in the early 1980s by one particular contract laboratory.

Flunixin

5. The available mutagenicity data were relatively old and of limited value. Negative results were reported in *Salmonella typhimurium*[2] and in an *in-vivo* micronucleus assay in BS1 mice using intraperitoneal administration of two doses separated by 24 hours (200 mg/kg bw reduced to 150 mg/kg bw) and harvested 6 hours after the last dose[3]. The DNA damage assay in *Escherichia coli* p3478 repair deficient strain showed an increase in DNA damage at one interim concentration, but this finding was not seen in a repeat test[4]. A positive result was claimed in a mitotic gene conversion assay in *Saccharomyces cerevisiae*[5].

6. Overall Members agreed that there was limited evidence that Flunixin was mutagenic *in-vitro* but there was no evidence to suggest that Flunixin had mutagenic potential *in-vivo*. Members felt the *in-vitro* mutagenicity data on this compound were inadequate and considered this should be raised with the CVMP.

Meglumine

7. Negative results were reported in an old plate incorporation assay in a limited number of *Salmonella typhimurium* strains[6]. A positive result had been reported in a bone-marrow micronucleus assay in BSI mice using intraperitoneal administration of 500 or 1000 mg/kg bw suspended in 0.25% methylcellulose (two doses given 24 hours apart and harvest 6 hours after last dose)[7]. A repeat test using 1000 mg/kg bw was also positive. Members queried how the repeat test could have been undertaken on the same day as the initial test. It was noted that negative results were obtained in a separate *in-vivo* micronucleus assay using intraperitoneal administration of two doses given 24 hours apart at up to 600 mg/kg bw of meglumine to CD1 mice[8]. Members noted that a clear positive control response had only been seen in males and not females in this latter assay and that the sampling regimen (24 and 48 hours after last dose) differed from the study in BS1 mice.

8. A number of in-vivo bone marrow micronucleus assays were recently undertaken for the Committee using a variety of single/double intraperitoneal dosing regimens at dose levels of 500 mg/kg bw and 1000 mg/kg bw and sampling for micronuclei at 6 or 24 h after the last dose[9]. There was some evidence for an effect 6 h after the second dose in one study using Alpk:ApfCD-1 mice, but no definite conclusions could be reached as the result was not repeatable, there was considerable individual animal variation and the observed effect could have been complicated by toxicity.

9. The Committee agreed that there was a need to further evaluate the potential mutagenicity of meglumine. This is discussed in the COM evaluation section below.

Flunixin meglumine

10. Members agreed that the mutagenicity data on Flunixin-meglumine (ie the mixture used in veterinary medicines) was difficult to assess. Negative results were reported in an old plate incorporation assay using a limited number of *Salmonella typhimurium* strains[10]. Negative results were also reported in an *in-vitro* UDS assay using rat hepatocytes[11]. Positive results were documented in a mitotic gene conversion assay in *Saccharomyces cerevisiae*[12,13]. There was also evidence for DNA damage in two separate tests in *Escherichia coli* p3478 repair deficient strain but not in a third test[14,15,16]. However there was limited evidence for a mutagenic effect in three separate mouse lymphoma assays[17,18,19] and evidence for an equivocal response in a cytogenetics test in Chinese Hamster Ovary cells[20].

11. Overall the Committee agreed that there was limited evidence that Flunixin-meglumine was an *in-vitro* mutagen. Members noted that negative results had been documented in an *in-vivo* micronucleus assay in BS1mice using intraperitoneal administration of two doses (up to 80 mg/kg bw) separated by 24 h and sampling 6 h after the last dose. Members were concerned with regard to the adequacy of the selection of dose levels[21] In addition the inconclusive *in-vivo* data on meglumine suggest that a definite conclusion regarding Flunixin-meglumine cannot be reached.

COM Evaluation

12. The Committee noted the poor quality of mutagenicity data on Flunixin, meglumine and Flunixin-meglumine. Members noted that while negative results obtained in carcinogenicity bioassays with Flunixin-meglumine in the rat and the mouse provided some reassurance with regard to Flunixin, they were not informative with regard to meglumine.

13. The Committee was aware of several *in-vivo* bone-marrow micronucleus assays using meglumine but noted that the results were inconsistent. Thus there was evidence in two separate studies (in BS1 and Alpk:ApfCD-1 mice) for a mutagenic effect following intraperitoneal dosing of 1000 mg/kg bw following a treatment regime of two doses of meglumine (24 hour apart) with a sampling time of 6 hours after the second dose (ie 30 hours after the first dose)[7,9]. One of these studies had been undertaken by a Committee member. However this result was not repeated in two recent bone marrow micronucleus assays in mice which had been conducted for the Committee which attempted to repeat these findings using an equivalent treatment regime[9].

14. The Committee considered a number of options for further investigating the mutagenicity of meglumine. The Committee agreed the most appropriate way forward would be to request additional *in-vitro* assays with meglumine to properly assess whether meglumine had mutagenic potential *in-vitro* before considering the need for any further *in-vivo* mutagenicity studies with meglumine. Thus data were required from an *in-vitro* chromosomal aberration assay and a mouse lymphoma assay, which together with the data currently available for a test in bacteria using Salmonella, would complete the in-vitro package to modern standards given in the COM guidance. In this case the weak and inconsistent induction of micronuclei in mice after two treatments with meglumine 24 hours apart with sampling 6 hours after the last treatment would be disregarded as strain-or-system specific effects of no genotoxic relevance if these two additional *in-vitro* tests were negative.

15. Thus negative data were available for a test in bacteria using Salmonella, but data were required from an *in-vitro* chromosomal aberration assay in mammalian cells and a mouse lymphoma assay to complete the *in-vitro* package to modern standards given in the COM Guidance[22]. If these were negative, there would be no concerns regarding meglumine.

Consideration of presentation by Schering-Plough on mutagenicity testing strategy (6 February 2003)

16. Schering-Plough (referred to as the data holder in this statement) suggested that two further *in-vivo* mutagenicity studies be carried out with meglumine, namely a bone-marrow chromosome aberration assay in rats and a bone-marrow micronucleus test in rats.

17. Members considered the presentation and concluded that provision of two adequately conducted in-vitro tests as outlined in COM Guidance with negative results would obviate the need for any further *in-vivo* testing. The purpose of these assays would be initial screening of meglumine and its metabolites *in-vitro*. The data holder accepted this proposal.

18. COC Members considered some additional data[23] provided by the data holder on the conduct of the carcinogenicity studies in the rat and mouse using Flunixin-meglumine and drew conclusions with regard to the adequacy of these studies for the assessment of Flunixin-meglumine and meglumine (see paragraph 12 above). The COM noted comments from the data holder on the rapid dissociation of Flunixin-meglumine into Flunixin and meglumine and the suggested fast metabolism of meglumine. The COM agreed that it would be useful for the data holder to consider undertaking appropriate ADME studies with meglumine using radiolabelled material.

COM conclusion and recommendations

19. The COM concluded:

i. The mutagenicity data on Flunixin, Flunixin-meglumine and meglumine are relatively old and the studies have not been conducted to contemporary standards.

ii. It is therefore difficult to draw any definite conclusions on the mutagenicity of these chemicals. A number of prudent conclusions were agreed:

a. For Flunixin (a non-steroidal anti inflammatory veterinary medicine) there was limited evidence that Flunixin was mutagenic *in-vitro* but there was no evidence to suggest that flunixin had mutagenic potential *in-vivo*. Members felt the *in-vitro* mutagenicity data on this compound were inadequate and considered this should be raised with the CVMP.

b. For Flunixin-meglumine, (Flunixin in the form of the meglumine salt) there is limited evidence for a mutagenic effect *in-vitro*. In addition the inconclusive *in-vivo* data on meglumine suggest that a definite conclusion regarding flunixin-meglumine cannot be reached.

c. For meglumine, the available *in-vivo* mutagenicity data are inconsistent with some positive and negative results. It is **recommended** that additional *in-vitro* mutagenicity tests conducted to modern standards are undertaken with meglumine to assess whether meglumine has any mutagenic potential using the approach recommended in the COM Guidance. Negative results are available for a gene mutation assay in *Salmonella*. Therefore these additional tests should comprise:

i. An *in-vitro* chromosomal aberration test in mammalian cells.

ii. An *in-vitro* mouse lymphoma assay.

COM/03/S4 March 2003

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5. Mitotic gene conversion; Sachromyces cerevisiae – flunixin. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref A15345.

6. Ames Test – S typhimurium; N-methyl-D-glucamine. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref D15346.

7. Micronucleus-N-methyl-D-glucamine. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref 15347.

8. Mouse micronucleus – meglumine. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref D15348.

9. Anon. Activity of methylglucamine in the mouse bone marrow micronucleus assay. Report submitted to April 2002 COM meeting.

10. Ames Test – S typhimurium; flunixin meglumine. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref 15335.

11. Assessment of genotoxicity in an unscheduled DNA synthesis assay using adult rat hepatocyte primary cultures. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref A18272.

12. Mitotic gene conversion; Sachromyces cerevisiae – flunixin meglumine. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref A15340.

13. Mitotic gene conversion; saccromyces cerevisiae – flunixin meglumine. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref A15341.

14. DNA polymerase deficient assay; Escherischia coli; flunixin meglumine. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. D15338.

15. Evaluation of flunxin meglumine in the bacterial DNA repair test. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. D15339.

16. SCH 14714 NMG (BanamineR) DNA repair test with Escherichia coli. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref A 18270.

17. Evaluation of SCH 14714 NMG for mutagenic potential using L51787 Tk +/- cells. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref D14318.

18. Mouse lymphoma – flunixin meglumine. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref D 15336.

19. Mouse lymphoma mutation assay – Banamine. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref A18271.

20. Chromosome aberration-Chinese hamster. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref A18463.

21. Micronucleus-flunixin meglumine. Flunixin Meglumine MRL Submission Dossier Study Reports, Volume 13. Ref D 15337.

22. Committee on Mutagenicity (2000). Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment. Guidance on a Strategy for Testing of Chemicals for Mutagenicity.

23. Schering-Plough (2003). Additional carcinogenicity data provided to COM in confidence at meeting of 6 February 2003.

Joint statement on review of malathion

Background

1. Malathion is an organophosphorous insecticide. It has been marketed in the UK for use in agriculture and horticulture since 1956. There were three products with approvals for use in agriculture and horticulture, home garden and use in pigeon lofts at the time when this review was initiated in January 2002. A number of products containing malathion are also licensed as human medicines for use in the control of head lice.

2. The Advisory Committee on Pesticides is reviewing the available toxicological information on malathion as part of its ongoing review of organophosphorous compounds. The ACP asked for advice from COM and COC on mutagenicity and carcinogenicity at its 289th meeting on 17 January 2002. There are inconsistent results in mutagenicity studies (both *in-vitro* and *in-vivo*) and there is evidence for the mutagenic activity of some impurities which may be present in some batches of technical malathion. There is also some limited evidence for tumourigenicity in rats (in particular the occurrence of benign nasal tumours in animals given high oral doses of technical grade malathion in the diet).

3. The Chairs of COM and COC agreed that a joint statement was required in view of the need for a full review of all mutagenicity and carcinogenicity data.

4. The COM undertook an initial consideration of the in confidence mutagenicity data provided by the pesticide data holder[1-5] and available published information provided by the data holder[6-55] at its 25 April 2002 meeting. A number of additional published papers on malathion[56-61] and impurities present in technical grade malathion were also considered at this meeting[62-65]. The COC reviewed the available carcinogenicity data on malathion which included in confidence reports (provided by the pesticide data holder regarding two studies in rats one in mice)[66-68] and published reports of long-term bioassays in rats[69,70] and mice[69] at its 27 June 2002 meeting. Three long-term bioassays using malaoxon (the principle metabolite of malathion and also present in technical grade malathion as an impurity) were available which included two in rats[71,72] and one in mice[71]. In addition the Committee also considered in confidence reports of Peer Reviews of the histology slides from the 1993-96 malathion bioassay in F344 rats[73,74], the 1992-94 bioassay in B6C3F1 mice[75] and some additional supplemental information for the 1992-1994 bioassay in B6C3F1 mice[76] and the 1993-1996 bioassay of malaoxon in F344 rats[77] provided by the Pesticide Data holder. A number of additional follow up reports from the contract laboratory concerning the 1993-96 bioassay of malathion in F344 rats were also reviewed[78-81]. In addition the pesticide data holder submitted a response to questions from COC secretariat which provided an overall summary of the histology of the nasal tissue in animals with tumours and additional evaluation of the historical control data on nasal tumours in F344 rats and possible mechanisms for nasal tumours induced in F344 rats fed high doses of technical grade malathion[82]. A published Peer Review of a number of published carcinogenicity bioassays was also available[83]. The COC also considered expert reports from the EPA[84] and a Scientific Advisory Panel[85] established by EPA to review malathion. At its meeting of 10 October 2002 the COM considered some additional information provided in confidence by the pesticide data holder (a report of one additional in-vivo study[86] and information on the potential for variation in impurities between different sources of malathion[87]) together with number of published studies not previously reviewed[88-92]. The in confidence reports provided by the pesticides data holder contained appropriate Good Laboratory Practice and Quality Assurance statements.

Introduction to review

5. The Committees had access to sections of the draft risk assessment prepared by the Pesticides Safety Directorate (PSD) for the ACP. This include evaluations of the absorption, distribution, metabolism and excretion and toxicology data on malathion. Information on the manufacturing process used by the pesticide data holder and the impurities present in commercial technical material was presented.

6. The Committees noted that malathion is an organophosphorothiolate compound and required activation to malaoxon which inhibits cholinesterases in mammals. It is rapidly absorbed orally and is rapidly metabolised and excreted (mainly in the urine) as metabolites. Dermal absorption studies in human volunteers suggested a moderate absorption (6-15%) depending on formulation and malathion concentration. The available information from a wide range of toxicity studies (including acute, sub acute, sub chronic and chronic studies and reproduction studies in a range of species) demonstrated that inhibition of cholinesterase is the most sensitive toxicological effect in all available studies. The toxicity of malathion can be influenced by the presence of impurities in the technical material. Thus, for example, data presented in the PSD draft risk assessment showed that isomalathion and OOS-trimethyl phosphorothioate can inhibit the detoxification (via carboxyesterases and glutathione) of malaoxon resulting in a potentiation of cholinesterase effects of this compound. It was notable that the acute toxicity of technical grade malathion differed between two manufacturing sources. In answer to questions raised by the COM, the pesticide data holder reported that the levels of impurities would vary according to quality of raw materials, reaction parameters and purification steps. In addition it was also reported that the level of one impurity (isomalathion) could increase during storage of technical grade malathion. Thus the possibility that impurities may have mutagenic potential needed to be considered. The Committee agreed that the specification of the test materials used in mutagenicity studies undertaken by the pesticide data holder had been adequately demonstrated. The evidence provided by the Pesticide Data Holder suggested that the impurities present in technical grade malathion from other manufacturers would be qualitatively similar but it was not possible to draw any conclusions on the levels of the individual impurities in the test materials used in published mutagenicity studies.

COM review of mutagenicity

In-vitro data on malathion

7. It was agreed that the primary objective of the COM review was to consider the mutagenic potential of commercially supplied (e.g. technical grade) malathion. Technical grade malathion used in many of the reported mutagenicity tests had a purity of between 93-96%, whilst in a small number of published studiesthe authors reported using highly purified malathion ($\geqslant$ 99%)[8,38,39,51]. The COM agreed that malathion was a DNA methylating agent[56]. N7-Methyl guanine was the main identified adduct but others could not be excluded. There were also data to show that iso-malathion (an impurity formed during manufacture and storage) and OOO-trimethylphosphorothioate (a process impurity found in some batches of technical grade malathion) could alkylate nitro-benzyl-pyridine *in-vitro*[62]. The Committees noted the proposed metabolism pathway in the draft risk assessment document prepared by the Pesticides Safety Directorate (PSD) and agreed that there were a number of malathion metabolites identified in metabolism studies, which could theoretically methylate DNA.

8. The Committee agreed that there was no evidence for mutagenicity of technical grade malathion in bacteria. The results in a number of tests in *Salmonella typhimurium* and *Escherichia coli* were negative[1,14,21,24,30,31]. The significance of enhanced SOS DNA repair activity in *Escherichia coli* was unclear[50]. The COM noted that only brief details of the studies in yeast were available, [21,31] but considered that further evaluation of the data would be of limited value in view of the clear evidence for in vitro mutagenicity in mammalian cells (see next para).

9. Regarding *in-vitro* assays in mammalian cells, the COM agreed that a cytogenetics assay with technical grade malathion conducted by the pesticide approval holder, was positive in the presence and absence of endogenous metabolic activation at the high dose (which was moderately cytotoxic)[4]. The Committee reviewed the available published literature and agreed that details of purity of malathion used was absent in several studies. However, the COM agreed that positive results had been obtained in published studies in human lymphocytes, where malathion was of technical grade or of higher purity (≥ 99%)[23,27,51]. Activity was reported both in the presence and absence of exogenous metabolic activation. Additionally, technical grade[23,27] and purified malathion (≥ 99% pure)[38,39] had been shown to induce Sister Chromatid Exchanges in mammalian cells (CHO cells) and human lymphocytes and fibroblasts. The COM also noted that evidence for a gene mutation in the *hprt* locus in human lymphocytes had been published[41]. The authors of this study noted that positive results had also been reported for different batches of technical grade malathion, which had contained slightly different levels of impurities. In a subsequent publication, these authors had provided limited information to suggest that malathion might induce specific mutations in the hprt gene in human lymphocytes. There was evidence for an increase in mutations at G:C base pairs and at GG dinucleotides in *hprt* gene in malathion treated cells compared to unexposed cells[89]. The Committee agreed that the mouse lymphoma assay, recently conducted by the pesticide data holder using technical grade material, had also given positive results in the presence and absence of exogenous metabolic activation after a short 4-hour exposure period[3]. However, a second trial using a 24-hour exposure period was negative (COM Members commented that the control mutation frequency in this study was relatively high and this might mask a weak positive response).

10. The COM agreed that the UDS assay in isolated hepatocytes with technical grade material, which had been submitted by the pesticide data holder, was negative[2]. The Committee noted that although the top dose level (0.16 μl/ml) had produced evidence of cytotoxicity, the dose might have been too low to detect any DNA damage induced by impurities rather than by malathion itself. The Committee noted that a recent COMET assay with malathion (98% pure) and using human peripheral blood lymphocytes had yielded negative results[12]. However, no exogenous metabolic activation had been used and the exposure period (1hour) used in this study was shorter than expected for an adequate regulatory study (ca 3 or 24-h).

11. Overall the COM agreed that the available evidence was consistent with the conclusion that technical grade malathion (including its metabolites and impurities) induced the mutagenicity observed in *in-vitro* studies.

In-vitro data on malaoxon

12. The Committee reviewed the available *in-vitro* mutagenicity data on malaoxon, the principle metabolite of malathion. Malaoxon had mutagenic activity in two mouse lymphoma assays in the absence of exogenous metabolic activation, and induced SCEs in CHO cells[64,65]. The test material used in these assays was approximately 94-96% pure. Malaoxon (98% pure) had also induced DNA damage in human peripheral blood lymphocytes in one assay[63].

13. The committee considered that the mutagenic activity seen *in-vitro* with technical grade malathion could, in part, be due to the metabolism of malathion to malaoxon.

In-vivo data on malathion

Studies submitted by the pesticide data holder

14. The Committee agreed that the oral rat bone marrow clastogenicity study, conducted by the pesticide data holder using high doses of technical grade material, had been adequately conducted and was negative[5].

15. The Committee had reservations regarding the adequacy of the oral *in-vivo* liver UDS assay in rats submitted by the pesticide data holder[86]. Members were concerned with regard to the apparent positive response in animals 24 and 25 in the assay. The Committee did not accept the rationale provided by the contract laboratory that these positive responses were caused by technical errors (staining errors, the timing of dosing relative to other rats and/or use of control rats sampled 2-4 hours after dosing as controls for treated rats sampled 12-16 hours after dosing). Members also considered it feasible that a positive control substance had been dosed to these animals by mistake or there had been a mislabelling of the animals. Whilst such explanations may be plausible, there was a lack of direct evidence linking the positive effects in the two rats to any experimental error. However, it was noted that no evidence of mutagenicity was found in a repeated 12-16 hour sampling time experiment. Overall the COM agreed that no weight could be attributed to the study and that it should be repeated. Negative results in such a repeat study would remove any concerns that technical grade malathion was mutagenic to rats when dosed orally.

Published Studies

16. The Committee was aware that there were a large number of published *in-vivo* studies which reported positive results for malathion. No definite conclusions could be drawn from the majority of these studies because the test material used was not of technical grade or the purity could not be determined and/or the methods used were inadequate by current standards[6,8,18-20,28,33,42,57,90]. The Committee was aware that there were some studies which reported negative findings *in-vivo* with technical grade malathion[17,46,52,53] but agreed that it was important to consider in detail the available studies where positive finding had been reported and where there was sufficient information reported to comment on the results[18,57,90].

Oral administration

17. Giri et al (2002)[57] documented limited evidence for an increase in chromosomal aberrations in bone-marrow of groups of 3 Swiss mice (sex not given) given either a single oral dose of 5 mg/kg bw or five daily doses of 2 mg/kg bw. A fixation time of 24 hours after the last dose was used. The test material used was a technical grade material manufactured in India[93]. The Committee agreed that in view of the lack of lack of evidence for carcinogenicity in the long-term feeding carcinogenicity bioassay in mice, that there was no need for a further oral study in mice at this juncture.

Dermal administration

18. The Committee considered the study by Dulout *et al.* (1982)[90] which had used both the dermal and intraperitoneal routes of dosing in bone-marrow micronucleus assays in mice. Members agreed it was difficult to explain why such a strong positive response had been documented following dermal application compared to intraperitoneal dosing. The test material (a technical grade material manufactured in Argentina) had been dissolved in corn oil which would have led to residual vehicle on the skin. Members noted that dermal absorption in human volunteers ranged from 5% to 15% depending on formulation and dilution with water. It was not possible to quantify the extent of dermal absorption in this study. There would have also been some oral ingestion arising by grooming of the animals, but his could not be quantified. Members noted that there was no evidence for a dose response following dermal administration in this study, but felt that the results could not be refuted and thus there was a need for a repeat study in mice.

Intraperitoneal administration

19. The Committee agreed there was evidence from several studies for a mutagenic effect of technical grade malathion in the bone-marrow of mice given the test material by intraperitoneal administration[18,57,90]. The Committee agreed that these data suggested technical grade malathion had mutagenic activity *in-vivo* in mice and reinforced the need to evaluate dermal exposure to technical grade malathion adequately.

20. Overall the Committee concluded that malathion was mutagenic *in-vivo* in mice dosed by the intraperitoneal route, and possibly also by the oral and dermal routes. Conversely, there was no evidence of *in-vivo* mutagenicity in rats dosed orally. These data may indicate a species-specific *in-vivo* mutagenicity in mice.

Studies in humans exposed to malathion and other pesticides

21. The Committee discussed the available studies in humans exposed to malathion[14,29,35,44,47,49,54]. It was noted that an increase in the incidence of micronuclei had been reported by the Californian Health Department, in a preliminary study of 13 applicators who used malathion in 1992[14,54]. This result was not confirmed by a further study undertaken by the same group of researchers in 1993[14,54]. Overall the Committee felt that no conclusions could be drawn from this study. The Committee considered that no conclusions could be derived from the other available published studies relating to monitoring of human exposure for mutagenic effects. The Committee concluded that appropriately designed studies of pesticide applicators using malathion, might be informative with regard to mutagenic activity in humans.

Response of data holder to COM Evaluation (19/11/02 and 2/01/03)

22. The data holder provided some additional data on the 19 November 2002 on the *in-vivo* liver UDS assay in rats[86], namely additional details of the conduct of the study and results in animals 24 and 25 and photomicrographs of slides from animal 24 and from a negative and positive control animal. It was noted that additional repeat investigations had been included in the study which had been triggered when technical difficulties concerning animals 24 and 25 had been noted. The data holder provided an additional *in-vivo* intraperitoneal micronucleus assay in the mouse[94]. The data holder provided comments on the dermal *in-vivo* micronucleus assay in mice published by Dulout *et al*[90]. Additional photomicrographs and comments on the conduct of the rat liver UDS assay were provided by the pesticide data holder on 2 January 2003.

COM comments

23. Regarding the new data on the rat liver UDS assay, COM members considered the additional data submitted on the 19/11/02 and on 2/01/03 and agreed that the assay was flawed. The arguments regarding the background staining of the slides did not match with the reported positive NNG values for animals 24 and 25. Overall members could not endorse this assay. The lack of evidence for a carcinogenic effect in livers of rats dosed with malathion is noted. However considering the clear mutagenicity of malathion *in-vitro* and in some other *in-vivo* assays, an adequate, technically defensible assay is considered appropriate.

24. Members noted the new intraperitoneal bone marrow micronucleus test which gave negative results and provided some reassurance, although the specification of the test material was uncertain and only 1000 PCEs had been scored[94].

25. COM members acknowledged there are considerable problems with the conduct of study reported by Dulout *et al.* 1982[90]. However members felt that the pesticide data holder had not provided a rationale to suggest it was a false positive response and agreed that a repeat dermal assay was appropriate.

26. The COM considered other comments from the Data holder regarding the interpretation of human studies and the assessment of *in-vitro* studies with malathion. The Committee agreed that no alterations with regard to these areas of the statement were required.

COM conclusions

27. The Committee agreed the following conclusions:

    i. Technical grade malathion is mutagenic *in-vitro* in human lymphocytes and mammalian cells (mouse lymphoma cells). The observed mutagenic activity is due to malathion, its metabolites and its impurities.

    ii. The weight of evidence suggests technical grade malathion is not mutagenic following oral administration to rats. There is a need for a further *in-vivo* oral rat liver UDS assay with technical grade material supplied in the UK. Negative results in such an assay would provide full reassurance with regard to oral exposure of rats to technical grade malathion. There is good evidence for a mutagenic effect of technical grade malathion given intraperitoneally to mice, and some limited evidence for mutagenicity in mice with oral or dermal administration. The Committee recommends that a further study using dermal administration in mice would be appropriate to adequately assess the potential for mutagenicity by this route of exposure as this is particularly relevant to the route of human exposure.

    iii. There are a number of studies of humans exposed to malathion which have investigated potential mutagenic effects. There are limitations in all of these studies predominantly due to small numbers of subjects, study design and co-exposure to other chemicals which might have mutagenic effects. No conclusions could be drawn from the available data. Further monitoring of populations exposed to malathion might be informative with regard to potential mutagenic activity in humans.

COC review of carcinogenicity

28. The Committee reviewed the conduct, adequacy and results from the available carcinogenicity bioassays in rats and mice where malathion or malaoxon had been administered in the diet.[66-72] In reviewing these studies, the COC agreed that particular reference should be made to the evaluation of nasal tissue. The Committee commented that nasal tissue was generally not examined in carcinogenicity bioassays until around 1990. The Committee made the following comments on each of the long-term bioassays.

Malathion bioassays in rats

29. *NCI 1978, Osborne Mendel rat*[69]. The study authors had reported that there was an increase in thyroid follicular cell carcinomas/adenomas in females in the high dose group. A peer review of pathology had concluded that there was no significant increase in thyroid tumours[83]. The COC noted there was no examination of nasal tissue, non-neoplastic pathology was reported in females but not males, there were no full necropsies of decedents and the control groups were too small. Overall it was agreed that this study was inadequate by contemporary standards and no conclusions could be drawn.

30. *FDRL 1980, Sprague Dawley Rat*[66]. The study authors had not reported any evidence for a treatment related carcinogenic effect. The committee noted that there was no examination of nasal tissue. With this limitation noted, the committee considered the study had in general been adequately undertaken.

31. *NCI 1979, Rat F344*[71]. There was an increase incidence in phaeochromocytomas in low dose but this was not found in high dose males, and the absence of adrenal medullary hyperplasia further questioned the significance of this finding. There was limited evidence for increased leukaemia in low dose males. It was agreed that this study provided no consistent evidence for a carcinogenic effect. The COC noted that examination of nasal tissue had not been undertaken in this study, but agreed that overall the study had in general been conducted adequately.

32. *HLS 1993-1996, Rat F344*[72]. The COC agreed that this bioassay was conducted to modern standards and was the critical carcinogenicity study in the rat. Dietary levels used were 100 ppm (reduced to 50 ppm at month 3), 500 ppm, 6000 ppm and 12000 ppm. (Averaged dose levels (mg/kg bw/day) in males and females were respectively; 4/5, 29/35, 359/415, 739/868 mg/kg bw/day). The COC considered that the top two dose levels in males exceeded the Maximum Tolerated Dose (MTD) level. This was based on reduced mean body weight (-16.8% at month 18 in the 12000 ppm group and -11.1% at 6000 ppm) and statistically increased mortality compared to control animals. In females the MTD was exceeded at 12000 ppm (based on reduced mean body weight at 24 months; -16.8% and significant mortality compared to controls) but was not exceeded at 6000 ppm (mean reduction in body weight$<$ 5.1% compared to controls, 62% survival to termination). The study investigators had documented two nasal tumours in males (an olfactory epithelial adenoma in one animal at 6000 ppm and an olfactory epithelial carcinoma in one animal at 12000 ppm). The COC noted that an independent peer review of the nasal pathology had been undertaken[73]. The COC concluded that the report of the peer review was satisfactory and agreed that the classsification of tumours used in this review should be used in the evaluation of this cancer bioassay. Following the peer review of the pathology, a consensus agreement had been reached that there were three nasal respiratory adenomas (one male and one female) at 12000 ppm and one female at 6000 ppm) and an olfactory epithelial adenoma in one male at 6000 ppm. These tumours were associated with severe non-neoplastic pathology in the nasal tissue at 6000 and 12000 ppm (including epithelial degeneration, hyperplasia and inflammation). The peer review also documented four tumours of the oral palate. The data holder had provided a concise summary of the neoplastic pathology in animals with nasal tumours[82]. The COC observed that based on the relatively small laboratory historical control data, the incidence of nasal tumours was highly statistically significant. However it was evident from the EPA review and information supplied by the data holder that the background incidence of nasal tumours was unclear and depended upon the number of nasal sections used in bioassays[82,84]. In modern bioassays this would comprise 5 sections

whilst only two sections were used in the bioassays in the laboratory historical control database. The historical control range from 20 bioassays undertaken as part of the NTP program was 0-2% in both males and females. The Committee considered that overall there was evidence of a tumourigenic response to malathion in the nasal tissue of the rat but the observed tumours were induced in tissue which was subject to severe ongoing nasal inflammation. The COC agreed there was an increase in liver tumours at the two highest dose levels and that this was likely to be due to an effect of treatment on the background pathology of ageing animals.

Malathion bioassays in mice

33. *NCI 1978, Mouse B6C3F1*[69]. The COC observed that there was no examination of nasal tissue, there were no full necropsies of decedents and the control groups were too small. A statistically significant increase in combined hepatocellular carcinoma and neoplastic nodule were reported in males animals by trend analysis. No increase was reported when analysed by pair wise comparison or by life-time analysis. The Committee commented that the terminology used to describe histological lesions of the liver was now considered outdated and that there was a high incidence of hepatocellular carcinoma in historical control groups. The committee agreed that by contemporary standards this bioassay was inadequate. The Committee observed the evidence of coughing and wheezing in most high dose animals from week 72 which provided some evidence for an effect on the respiratory tract.

34. *HLS 1992-1994, Mouse B6C3F1*[68]. The COC agreed that this bioassay had been conducted to modern standards. Dietary levels of 100 ppm, 800 ppm, 8,000 ppm, and 16000 ppm were used. (Averaged dose levels (mg/kg bw/day) in males and females were respectively; 17.4/20.8, 143/167, 1476/1707, 2978/3448 mg/kg bw/day) The COC agreed that the Maximum Tolerated Dose level was exceeded at 16 000 ppm in both males (-20 % reduction in mean body weight compared to controls at week 78) and in females (-16% reduction in mean body weight compared to controls at week 78). The MTD was exceeded in males at 8000 ppm (-14% reduction in mean body weight compared to controls at week 78). The COC considered that the 8000 ppm dose level in females equated to the MTD (reduction in mean body weight for females was -9.7%). The study investigators reported the finding of hepatocellular adenoma, hepatocellular hypertrophy and increase liver weight in most mice (male and female) at 8000 ppm and 16000 ppm. A Peer review of histology in male mice essentially confirmed the liver findings reported by the study investigators[75]. The Peer review group also identified significant and excessive non neoplastic pathology of the nasal tissue similar to that reported in F344 rats. The Committee agreed that the liver tumours were treatment related.

Malaoxon bioassays in rats

35. *NCI 1979, Rat F344*[71]. A statistically significant increase in thyroid C-cell adenomas and carcinomas were reported in females at the top dose. A Peer review had been undertaken[83]. The Committee agreed with the conclusion reached by the peer review group that the evidence for a carcinogenic effect in the thyroid was equivocal. The Committee noted that examination of nasal tissue had not been undertaken in this study. The Committee noted the high dietary level was 1000 ppm, but agreed that, based on the preliminary 13 week study (no effects on body weight or histology at 2000 ppm), a higher dose level might have been used.

36. *HLS 1993-1996, Rat F344*[72]. The COC agreed that this bioassay had been conducted to modern standards. Dietary levels of 20 ppm, 1000 ppm, and 2,000 ppm were used. (Averaged dose levels (mg/kg bw/day) in males and females were respectively; 1/1, 57/68, 114/141 mg/kg bw/day) The Committee considered that the Maximum Tolerated Dose level had not been exceeded in this study (-1.4% to -7.1% reduction in body weight compared to controls in males and -4.0% to -8.8% in females at termination). However a dose related increase in mortality was reported in males. A significant dose-related trend in mononuclear cell leukaemia was reported in males but no statistically significant increase was evident in pair wise comparisons with controls. The incidence of this tumour was within the historical control for the laboratory. A significant increase in testicular interstitial cell tumours was reported at 2000 ppm when the data were corrected for time to tumour identification. However the observed increase was within the historical control incidence. There was thus no evidence for any treatment related increase in neoplasia. The study investigators found significant non-neoplastic pathology of the nasal tissue and respiratory tract. This included significant purulent inflammation of the lungs. There was evidence of food particle deposition in many animals in this study.

Malaoxon bioassay in mice

37. *NCI 1979, Mouse B6C3F1*[71]. The COC noted that examination of nasal tissue had not been undertaken in this study. The Committee noted the high dietary level was 1000 ppm, but agreed that, based on the preliminary 13 week study (no effects on body weight at 2000 ppm), a higher dose level might have been used. No treatment related neoplasia were reported.

Overall malathion carcinogenicity evaluation

38. The COC agreed that the assessment of the significance of the observed nasal tumours in F344 rats in the modern study undertaken by HLS USA was the critical neoplasm for discussion. The Committee agreed that the finding of liver tumours in F344 rats and B6C3F1 mice in the modern studies should also be evaluated in detail. The findings reported in earlier studies undertaken in the 1970's and 80's contributed little weight of evidence to the assessment of malathion.

Nasal tumours in F344 rats

39. The COC noted that tumours of the nasal tissue in F344 rats were rare and that there were difficulties in assessment particularly with regard to the adequacy of historical control data from the laboratory. The COC noted the additional sectioning and microscopic evaluation of additional slides from the HLS USA study suggested that the US NTP historical control data for F344 rats might be more appropriate. Overall it was agreed that a tumourigenic response had been documented in this study. The COC observed that the observed tumours were all benign and included an olfactory epithelial adenoma arising from the Bowmans' gland in a male fed 6000 ppm and a respiratory epithelial adenoma in a male fed 12000 ppm. In females one animal at 6000 ppm and one at 12000 ppm had respiratory epithelial adenomas. All tumours were well defined and there was no evidence of pleomorphism or atypia[82]. The Committee considered that the significance of these tumours had to be assessed against a background of severe inflammation, which exceeded that found in inhalation carcinogenicity studies with chemicals that were directly irritant to the nasal tissue. It was unclear from the HLS malathion

study in F344 rats to what extent inhalation of food particles contributed to the induction of this inflammatory response in this study. The Committee agreed that direct irritant effects on the nasal passages was possible when animals were fed powdered diets. It was noted that the food pots used in the malathion study had some degree of covering which might have limited inhalation of food particles. It was noted that there was clear evidence to show the effect of food particles in the induction of localised inflammation of the nasal passages and the lungs in the malaoxon bioassay in F344 rats undertaken at the same laboratory as the critical malathion F344 rat bioassay. The Committee considered that the proposal from the data holder that de-esterification of malathion in nasal tissue to form acids was also potentially plausible. It was noted that nasal tissue would have appropriate metabolic capacity to metabolise malathion to its corresponding diacid metabolites. The Committee discussed the possibility of a genotoxic mechanism in the induction of nasal tumours. However the weight of evidence including the formation of tumours only at excessive doses, the evidence for severe prolonged localised inflammation in the target tissue, and the lack of multi-organ response suggested that a non-genotoxic mechanism was probable in this instance.

Liver tumours in F344 rats and B6C3F1 mice

40. The Peer review pathology report of the Huntingdon Life Sciences bioassay in F344 rats conducted between 1993 and 1996 reported a consensus incidence of 5/70 (7.1%) hepatocellular adenomas in females at a dietary level of 12000 ppm (ca 868 mg/kg bw/day)[74]. The historical control incidence for the laboratory was 0-5.4% (n= 254). The Peer review report noted that there were a number of non-neoplastic microscopic findings in the five animals with tumours, which included hypertrophy, congestion and vacuoloation. The COC agreed that the time of death and liver pathology seen in these animals would appear to be generally consistent with ageing pathology in this strain of rat. There was a significant increase in mortality in the female high dose group from month 18 to 24 (above that seen in controls) resulting in limited numbers of animals available at terminal sacrifice and thus the possibility existed that there might be some animals which could have developed liver tumours being missed. However there was no evidence of a dose response in females and only one hepatocellular adenoma was found in treated males (in a high dose male). All the available pathology evidence suggested that these tumours are most likely to be an effect of malathion on the background pathology of ageing F344 rats and are unlikely to be of significance for human health.

41. The COC commented that the increased incidence of hepatocellular adenomas in male and female B6C3F1 mice was associated with clearly overt increases in liver weight and hepatocellular hypertrophy and are unlikely to be significant to humans. It was suggested that, given the evidence for hepatocellular hypertrophy induced at doses exceeding the MTD, that it was highly likely that these tumours were induced through a non-genotoxic mechanism of technical grade malathion.

Overall evaluation of malaoxon

42. The Committee concluded that there was no evidence for a carcinogenic effect of malaoxon, the main metabolite of malathion.

COC conclusion

43. The COC drew the following overall conclusion:

    i. The COC agreed that technical grade malathion had been tested in four long-term dietary bioassays in rats and two long-term dietary bioassays in mice. The most recent studies undertaken in F344 rats (1993-96) and in B6C3F1 mice (1992-94) were adequate for the evaluation of carcinogenicity. There is evidence for tumourigenicity in the nasal tissue and liver (females only) of F344 to rats fed malathion. The nasal tumours were associated with severe ongoing inflammation, which is most likely involved in the mechanism of tumourigenesis. There was evidence for liver tumours in female F344 rats and male and female B6C3F1 mice. The weight of evidence suggested that these liver tumours were induced through a non-genotoxic mechanism and were not relevant to human health.

Overall conclusion: Mutagenicity and Carcinogenicity of Malathion

44. The Committees agreed the following overall conclusion:

    i. Technical grade malathion is mutagenic *in-vitro*. The observed activity is due to malathion, its metabolites and impurities.

    ii. The evidence from mutagenicity studies suggests technical grade malathion is not mutagenic in rats by the oral route. There is a need for a further *in-vivo* oral rat liver UDS assay with technical grade material supplied in the UK. Negative results in such an assay would provide full reassurance with regard to oral exposure of rats to technical grade malathion.

    iii. There is good evidence from published studies that technical grade malathion is mutagenic in mice following intraperitoneal administration and some limited evidence for mutagenicity in mice following oral and dermal administration. These data may indicate a species-specific *in-vivo* mutagenicity in mice. The Committee recommends that a further study using dermal administration in mice is appropriate to adequately assess the potential for mutagenicity by this route of exposure as this is particularly relevant to humans.

    iv. Technical grade malathion has been shown to induce benign tumours at high oral doses which were above the Maximum Tolerated Dose in rats (nasal tissue and liver (females only) and in mice (liver) following administration via the diet in long term bioassays. The nasal tumours were associated with severe ongoing inflammation, which is most likely involved in the mechanism of tumourigenesis. The weight of evidence suggested that the liver tumours were not relevant to human health. The Committees concluded that the nasal and liver tumours were induced by non-genotoxic mechanisms.

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Statement on 2-phenylphenol and its sodium salt

Introduction

1. 2-Phenylphenol and its sodium salt are broad spectrum fungicides that are approved in the UK for use as wood preservatives. They are also used as surface biocides in a number of areas. The COM have advised on the mutagenicity of these compounds, specifically in the context of the mechanism of the bladder tumours seen in male rats fed high doses of these compounds, on a number of occasions, the most recent being in 1997. Significant new data are now available. Furthermore an EU review of the use of 2-phenylphenol and its sodium salt in wood preservation is shortly to be initiated under the Biocidal Products Directive (98/8/EC). It would thus be timely to update the COM view on the mutagenicity of these compounds. This information would be helpful in developing the UK position with regard to the EU in the context of the Biocidal Products Directive.

2. The advice of the COM was thus sought on the mutagenicity of 2-phenylphenol and its sodium salt, and specifically whether the induction of bladder tumours at high dose levels in male rats in the chronic bioassay is likely to have arisen from a genotoxic mechanism. If this were the case the risk assessment would need to adopt a non-threshold approach.

Background

3. The COM gave detailed consideration to a comprehensive review of the mutagenicity of 2-phenylphenol in 1992 and agreed the following conclusions:

    i. Data from several assays to investigate the ability of 2-phenylphenol or its sodium salt to produce gene mutation in *Salmonella typhimurium* were consistently negative.

    ii. Positive results were obtained in *in-vitro* metaphase analysis studies in CHO cells*. Mouse lymphoma assays in the presence of exogenous metabolic activation also gave positive results in the form of small colonies. These results suggested that a metabolite of 2-phenylphenol had clastogenic potential. These findings conflicted with an earlier *in-vitro* metaphase analysis in a single study.

    iii. Negative results were obtained in bone marrow assays for clastogenicity *in vivo* and also in germ cells (dominant lethal assay), indicating that any possible clastogenic potential was not expressed in the whole mammal.

    iv. Conflicting results appeared to be obtained in *in-vivo* assays for effects on DNA in bladder epithelium, the target tissue of concern. The Committee therefore wished to see data from a more sensitive *in-vivo* method to investigate adduct formation. There was insufficient evidence to recommend a departure from the use of the safety factor approach for regulation of this compound at the present time.

*Not included in the 1992 conclusions. The positive results seen in the in vitro metaphase study in CHO cells were in the presence of an exogenous metabolic activation system.

4. In the light of this advice the Advisory Committee on Pesticides (ACP) requested further data relating to DNA adduct formation in the urinary bladder epithelium of the male rat with particular emphasis on the dose response relationship at levels known to produce hyperplastic and neoplastic changes in male rats.

5. In 1997 industry provided these data to the registration authority (HSE) which was referred to COM for an opinion. The following conclusions were drawn by the COM:

   The Committee expressed concern regarding the limitations of methodology used in the study entitled "^{32}P-post-labelling study of technical grade 2-phenylphenol to examine the potential for the formation of DNA adducts in the urinary bladder of the male rat." The Committee requested additional analyses of the bladder epithelial samples from this study using an appropriate sensitive adduct enrichment method for the detection ^{32}P-postlabelled adducts (namely both nuclease P_1 and butanol extraction) and appropriate control experiments to evaluate the fate of 2-phenylphenol DNA adducts during the extraction and enrichment procedures.

6. The ACP considered the COM opinion, together with additional data from specialist feeding studies/carcinogenicity bioassays in 1998 and concluded that the post-review data requirements had been met and confirmed that a threshold based approach remained relevant for risk assessment purposes.

Consideration of new data published since the comprehensive review in 1992

7. A number of papers relating to the metabolism of 2-phenylphenol and its sodium salt and the production of reactive species were considered together with DNA adduct studies in rat bladder epithelium, mutagenicity data on the quinone metabolites and other data relevant to the mutagenicity of 2-phenylphenol. This new information is summarised below.

Metabolism

8. The metabolic profile of 2-phenylphenol is now well established[1, 2, 3]. Recent data on the metabolism of 2-phenylphenol in the rat and the mouse confirms that at relatively low dose levels (around 20mg/kg bw/day) the compound is metabolised and excreted in the urine as sulphate, or to a lesser extent, the glucuronide conjugate[1]. At higher doses increased levels of conjugates of PHQ were present, but very little non-conjugated PHQ or PBQ. Studies on the comparative metabolism in the rat and the mouse indicate only minor differences[2]. It is difficult to see how these could explain the marked species difference in the induction of bladder tumours in the male rat and not the mouse. It is possible that localised deconjugation of PHQ glucuronide (or sulphate) may play a role at high dose levels in the male rat[2].

Formation of DNA adducts in male rat bladder epithelium

9. Further data are now available on the ability of 2-phenylphenol or its sodium salt to induce DNA adducts in the rat bladder *in vivo*. The earlier studies had given conflicting results, with negative results using radioactivity detection following a very high acute exposure with ^{14}C-2-phenylphenol, but positive results using ^{32}P-postlabelling in a subchronic dietary study using a single high dose level (2%) and examining total bladder DNA (rather than epithelial DNA)[4,5].

10. The study considered by the COM in 1997, and criticised because of its insensitivity, has now been published, and a rationale for the chosen methodology was given[6)] The Committee did not agree with this and maintained their view that an appropriate sensitive enrichment method for the detection of ^{32}P-post-labelled adducts should have been used. However a study to investigate both DNA and protein binding in the liver, kidney and bladder of male F344 rats has now been published using the highly sensitive AMS technique[7]. An extensive dose response was investigated, (6 single doses by gavage over the range 15-1000mg/kg 2-phenylphenol). A clear dose-related and essentially linear response was seen with protein binding in the liver and kidney, but protein binding was seen in the bladder only at high dose levels (about 500mg/kg and above). There was no evidence for DNA binding at any dose level in bladder tissue.

11. The Committee agreed that the weight of evidence from *in-vivo* studies is now sufficient to conclude that 2-phenylphenol does not produce significant DNA binding in the male rat bladder. However most of the available data were from short term studies and the one, limited, subchronic study had provided some evidence of adduct formation in bladder DNA. The Committee thus felt that the possibility of prolonged high level exposure producing DNA adducts could not be entirely discounted.

Genotoxicity of PHQ and PBQ

12. A number of studies have investigated the ability of the PHQ or PBQ metabolites to induce chromosome damage, micronuclei or DNA damage in HL60 or V79 cells[8,9,10]. There was also some evidence for the induction of aneuploidy[8]. Positive results were obtained, sometimes only in the presence of arachidonic acid supplementation. Members noted that these cells were readily stimulated to generate an oxidative environment. The relevance of positive data with high concentrations of metabolites rather than the parent compound, was felt to be questionable. Members felt that it was not possible to exclude oxidative damage arising from PBQ or PHQ contributing to the induction of bladder tumours at high dose levels, but they agreed that such effects would not be expected to occur at low dose levels.

Additional In-vivo Mutagenicity Data

13. Members considered data from a study to investigate micronucleus induction cell proliferation and hyperdiploidy in bladder epithelium cells of rats treated with 2-phenylphenol at 2% in the diet[11]. An increase in micronuclei, but no effect on chromosome number, was reported. However, in view of the limitations of this study, particularly the failure to be able to distinguish micronuclei from cell debris, it was felt that no conclusions could be drawn from the reported micronucleus induction.

14. The Committee also considered the results obtained in a study in rats to investigate DNA damage in stomach, liver, kidney, bladder, brain and bone marrow using a modified version of the COMET assay based on isolated nuclei[12]. The results were suggestive of high dose activity in the stomach, liver, kidney and lung but not the bladder. There were concerns however as to whether the method used at that time would have adequately distinguished between genotoxicity and cytotoxicity, and it was felt that no definite conclusions could be drawn.

Conclusions

15. The Committee agreed the following overall conclusions regarding the mutagenicity of 2-phenylphenol and its sodium salt:

    i. Data from several assays to investigate the ability of 2-phenylphenol or its sodium salt to produce gene mutation in Salmonella typhimurium were consistently negative.

    ii. Positive results were obtained in *in-vitro* metaphase analysis studies in CHO cells and also in mouse lymphoma assays in both cases in the presence of an exogenous metabolic activation system. The induction of small colonies in the latter assay is consistent with the compound having clastogenic potential.

    iii. Phenylhydroquinone (PHQ) and phenylbenzoquinone (PBQ), which are metabolites of 2-phenylphenol, PHQ usually present as conjugates, have been shown to produce oxidative DNA damage and single strand DNA breaks *in-vitro* using HL60 and V-79 cells.

    iv. Negative results were obtained in bone marrow assays for clastogenicity *in-vivo* and also in germ cells (dominant lethal assay), suggesting that any possible clastogenic potential was not expressed in the whole mammal.

    v. The weight of evidence from *in-vivo* studies to investigate 2-phenylphenol binding to DNA in the male rat bladder was negative: a recent study using a highly sensitive AMS techniques was particularly important in this regard. However the possibility of prolonged high level exposure producing DNA adducts cannot be entirely discounted.

    vi. Although a contributory role of oxidative DNA damage cannot be excluded when considering the mechanisms of bladder tumour induction in the male rat, this would not be expected to occur at low dose levels.

    vii. The committee concluded that it would be reasonable to adopt a threshold based risk assessment for 2-phenylphenol and its sodium salt.

COM/03/S3 – June 2003

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Statement on guidance on considering high dose positive *in-vivo* mutagenicity data in the bone marrow assays that may not be biologically significant with regard to considering a chemical to be an *in-vivo* mutagen

Introduction

1. The advice of the COM previously has been that compounds that are clearly positive in any *in-vivo* mutagenicity assay should be regarded as mammalian mutagens (and hence potential genotoxic carcinogens)[1]. (http://www.doh.gov.uk/com/guidance.pdf). Furthermore the COM consider that for such compounds it is prudent to assume that there is no threshold for their mutagenic activity, unless appropriate mechanistic data can be provided to identify a threshold related mechanism, e.g. induction of aneuploidy, where the site of initial action is not DNA but the spindle apparatus[2]. (http://www.doh.gov.uk/comivm.htm). Such mechanistic data are rarely available.

2. This approach has major implications with regard to risk assessment of such chemicals, with the assumption that there is no 'safe' level and that any exposure results in some increased health detriment, albeit this may be very small.

3. In practice this means that much weight is placed on the *in-vivo* genotoxicity assays recommended by the COM in their strategy of testing, in particular on one of the bone marrow assays for clastogenicity (usually the micronucleus test) which is usually the initial assay investigated. A positive in one of these assays results in the compound being considered as an *in-vivo* mutagen. It is thus especially important that such decisions are based on sound data, and that the positive results truly reflect *in-vivo* mutagenic activity. In some cases such positive results in the bone marrow assays have only been seen at high dose levels associated with severe toxicity including lethality, giving rise to concern at the relevance of the results. This is particularly true if the overall weight of evidence from the *in-vivo* data, including the results of carcinogenicity bioassays, is not supportive of the compound being an *in-vivo* genotoxin.

4. Furthermore it is now recognised that in the case of the *in-vivo* micronucleus test, micronuclei can be induced by mechanisms such as hypothermia, hyperthermia, stimulation of erythropoiesis and possibly indirectly due to severe cytotoxicity in the bone marrow. In such cases the positive results, if not supported by other *in-vivo* data, may be regarded as artefacts, which do not reflect mutagenicity of the compound itself in animals.

5. Draft COM guidance on the interpretation of *in-vivo* bone marrow mutagenicity data that may give rise to concern with regard to being a 'false positive' is provided in this document.

Guidance on 'false positive' in-vivo mutagenicity data

6. The COM guidance on a strategy for testing chemicals for mutagenicity is based on the identification of compounds with mutagenic potential on the basis of a small number of well conducted *in-vitro* tests. This is followed by establishing whether activity seen *in-vitro* can be expressed *in-vivo* in somatic cells, to establish whether the compound can be regarded as an *in-vivo* somatic cell mutagen and hence a potential carcinogen. In the initial *in-vitro* testing it is recognised in the guidelines that artifactual positives may be obtained in the mammalian cell assays which do not reflect intrinsic mutagenic activity, and that this uncertainty may be resolved by *in-vivo* assays, and specifically the bone marrow assays for clastogenicity. Until recently there has been little consideration as to whether artifactual positive results can also be obtained in the *in-vivo* bone marrow assays.

7. However it is now known that effects such as hyperthermia[3,4] or hypothermia[5,6], which can be produced as a secondary toxic effect at high dose levels with certain compounds, can produce micronuclei in bone marrow cells *in-vivo*. Another possible mechanism is the induction of erythropoiesis. This was demonstrated following unexpected positive results in the bone marrow micronucleus test with a series of recombinant human erythropoietin products; similar effects were later shown with native erythropoietin[7,8,9]. The induction of micronuclei by such non-DNA reactive compounds has been shown to be due to the accelerated proliferation and differentiation of erythrocytes and promotion of early release of PCEs resulting in the micronucleated PCEs.

8. Another possible mechanism is excessive toxicity at high dose levels, associated with severe toxicity including lethality, i.e. higher levels than recommended in the current OECD guidelines. Such 'high dose' studies may possibly give positive results due to the severe toxicity seen in the bone marrow. Thus there are a number of reasons why the *in-vivo* data may not indicate that the compound is an *in-vivo* mutagen but further information would be needed to draw any definite conclusions.

9. In considering whether positive results in *in-vivo* bone marrow mutagenicity data may be caused by factors other than mutagenicity of the compound itself, or its metabolites, it is important to consider all the available data regarding the mutagenic profile of the chemical and whether the hypothesis that it is not an *in-vivo* mutagen is biologically plausible. This will include information on structure-activity relationships, data from *in-vitro* mutagenicity assays, other *in-vivo* mutagenicity data, information on toxicokinetics of the substances and any available data from carcinogenicity bioassays. Each compound needs to be considered on a case-by-case basis.

10. An important consideration with regard to data from bone marrow assays for clastogenicity (metaphase analysis or micronuclei) is the highest dose level used. The guidance in the current OECD guidelines is that the top dose should produce signs of toxicity such that higher dose levels, based on the same dosing regime, would be expected to produce lethality (or for non-toxic compounds it should be set at a limit dose of 2 grams/kg for single doses and 1 gram/kg for multiple dosing regimes)[10]. In the past considerably higher dose levels have been used, and on occasions the relevance of such data needs to be assessed. In the most extreme case, when positive results have been

obtained only at highly toxic or lethal dose levels, the Committee believe that the results obtained are confounded by toxicity and cannot be interpreted with any certainty in these circumstances; it is thus necessary to repeat the test using lower, more appropriate dose levels. It may, however, be possible to utilise the available data from the lower dose levels, together with other relevant information, to enable the isolated high dose *in-vivo* positive data to be discounted.

Consideration of carcinogenicity data (if available)

11. The implications of mutagenicity data with regard to carcinogenicity was considered in some detail in the 1989 COM guidelines for the testing of chemicals for mutagenicity and also in the 1991 COC guidelines on the evaluation of chemicals for carcinogenicity[11,12]. At that time both committees concluded that it was reasonable and prudent to regard compounds that were mutagens *in-vivo* in the somatic cell assays recommended by the COM to also have carcinogenic potential even though such potential had not been examined or had not been demonstrated in formal carcinogenicity tests.

12. The updated COM guidelines on a strategy for testing chemicals for mutagenicity published in December 2000 endorsed the view that *in-vivo* somatic cell mutagens should be regarded as potential carcinogens[1]. These conclusions have implications with regard to the testing of chemicals for carcinogenicity. The need to use large numbers of animals in carcinogenicity bioassays on compounds that were clear *in-vivo* somatic cell mutagens is unlikely to be justified; this is recognised in the 1991 COC guidelines. The COC is currently updating its guidance on risk assessment of carcinogens.

13. These conclusions also have implications regarding how carcinogenicity bioassay data that are not supportive of a compound being a genotoxic carcinogen may be used to assist in the interpretation of *in-vivo* mutation data when there is concern about the relevance of such data.

14. On occasions in the past the COM have used negative data from carcinogenicity bioassays in this way. An example was the chemical incapacitant CS (2-chlorobenzylidene malonitrile). This compound is mutagenic *in-vitro* inducing both clastogenic and aneugenic effects in mammalian cells. Although there were negative results from *in-vivo* bone marrow micronucleus assays it was noted that no data were available to indicate whether adequate amounts of CS, or short-lived reactive metabolites, reached the target organ. Data from DNA binding studies in the liver and kidney did not help with regard to concerns in tissues of initial contact due to the direct acting mutagenic potential. However the COM took into account the negative carcinogenicity bioassay data from the NTP programme comprising inhalation studies in the rat and the mouse[13]. The advice from the COC was that these assays had been adequately conduced and were negative. The COM agreed these negative data provided reassurances that the *in-vitro* effects seen with CS do not occur *in-vivo* at the site of initialcontact in animals. (Full information can be found at www.doh.gov.uk/cot/csgas/htm.)

15. Thus consideration of the results from carcinogenicity bioassays, if available, is important when assessing the overall weight of evidence as to whether the compound should be regarded as an *in-vivo* mutagen. In this regard an assessment needs to be made as to whether the carcinogenicity data are consistent with the compound being a genotoxic carcinogen. Alternatively the tumour profile may be

suggestive of a non-genotoxic mechanism of chemical carcinogenesis, or that the chemical is not carcinogenic. Consideration may need to be taken of any difference in dose levels used but it must be recognised that the difference in duration of the mutagenicity and carcinogenicity bioassays will severely limit the utility of any comparison. However, consideration of the available information on the toxicokinetics of the compound will often be useful.

16. It is not possible to give more than generic guidance in this area, as each compound will need to be considered on a case-by-case basis. Expert judgement will be needed with particular consideration of a number of key points that are summarised in the conclusions.

Conclusions

17. The following points need to be considered when deciding whether positive *in-vivo* bone marrow clastogenicity data can be discounted with regard to considering a compound as an *in-vivo* mutagen:

    i) The totality of the relevant data relating to the specific compound in question needs to be considered, including any information on its chemical reactivity, toxicokinetics, its mutagenic profile (*in-vitro* and *in-vivo*), and also any available data from carcinogenicity bioassays to enable an assessment to be made as to whether the compound appears to be a genotoxic carcinogen.

    ii) Positive bone marrow *in-vivo* mutagenicity data from dose levels that are associated with high levels of toxicity or lethality (i.e. above the maximum dose level recommended in the current OECD guidelines) cannot be interpreted with any certainty because of the confounding effects of toxicity. If adequate information is not available at lower, non-lethal dose levels then retesting is necessary.

    iii) It is also important to consider whether there is any evidence for a plausible mechanism to support the contention that the observed positive results in the bone marrow assays at high dose levels may be secondary to other non-genotoxic effects rather than being a mutagenic effect of the compound (or its metabolites). Examples of such mechanisms include (but are not limited to) hypothermia, hyperthermia, and erythropoiesis.

    iv) Data from adequately conducted carcinogenicity bioassays, if available, provide important information to help in the assessment of the significance of such high dose bone marrow mutagenicity results. Such data may indicate that the carcinogenic profile of the chemical is consistent with either a genotoxic or a non-genotoxic mechanism, or that the compound is not carcinogenic.

    v) Only generic advice can be given in this area and it should be emphasised that each compound needs to be considered in a case-by-case basis. However consideration of the above factors, with expert judgement, may provide sufficient evidence to conclude that the positive *in-vivo* bone marrow data at high dose levels was due to a non-genotoxic effect. A threshold based risk assessment may thus be appropriate.

COM/03/S5 November 2003

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2003 Membership of the Committee on the Mutagenicity of Chemicals in Food, Consumer Products and the Environment

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Declaration of COM Members' Interests during the period of this report

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof P B Farmer (Chairman)	Abbey National	Share Holder	NONE	NONE
	Bradford & Bingley	Share Holder		
	Celltech	Share Holder		
	Friends Provident	Share Holder		
	Torotrak	Share Holder		
Dr D Burlinson	GlaxoSmithKline	Salary Employee	NONE	NONE
		Share Option Holder		
		Shareholder		
Dr G Clare	Huntingdon Life Sciences	Employee salary	NONE	NONE
	AstraZeneca	Share Holder		
	HBOS	Share Holder		
Dr J Clements	Covenance	Employee Salary	NONE	NONE
		Share Option		
		Share Holder		
Dr D Gatehouse	Covenance	Salary Employee Consultant	NONE	NONE
	Friends Provident	Share Holder		
	GlaxoSmithKline	Pension Share Option Holder Share Holder		
Dr N Gooderham	Abbey National	Share Holder	GlaxoSmithKline	BBSRC Collaborative Studentship
	Friends Provident	Shareholder		
	Game	Share Holder		
	ML Laboratories	Share Holder		
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	Protherics	Share Holder		
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	Company	Interest	Company	Interest
Ms M Langley	BT	Share Holder	NONE	NONE
	Business Consolidating Services	Director		
	Ciebel	Share Holder		
	CREE Research	Share Holder		
	Cyber Care	Share Holder		
	Eshelon	Share Holder		
	HBOS	Share Holder		
	MMO2	Share Holder		
	Quelcom	Share Holder		
	Wibex	Share Holder		
Dr I Mitchell	Kelvin Associates	Associate Consultant	NONE	NONE
	IM Enterprises	Director/Creditor		
	GlaxoSmithKline	Pensioner		
		Option and		
		Share Holder		
	Bass	Consultant		
	Cable & Wireless	Share Holder		
	Cadbury Schweppes	Share Holder		
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	RTZ	Share Holder		
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	Unilever	Share Holder		
	Vodafone	Share Holder		
	Whitbread	Share Holder		
	British Telecom	Share Holder		
	Centrica	PEP Holder		
	Scottish Power	PEP Holder		
	Shire	PEP Holder		

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Dr E M Parry	Invesco	PEP Holder	NONE	NONE
	Fleming	PEP Holder		
	Legal & General	PEP Holder		
Prof D Phillips	Abbey National	Share Holder	NONE	NONE
	BG Group	Share Holder		
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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 2003, the Committee provided advice on a wide diversity of topics including polycyclic aromatic hydrocarbons in air pollution (and in particular the highly potent compound dibenzo(a,l)pyrene), 1,3-dichloropropan-2-ol (a potential contaminant of drinking water), and impurities present in the pesticide 1-methylcyclopropene. The Committee also updated its view on intrahepatic cholangiocarcinoma, assessed the risks associated with exposure to low levels of air pollution and reviewed a paper by Enstrom JE and Kabat GC (British Medical Journal, volume 326, 1057-1066, 2003) on environmental tobacco smoke and lung cancer.

The Committee has an ongoing responsibility to provide Government Department's and Regulatory Authorities with advice on developments in procedures for the evaluation and risk assessment of carcinogens. During this year, the Committee provided advice on the proposed approach from the U.S. Environmental Protection Agency (EPA) on the supplemental data necessary for assessing susceptibility from early life exposure to carcinogens and on the hypothesis for the occurrence of "U" shaped-dose response curves (Hormesis). The Committee has commented on the proposed "Biobank" project.

The Committee discussed a number of proposals for greater openness and it is hoped to agree procedures for open meetings during 2004.

During 2003, the Committee also said farewell to three highly respected members. Professors Cooper, Renwick and Williams have given many years of highly valuable service to COC. I wish to record my thanks for the quality of their scientific advice and commitment to public health during their terms of office with COC.

Professor P.G. Blain (Chairman) CBE
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Dibenzo(a,l)pyrene in air pollution

3.1 In 1995, at the request of MAFF and the Department of Environment, the COC agreed a hazard-ranking scheme for the carcinogenicity of 25 polyaromatic hydrocarbons (PAHs). It was based on classification into one of 5 categories. The COC in 1995 had accepted the principle that the carcinogenicity of PAHs was additive. Advice on dibenzo(a,l)pyrene (DB(a,l)P) had not been requested in 1995. Since then, air pollution monitoring in the UK had detected the presence of dibenzo(a,l)pyrene in a number of samples. An assessment of the relative carcinogenic potency of DB(a,l)P compared to benzo(a)pyrene which claimed that this was about 100 times greater, had been published in an Environmental Health Criteria Document (International Programme on Chemical Safety).

3.2 The COC was asked to consider the mutagenicity and carcinogenicity data on dibenzo(a,l)pyrene and to consider to what category of the COC hazard ranking scheme of PAHs dibenzo(a,l)pyrene could be assigned. The COC agreed that the *in-vitro* mutagenicity tests and information on *in-vivo* DNA adduct formation was consistent with dibenzo(a,l)pyrene being an *in-vivo* mutagen. Members also agreed that dibenzo(a,l)pyrene was carcinogenic in mice and rats. Dermal application to mice produced tumours at a number of sites (including the skin, lung, and malignant lymphoma of the spleen and malignant lymphoma with multiple organ involvement) and intraperitoneal administration to rats produced lung tumours. Intramammary instillation in rats resulted in mammary tumours. Dibenzo(a,l)pyrene also acted as an initiator in mouse skin carcinogenicity promotion assays. The COC therefore considered that dibenzo(a,l)pyrene should be assigned to group A of its hazard ranking scheme for PAHs. This category includes chemicals for which 'there is a high level of concern about a carcinogenic hazard for humans because the compound is an *in-vivo* mutagen and/or a multi-site carcinogen in more than one species.'

3.3 Regarding potency, the Committee agreed that dibenzo(a,l)pyrene was a very potent genotoxic carcinogen and that potency varied depending on factors such as species, route of administration, dose and site of tumour produced. From the available data where a comparison could be made, members considered that the dibenzo(a,l)pyrene carcinogenic potency was likely to be in the range of 10-100 times more potent than benzo(a)pyrene depending on the tests system used. The measurement of DNA adducts (Time Integrated DNA Adduct Levels: TIDAL) following intraperitoneal dosing of DB(a,l)P showed that, in comparison to other PAHs, DB(a,l)P bound much more extensively to DNA, due presumably to the higher reactivity of its diol-epoxide metabolite(s). This might explain its greater carcinogenic potential.

3.4 The Committee agreed that there were insufficient data available on dibenzo(a,l)pyrene to draw any conclusions on the relative potency compared to benzo(a)pyrene by the inhalation route of exposure. The committee agreed to reconsider the topic of the relative carcinogenic potency of PAHs by the inhalation route of exposure at a future meeting. The Committee reached the following overall conclusion;

"Dibenzo(a,l)pyrene should be considered as a highly potent genotoxic carcinogen in experimental animals. There is a need for further consideration of the potential importance of exposure to dibenzo(a,l)pyrene and other highly potent carcinogenic polycylic aromatic hydrocarbons in air pollution."

3.5 A statement is appended at the end of this report.

1,3-Dichloropropan-2-ol (1,3-DCP):

3.6 1,3-Dichloropropan-2-ol (1,3-DCP) is a member of a group of chemicals called chloropropanols, which also includes 3-chloro-1,2-propanediol (3-MCPD) 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. The COC considered the 1,3-DCP in 2001 and in particular a carcinogenicity bioassay in rats which had identified treatment-related tumours in several organs. Taking into account the advice of the COM on this compound, the Committee concluded that "*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.*"

3.7 The COM considered two new in-vivo mutagenicity studies on 1,3 DCP namely a bone marrow micronucleus assay and an unscheduled DNA synthesis (UDS) assay during its 2003 meetings. In the light of these new data, the COM had now concluded that it would be appropriate to consider that 1,3-DCP was not an *in-vivo* mutagen.

3.8 The COC was asked to review its previous conclusions on the tumours induced by 1,3 DCP in rats. The Committee noted its previous opinion that the 1,3-DCP induced tumours of the kidney and thyroid could have been secondary to sustained cell proliferation. Members also agreed that there was evidence of a hepatotoxic effect at doses below those producing a significant increase in combined hepatocellular adenoma and carcinoma.

3.9 The Committee then considered possible modes of action of 1,3-DCP in inducing tumours of the tongue. Members agreed that a significant increase in the incidence of papillomas and carcinomas had been identified in the tongue at the high dose level in both males and females. It was noted that this dose level exceeded the Maximum Tolerated Dose level in that there was an increase in treatment related mortality and hepatotoxicity in this group of animals. The Committee agreed that 1,3-DCP was an irritant and had produced irritant effects in gastric mucosa of treated rats, but there were no suitable data on the potential for 1,3-DCP irritation of the tongue. Members noted that at the time of conduct of the bioassay (1986) it was not routine to examine the tongue histologically. It was agreed however, that since the compound had been given in the drinking water in the bioassay, chronic irritation was a plausible hypothesis for the induction of the tumours in the tongue. Members noted that there was evidence suggesting that bacteria metabolised 1,3-DCP to the genotoxic carcinogen epichlorohydrin. It was possible that bacteria present in the oral cavity might produce epichlorohydrin when exposed to 1,3-DCP and it was not known whether this would be rapidly detoxified in the oral cavity or tongue.

3.10 Members considered that although there was no precedent for site-specific tongue tumours arising from bacterial activation of a compound, the suggestion regarding 1,3-DCP was plausible. It was noted that chemical induction of tumours of the tongue in rats was relatively rare, the only examples that could be recalled readily were a variety of nitrosamine compounds. Members considered the possibility of further work to fully discount a genotoxic mode of action for the tongue tumours in rats. It was agreed that information on contact-irritancy, cell proliferation and formation of ^{32}P-postlabelling adducts in animals treated with suitably high doses of 1,3-DCP would provide appropriate information.

3.11 A revised statement would be drafted in due course.

Environmental tobacco smoke (ETS) and lung cancer: consideration of paper by Enstrom JE and Kabat GC (2003). British Medical Journal volume 326, 1057-1066

3.12 The COC undertook a detailed review of the evidence regarding the association between exposure to environmental tobacco smoke (ETS) and lung cancer during 1997. The review was requested by the Scientific Committee on Tobacco and Health (SCOTH). The full transcript of the COC statement can be found in the 1997 Annual report on the COC internet site(http://www.doh.gov.uk/coc/1997ar.pdf)

3.13 The Committee agreed there was evidence that exposure to ETS of individuals in the Cancer Prevention-1-cohort could not be adequately assessed and thus no definite conclusions could be drawn with regard to the study by Enstrom and Kabat.

3.14 The Committee reviewed a number of additional publications. Overall it was concluded that the study of Enstrom and Kabat should be reviewed with caution in view of inadequacies in assessment of exposure to ETS in the investigation.

3.15 A statement is appended at the end of this report.

Impurities in the pesticide 1-methylcyclopropene

3.16 1-methylcyclopropene (1-MCP) is a new pesticide active ingredient (growth regulator) being assessed by the Pesticides Safety Directorate (PSD) under EU Directive 91/414/EEC. 1-MCP blocks the effects of ethylene release in apples thus preventing over-ripening and softening. The Committee was asked for advice on the risks posed by the potential for exposure to two impurities present in the active ingredient namely impurities 1 and 2 which were presumed by the Advisory Committee on Pesticides (ACP) to be genotoxic carcinogens on the basis of published mutagenicity studies and life-time bioassays in rats and mice using oral administration. The Department for the Environment, Food and Rural Affairs (DEFRA) asked for advice on the carcinogenic risk posed by the impurities. The COC was not asked to evaluate the active ingredient and data on this compound have not been reviewed. The COC has not been asked to evaluate the other two impurities present in 1-MCP.

3.17 The COC was provided with published information on the mutagenicity, carcinogenicity and metabolism of impurity 1 and impurity 2 and with details of the manufacture of 1-MCP, information on the formulation and product to be used in the U.K and estimates of potential exposures to impurities present in 1-MCP during use. The data holder, Rohm and Haas company, provided an estimation of the 5% Bench Mark Dose for the most sensitive carcinogenic response for these two impurities which was for forestomach tumours.

3.18 The Committee's conclusions based on the questions posed by DEFRA are given below;

Whether a minimum risk levels for the impurities 1 and 2 can be derived

The COC concluded that pragmatic minimum risk levels could be established for impurity 1 and impurity 2. For impurity 1 this was equivalent to a daily oral does of 0.06 mg/kg bw/day. For impurity 2 this was equivalent to 1.28 mg/kg bw/day.

Whether exposures to the impurities 1 and 2 present any significant risks to consumers or operators

The COC noted that the maximum predicted exposures for operators and consumers will be below the proposed minimum by factors varying from approximately 3 to 5311. The risk of carcinogenicity posed by exposure to impurity 1 and impurity 2 is considered to be negligible.

Whether maximum levels of the impurities 1 and 2 should be proscribed

The COC agreed that maximum levels for these impurities should not result in doses exceeding the minimum risk level and restated that there was always a need to apply the "as low as reasonably practical" (ALARP) principle to genotoxic carcinogens.

3.19 A statement is appended at the end of this report.

Polycyclic Aromatic Hydrocarbons in air pollution

3.20 The COC has recently evaluated published carcinogenicity data on dibenzo(a,l)pyrene (DB(a,l)P) and had agreed that this compound was between 10-100 times more potent than benzo(a)pyrene depending on the test system used (see section 3.3 of the COC Annual Report). Thus a paper had been drafted which considered the potential impact of DB(a,l)P and other high carcinogenic potency PAHs on the existing approaches to risk assessment of PAHs in air pollution.

3.21 Three approaches to carcinogenic risk assessment have been advocated: the use of Potency Equivalency Factors (PEFs) (equivalent to Toxicity Equivalent Factors, TEFs, for general toxicity); the complete mixture method; and use of B(a)P as a surrogate carcinogen for all PAHs. It was possible that the B(a)P surrogate approach which is currently used to monitor for compliance with UK air pollution standard, might not be appropriate if high potency PAHs were present in air pollution and if the concentrations of these compounds varied significantly when compared to B(a)P. The COM had provided advice on the most suitable approach to measuring relative carcinogenic potency of PAHs using measurement of time integrated DNA adduct levels after intratracheal administration of low doses of PAH.

3.22 COC Members agreed with the suggested approach of using DNA adducts for ranking PAHs as a suitable surrogate for measuring carcinogenic potency by inhalation. It was noted that the potential for induction of PAH metabolism and differential repair of DNA adducts needed to be considered when interpreting results. However the approach represented a pragmatic method of providing ranking into broad groups. These results could be used with data on air levels (derived from a range of sites in the UK at different time intervals) to evaluate variation in carcinogenic potency of PAHs in air pollution. The outcome of this research would help review whether a PEF or B(a)P surrogate approach to the air quality standard was most appropriate for the risk assessment of PAHs in air pollution.

3.23 The Committee agreed that was important to find out the proportion and variation of high potency PAHs in the UK, as detected by air monitoring.

3.24 A paper is in preparation for submission to a peer review journal.

Update on consideration of Intrahepatic Cholangiocarcinoma

3.25 The Committee had considered this subject in 2000 and in 2001. Evidence for an increase in the mortality from intrahepatic cholangiocarcinoma over the period 1968-1998 had been provided by Professor Howard Thomas and colleagues (from the Department of Medicine at Imperial College of

Science Technology and Medicine). The Committee had advised that changes in diagnostic standards over time could have accounted for the reported increase and concluded that it was important to keep this topic under review. During 2001 more information was made available to the Committee including a prepublication report of additional investigations by Professor Thomas and colleagues. A statement had been agreed at the beginning of 2002. (http://www.doh.gov.uk/coc/intrahepaticst.htm).

3.26 Some additional information from Professor Thomas's group was considered by COC during 2003. This information was valuable in assessing the progress regarding the recommendations for research that had been identified by the COC in its statement. Members considered that the evidence for an increase in the incidence of IHCC was becoming more convincing, in particular, the evidence on trend now available from several countries. The COC considered the proposed case-notes study would not be useful in that it would only examine the accuracy of diagnosis as given on death certificates in the early 1990s and would not provide clarification on the possibility of diagnostic transfer.

3.27 Members commented on the published paper on DNA adducts (Khan et al, Gut (2003): 52, 586-591). This was considered to be a well-conducted study, although there was only a small number of controls. However, it was not possible to determine the cause of the adducts. They could be due to an exogenous genotoxic agent or an endogenous process, or they could be a marker of tumour development. Looking at tumour tissue and tumour-adjacent tissue from people with IHCC caused by liver fluke might help to clarify this. The Committee was unable to draw any conclusions about the relevance of the high levels of DNA adducts in tumour-adjacent tissue. The unpublished paper on P53 mutations was not considered to provide any support for causation by an exogenous genotoxic agent because the mutational spectra recorded showed no reproducible pattern.

3.28 The Committee will keep developments in this area under review.

Update on risks associated with exposure to low levels of carcinogenic air pollutants

3.29 At the November 2002 meeting of the Committee, Members considered a proposal to use the upper bound estimate derived from the one-hit model using data from epidemiology studies in exposed humans to set upper bounds of risk at low levels of exposure to air pollutants. Members were advised that the intention was to use this very conservative approach to advise on the practicality of risk management options for air pollutants and there was no intention to publish risk estimates based on this approach. The primary objective would be to assess the cost of reducing levels of air pollution to the exposures associated with the upper bound estimate of risk based on the one hit model. The COC was content with the approach provided that it was limited to chemicals for which there was good cancer epidemiology data and that data were used only as a guide when considering risk management options. Members felt, however that it was important to restate that extrapolation of risk estimates below the observed range was very problematic, as no model was completely satisfactory.

3.30 A statement on this topic was agreed during 2003 and is appended to this report.

Review of Committee Procedures

Horizon Scanning

3.31 The Code of practice published by the Office for Science and Technology (OST) on Guidance for Scientific Advisory Committees (http://www.ost.gov.uk/policy/advice/copsac/) encouraged Committees to develop strategies for the early identification of issues. This included i) "new issues", eg previously unidentified potential chemical mutagens/carcinogens which may represent a risk to public health and where advice is required, and ii) "new or unexpected developments in science."

3.32 The DH Toxicology Unit had provided a paper which updated the research priorities discussion and conclusions reached by COC in 1996 and also reviewed potential areas for further work such as cancers where there was evidence for an increasing trend in incidence (http://www.doh.gov.uk/coc/papers.htm). The chair thought that it would be valuable if the COC revisited horizon scanning for a short period at each future meeting.

3.33 As part of the discussion of this item, members commented on the proposal regarding the occurrence of "hormesis" (ie the occurrence of a "U" shaped dose-response curve at low dose level). A report of the discussion is given under Test Strategies and Evaluation (see section below).

3.34 Regarding other topics, members agreed a review of oesophageal cancer and the induction of DNA repair following exposure to low doses of carcinogens would be valuable. Members also considered evaluation of potential risks of chemical induced cancer in children had received considerable interest in the scientific literature and it would be appropriate for COC to form a view on the subject.

Further discussions on Openness of Committee Business

3.35 The COC currently publishes an agenda and many of its discussion papers before each meeting, whilst minutes of meetings and Committee statements are published when finalised. The Committee considered a draft protocol that set out procedures for conducting meetings in open forum. It is intended that after discussion by all three Committees had been completed, a finalised document would be agreed and published as part of a revised code of practice for openness.

3.36 The COC agreed in principle to conducting meetings in open forum. Members noted that it was important to manage applications for attendance according to the procedures set out in the draft protocol. Of particular importance was the need to seek information from attendees on their declarations of interest prior to the discussion of items. It was noted that observers at open COT meetings had usually attended for a specific item and it was agreed that, if this were so, then it was appropriate to undertake the proposed question and answer session with the relevant item. The Committee also raised a number of concerns regarding the need to avoid attributing comments to individual members and dealing with in-confidence data submitted to the Committee. The secretariat was asked to consider these further and report back to COC.

Test Strategies and Evaluation

Biobank project

3.37 The Committee heard a presentation on the aims, objectives and preparatory work undertaken to establish the Biobank project in U.K. Further information can be obtained from (http://www.ukbiobank.ac.uk/).

3.38 It was noted that the publication of the draft code for the human genome and new techniques for rapid sequencing Single Nucleotide Polymorphisms of DNA had opened the way for research to investigate the combined effects of genetics and lifestyle or environmental factors on common multifactorial diseases of adult life. Thus the strong scientific base in the UK, the population size and diversity and the almost universal coverage of the NHS indicated that large scale prospective research on disease occurrence and genetics was feasible in the UK. A key factor in the design of the study

would be including enough individuals so that sufficient clinical outcomes could accrue within a reasonable period of time. For this reason, the Biobank would be established with a minimum of 500,000 individuals aged between 45-69y with a follow-up period of 10 years. People registered with general practices would be asked to join the study. Participants would be asked to complete a self-administered questionnaire and interview with a research nurse, give a blood sample, and sign a consent form for participation and follow-up. Participating individuals would be flagged through the ONS and incident data on morbidity would be obtained through practice records and hospitalisation data. A key policy for participation was "opt-in", and that individuals would be able to leave the project at any time.

3.39 The Committee made a number of comments regarding the recruitment of individuals, ascertainment of disease status, and quality control of sample handling and analysis. A key concern of members was the limited information on chemical exposure that would be available for cohort members. This would limit the potential for studies to investigate the association between genotypes and susceptibility to chemical induced disease.

EPA risk assessment guideline: supplemental data for assessing susceptibility from early life exposure to carcinogens

3.40 The US Environmental Protection Agency had issued a draft consultation document on the assessment of susceptibility to carcinogens from early life exposures. The USEPA had argued that conventional bioassays underestimated risks (from the dose response slopes) arising from early life exposures for genotoxic carcinogens, but not for non-genotoxic carcinogens. These data led the USEPA to propose adjustment factors of 10 (for below 2 years of age) and 3 (for ages 3-15 years). The Committee's view was requested on this and the implications, if any, for the UK approach to risk assessment for chemical carcinogens and the methodology used.

3.41 Members felt that it was very difficult to draw any definite conclusions from the comprehensive EPA comparisons between results with conventional carcinogen bioassays and the studies involving neonatal/ perinatal exposure. This was because of the great variability in the study designs used for the neonatal/perinatal studies. Rarely were any pharmacokinetic data available to allow a comparison to be made between the systemic doses achieved by oral exposure in the conventional assays and the parental routes frequently used in the studies involving exposure of neonates. Furthermore there were only limited consideration of the mechanism involved and the target tissues (tamoxifen was given as an example of when the approach adopted was inappropriate due to differing tissue specificity).

3.42 It was agreed that there was some biological plausibility to the argument of increased sensitivity in the early life stages (due to factors such as differing metabolism and cell turnover), and the analysis did provide some limited data to support this. However, this was not always the case. It was pointed out that the document was concerned with the risk of cancer in later life, and whether early life stage exposure made this more likely. It did not address childhood cancer, only the lifetime risk of cancer following early life exposure.

3.43 As the COC does not recommend the use of slope of the dose response from animal bioassays to calculate human cancer risks (and the estimation of the tolerable exposure levels) it was agreed that the adjustment factors being proposed by the US EPA were not relevant to the UK. In the UK a risk management approach of reducing exposures to as low as reasonably practical for all age groups is adopted for genotoxic carcinogens.

3.44 The Committee considered that in some instances the data suggested that animal models that include perinatal exposure may be more sensitive; these difference were quantitative, and there was no evidence that the use of conventional cancer bioassays in animals would fail to detect chemical carcinogens.

3.45 The Committee agreed that these data supplied by the USEPA had very limited implications for the way carcinogenicity assessment was carried out in the UK.

Hormesis (The occurrence of "U" shaped dose dose-response curves)

3.46 Members were asked to comment on the recent publication from Calabrese EJ and Baldwin LA (Nature, vol 421, 691-692, 2003.) which provided an argument for the occurrence of hormesis (ie the occurrence of a "U" shaped dose-response curve at low dose level).

3.47 The Committee noted that although it was stated that there were up to 5000 examples of the hormetic effect in the published literature, it was not possible to assess this claim on the evidence available. It was noted that in the few studies on genotoxic carcinogens using group sizes sufficient to detect effects at 1% levels or below, the evidence was generally consistent with the absence of a threshold. Also, DNA adduct formation with genotoxic carcinogens was linear down to the lowest measurable dose levels. The dose-response data for non-genotoxic carcinogens was consistent with the occurrence of a threshold.

3.48 Members considered that there was no evidence available to justify the use of a hormetic approach to risk assessment for chemical carcinogens. It was agreed that the evidence for DNA repair following exposure to very low doses of genotoxic carcinogens warranted further review.

3.49 Overall members felt that the arguments presented by Calabrese and Baldwin should be considered further in the future. It was agreed that there might theoretically be a point of departure in the dose-response for a genotoxic carcinogen but it was not possible to identify any potential threshold with methods available. Members considered it prudent to reaffirm that for practical purposes genotoxic carcinogens should be presumed to have no threshold.

Ongoing reviews

Alcohol and Breast Cancer

3.50 The Committee has continued its consideration of the association between drinking alcohol and breast cancer. The research commissioned for the Committee from the Department of Epidemiology and Public Health, Imperial College, London has been finalised. The publication of the COC views on this work awaits publication of the research report in a peer-reviewed journal.

Organochlorine insecticides and risk of breast cancer

3.51 The Committee initiated a review at its September 2003 meeting. There is a large number of new epidemiological and other investigations to consider. The Committee will consider a draft statement at its April 2004 meeting.

Olfactory Neuroblastoma: Possible association in dentists and dental nurses

3.52 The Committee heard a presentation from a researcher on some preliminary data. Further consideration is expected at the April 2004 meeting.

Prostate Cancer

The Committee considered an overview report prepared by the DH Toxicology Unit at Imperial College, London. Further consideration of some aspects is expected at the April 2004 meeting.

Revised COC guidance on risk assessment of carcinogens

3.54 A draft document has been produced for consultation. Further consideration is expected at the June 2004 meeting. http://www.doh.gov.uk/coc/guideline03.pdf

Statements from COC published during 2003

Carcinogenicity of Dibenzo(a,l)pyrene

Environemental Tobacco Smoke (ETS) and lung cancer: Consideration of paper by Enstrom JE and Kabat GC (2003). British Medical Journal volume 326, 1057-1066.

Evaluation of carcinogenic impurities in the pesticide 1-methylcyclopropene,

Risks Associated With Exposures to Low Levels of Carcinogenic Air Pollutants.

Carcinogenicity of dibenzo (a,l) pyrene

Introduction

1. In 1995, at the request of MAFF and the Department of Environment, the COC agreed a hazard-ranking scheme for the carcinogenicity of 25 polyaromatic hydrocarbons (PAHs)[1]. It was based on classification into one of 5 categories. The COC in 1995 had accepted the principle that the carcinogenicity of PAHs was additive. Advice on dibenzo(a,l)pyrene (DB(a,l)P) had not been requested in 1995. Since then, air pollution monitoring in the UK had detected the presence of dibenzo(a,l)pyrene in a number of samples[2]. An assessment of the relative carcinogenic potency of DB(a,l)P compared to benzo(a)pyrene (claimed to be about 100 times) had been published in an Environmental Health Criteria Document (International Programme on Chemical Safety)[3].

2. The COC was asked to consider the mutagenicity and carcinogenicity data on dibenzo(a,l)pyrene and to consider to what category of the COC hazard ranking scheme of PAHs dibenzo(a,l)pyrene could be assigned. Additionally the committee was asked whether the relative carcinogenic potency of DB(a,l)P compared with benzo(a)pyrene could be determined from the available data and in particular whether it was possible to make any statement on the relative potency by the inhalation route of exposure. The Committee considered the available published mutagenicity and carcinogenicity data on DB(a,l)p at its November 2002 meeting.

Introduction to Dibenzo(a,l)pyrene; DB(a,l)P

3. Dibenzo(a,l)pyrene (DB(al)P) CAS Number191-30-0, Chemical Abstracts Name; Dibenzo(def,p)chrysene). There is very little information available on the chemical characteristics of this compound. Its Molecular weight is 302.38 D and its melting point is 162-164°C. Dibenzo(a,l)pyrene occurs in some products of incomplete combustion; it also occurs in fossil fuels[4]. It has been identified in mainstream cigarette smoke and products of coal gasification[4]. There are few data on levels in the environment. Some measurements have been reported for urban and industrialised areas of Holland and from air quality monitoring in Canada[5,6]. It is noted that in some early analyses dibenzo(a,e)fluroanthene has been mistaken for DB(a,l)P [4].

Evaluation of DB(a,l)P

4. The following paragraphs give an overview of the mutagenicity and carcinogenicity data on DB(a,l)P. This is followed by a discussion of the studies where the carcinogenicity of, and DNA adducts formed by DB(a,l)P have been compared to equivalent studies with other PAHs. This statement does not present a review of the large number of investigations which have considered the pathways of metabolic activation of DB(a,l)P, although some references to studies with pro-carcinogenic metabolites are made.

Mutagenicity

DB(a,l)P

5. DB(a,l)P was mutagenic *in-vitro* in *Salmonella typhimurium* TA 100 and TA 98 in the presence of exogenous metabolic activation (using Aroclor 1254 pre-treated rat liver S-9)[7]. Positive results have also been reported in an *in-vitro* cell transformation assay using CH310T1/2 cells, although the significance of these findings for mutagenicity are unclear[8].

6. No *in-vivo* mutagenicity studies were identified in a literature search but DB(a,l)P has been shown to act as an initiator in mouse skin promotion assays[6,10]. It is also noted that an increase in mutations of codon 12 and 61 of Ki-ras was documented in pulmonary adenomas from mice treated with single intraperitoneal doses of 0.3-6 mg/kg bw of DB(al)P[11]. The distribution of mutations at codon 12 (GGT-TGT, GGT-GTT and GGT-CGT) and of codon 61 (CAA-CTA, CAA-CGA and CAA-CAT) was different from those reported by the same investigators in pulmonary adenomas from control animals. (There are no concurrent *in-vitro* mutagenicity spectra data for DB(a,l)P.) Finally DB(a,l)P has also been shown to induce DNA adducts in rat mammary tissue following intramammary instillation using ^{32}P-postlabelling with adduct enrichment via nuclease P1[12].

7. Thus the available mutagenicity data in studies where DB(a,l)P has been used in conventional tests is very limited. (This concurs with the conclusion reached in the IPCS Environmental Health Criteria document 202 on PAHS[3].)

Metabolites of DB(a,l)P

8. The pathways leading to metabolic activation of DB(a,l)P are complex and not reviewed in detail in this statement. It has been suggested that stereospecific metabolism may be important with the fjord region syn-and anti-11,12-dihydrodiol 13,-14-epoxides (DB(a,l)PDE) being of most importance in the activation of BD(a,l)P to DNA reactive metabolites[13].

9. Investigations of pathways leading to activation using 3-methylcholanthrene pre-treated liver microsomes from Sprague-Dawley rats and CD-1 mice in Chinese hamster V79 cells showed a preferential steroeselective oxidation of (-) -11R,12R dihydrodiol of DB(a,l)P at the 13,14 position to form the diol-epoxide[14]. The authors noted that this finding was consistent with the results of mutagenicity studies in V79 cells using the individual steroeisomers.

Summary: Mutagenicity

10. DB(a,l)P and some of its diol-epoxide metabolites are mutagenic *in-vitro*. Other PAHs which have been shown to be metabolised to diol-epoxides have also been shown to be mutagenic *in-vivo*. DB(a,l)P is an initiator in mouse-skin promotion assays, binds DNA in mammary tissue when instilled directly into mammary tissue. There is also limited evidence that DB(a,l)P induces a specific mutational signature in Ki-ras in lung tumours in mice following intraperitoneal administration. Overall the data are consistent with DB(a,l)P being an *in-vivo* mutagen.

Carcinogenicity of DB(a,l)P

Dose levels in the following studies have been cited in the units used by the investigators which have included dosed expressed in terms of number of moles or weight of test material dosed. Thus dosages of DB(a,l)P have been reported in terms of nmoles/kg bw in several of the studies reported. 1 nmole of DB(a,l)P is approximately equal to 0.3 μg DB(a,l)P by weight. Additionally 1μmole of DB(a,l)P is approximately equal to 300 μg DB(a,l)P by weight (or 0.3 μg DB(a,l)P by weight.

Mouse

Dermal administration

11. DB(a,l)P was applied to shaved skin of groups of 22-27 female Swiss mice twice weekly (either 4 or 8 nmol in 100ul of acetone) for 40 weeks. DB(a/l)P was carcinogenic in this study inducing squamous cell carcinomas in 90% of mice at 8 nmol and 70% at 4 nmol[9]. Tumours were also recorded at other sites; these included adenoma of the lung, malignant lymphoma of the spleen and malignant lymphoma with multiple organ involvement. A dose level of 8 nmol was the maximum dose that could be applied without erythema occurring. These data are consistent with DB (a,l)P being a potent carcinogen in the mouse.

12. A single dose of 100 nmol DB(a,l)P was applied to the shaved skin of a group of 24, eight week old SENCAR mice. Mice were killed after 27 weeks. It was reported that 7 mice treated with DB(a,l)P each developed a skin tumour (4 papillomas and 3 squamous cell carcinomas)[10].

Intraperitoneal administration

13. Groups of 70 male A/J male mice (5-6 weeks old) were given a single intraperitoneal administration of DB(a,l)P in tricaprylin at 0.3, 1.5, 3.0, or 6.0 mg/kg bw. Approximately half of the animals were used at various time points up to 28 days for DNA adduct studies. Remaining animals (30-35/group) were subject to necropsy at 8 months post dose and the number of surface lung adenomas counted using a dissecting microscope. A dose level of 0.3 mg/kg bw resulted in 43% Tumour Bearing Animals (TBA), whereas 1.5 μg/kg bw resulted in 97% TBA and doses of 3 μg/kg be and above 100% TBA. The mean number of adenomas per mouse at 6 mg/kg was 16.1 ± 7.26[11]. Maximal levels of DNA adducts occurred between 5-10 days after injection followed by a gradual decrease. The Time Integrated DNA Adduct Levels (TIDAL) were linearly related to dose.

Rat

Intramammary instillation

14. Groups of 20 Sprague-Dawley rats (8 weeks old) were given intramammary doses of 0.25 or 1μmol/gland in 50μl of trioctanoin. Administration was to the nipple region of glands 2, 3, 4, 5 on the right and left sides. The development of tumours was monitored for the following 24 weeks. Animals with tumours >2cm were killed. Full necropsies were undertaken in all animals and mammary glands and any other abnormal tissues examined microscopically. All animals given DB(a,l)P developed mammary tumours (predominantly mammary epithelial adenocarcinmas with a smaller number of mesenchymal fibrosarcomas and squamous cell carcinomas of the skin). The number of tumours/TBA animal was 10.8/animal at 1μmol and 6.6/animal at 0.25 μmol[10]. (Lung tumours were not specifically assessed in this study).

Initiation-Promotion models

15. Groups of 24 female SENCAR mice (eight weeks of age) received a single dermal dose to shaved dorsal skin of 33.3, 100 or 300 nmols DB(a,l)P in 100µl acetone. One week later promotion treatments using 12-O-tetradecanoylphorbol-13-acetate (TPA, 3.24 nmol/100µl acetone, twice weekly) were begun. All mice treated with DB(a,l)P developed erythemas after the first application of TPA. Severity was proportional to DB(a,l)P dose. Promotion treatments were therefore stopped until the fourth week of the study. Promotion treatment was re-started and continued for 11 weeks. Animals killed after 16 experimental weeks[10].

16. In a second experiment, the same investigators undertook a further initiation-promotion study using dose levels of 4, 20, 100 nmols DB(a,l)P. In concurrent studies, initiation-promotion investigations were undertaken using DB(a,l)P 11,12 dihydrodiol and DB(a,l)P 8,9-dihydrodiol. Erythema was noted in the 100 nmol DB(a,l)P group after 10 days. Promotion was delayed till the third experimental week. Promotion treatments (as above) were undertaken for a further 24 weeks[10].

17. In the first experiment it was noted that tumours were already present in some animals at 100 and 300 nmol DB(a,l)P before promotion treatment was resumed in the 4th experimental week. An increase in the percent TBAs was reported compared to acetone control (No tumours seen in controls) of 96%, 92% and 100% at 33.3, 100 and 300 nmol respectively. The number of tumours/animal was increased in a dose-related fashion, 3.29, 5.29, 6.26 respectively. An increase in percent tumour bearing animals and numbers of tumours/animal was recorded in the second experiment at all dose levels of DB(a,l)P but the dose-response was noted to show an inversion. The authors considered this was most likely due to a more severe toxicity of DB(a,l)P in the second experiment. However it is noted that 92% of surviving animals at 4 nmol had tumours (6.96/animal). It was also documented that substantial carcinogenic response was recorded in initiation-promotion studies with DB(a,l)P11,12- dihydrodiol but not with DB(a,l)P 8,9-dihydrodiol using the same treatment regime as with DB(a,l)P[10].

18. In a subsequent study, the same research group investigated initiation-promotion of DB(a,l)P and DB(a,l) 11,12-dihydrodiol in female SENCAR mice using dermal application of 0.25 or 1nmol in 100µl acetone. Promotion, twice weekly for 27 weeks, was undertaken using a lower dose of TPA (2.16 nmol in 100µl acetone) in order to reduce sensitivity to erythema induced by these chemicals. DB(a,l)P induced 2.6 tumours/mouse and 0.79 tumours/mouse at 1 nmol and 0.25 nmol respectively. DB(a,l)P 11,12-dihydrodiol induced 0.17 tumours/mouse at 1 nmol but was reported to be virtually inactive at the lower dose level. Tumours were seen following treatment with 1 nmol DB(a,l)P in mice after 5 weeks of TPA promotion[9].

19. Initiation-promotion assays in mice using dermal application of enantiomerically pure 11,12-dihydrodiols of DB(a,l)P revealed that (-) -11R,12R trans-dihydrodiol of DB(a,l)P induced skin tumours in 93% of animals (4-5/animal) after a single induction dose of 10 nmole whereas the (+) -11S,12S trans-dihydrodiol of DB(a,l)P induced no tumours at 10 nmole and only 13% of animals had tumours following application of 20 nmoles[15]. The results of this study were consistent with the *in-vitro* mutagenicity studies in V-79 cells using these enantiomers[13].

Summary: Carcinogenicity

20. DB(a,l)P is carcinogenic in mice following dermal or intraperitoneal application. DB(a,l)P is also carcinogenic in rats following intramammary instillation. It acts as an initiator in mouse skin promotion assays. The 11,12-dihydrodiol also acted as an initiator in mouse skin promotion assays. These data are consistent with DB(a,l)P being a genotoxic carcinogen in experimental animals.

COC discussion: DB (a,l)P ranking under 1995 COC scheme

21. The COC agreed that the *in-vitro* mutagenicity tests and information on *in-vivo* DNA adduct formation was consistent with dibenzo(a,l)pyrene being an *in-vivo* mutagen. Members also agreed that dibenzo(a,l)pyrene was carcinogenic in mice and rats. Dermal application to mice produced tumours at a number of sites (including the skin, lung, and malignant lymphoma of the spleen and malignant lymphoma with multiple organ involvement) and intraperitoneal administration to rats produced lung tumours. Intramammary instillation in rats resulted in mammary tumours. Dibenzo(a,l)pyrene also acted as an initiator in mouse skin carcinogenicity promotion assays. The COC therefore considered that dibenzo(a,l)pyrene should be assigned to group A of its hazard ranking scheme for PAHs. This category includes chemicals for which 'there is a high level of concern about a carcinogenic hazard for humans because the compound is an *in-vivo* mutagen and/or a multi-site carcinogen in more than one species.'

COC Discussion: Relative potency of DB(a,l)P compared to Benzo(a)pyrene

22. Regarding potency, the committee agreed that dibenzo(a,l)pyrene was a very potent genotoxic carcinogen and that potency varied depending on factors such as species, route of administration, dose and site of tumour produced. From the available data where a comparison could be made, members considered that the dibenzo(a,l)pyrene carcinogenic potency was likely to be in the range of 10-100 times more potent than benzo(a)pyrene depending on the tests system used. The measurement of DNA adducts (TIDAL) following intraperitoneal dosing of DB(a,l)P showed that in comparison to other PAHs, DB(a,l)P bound much more extensively to DNA, due presumably to the higher reactivity of its diol-epoxide metabolite(s). This might explain its greater carcinogenic potential.

23. The Committee agreed that there were insufficient data available on dibenzo(a,l)pyrene to draw any conclusions on the relative potency compared to benzo(a)pyrene by the inhalation route of exposure. The committee agreed to reconsider the topic of the relative carcinogenic potency of PAHs by the inhalation route of exposure at a future meeting.

COC Conclusion

24. The Committee agreed the following overall conclusion regarding the carcinogenicity of DB(a,l)P:

    "Dibenzo(a,l)pyrene should be considered as a highly potent genotoxic carcinogen in experimental animals. There is a need for further consideration of the potential importance of exposure to dibenzo(a,l)pyrene and other highly potent carcinogenic polycylic aromatic hydrocarbons in air pollution."

COC/03/S5 – November 2003

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Statement on Environment Tobacco Smoke (ETS) and lung cancer: consideration of paper by Engstrom JE and Kabat GC (2003). British Medical Journal volume 326, 1057-1066.

Introduction

1. The COC undertook a detailed review of the evidence regarding the association between exposure to environmental tobacco smoke (ETS) and lung cancer during 1997. The review was requested by the Scientific Committee on Tobacco and Health (SCOTH). The full transcript of the COC statement can be found in the 1997 Annual Report[1] on the COC internet site (http://www.doh.gov.uk/coc/1997ar.pdf). The COC was asked to consider a new report of a cohort study2 at its June 26 2003 meeting.

Consideration of publication by Enstrom and Kabat British Medical Journal 17 May 2003, volume 326, 1057-1066[2,3].

2. The Committee considered the information presented in the paper, the editorial regarding this paper, commentaries published by the American Cancer Society, and the British Medical Association, and a number of papers published since the COC review in 1997.

3. The Committee agreed there was evidence that exposure to ETS of individuals in the Cancer Prevention-1 cohort could not be adequately assessed and thus no definite conclusions could be drawn with regard to the study by Enstrom and Kabat.

Additional evidence on ETS and lung cancer considered by the COC at 26 June 2003 meeting[4-9].

4. The Committee noted the results of an additional systematic review[4] published since the COC review in 1997 which were consistent with previous evaluations and suggested an increased risk in never smoking women exposed to ETS from spouses compared to never smoking women unexposed to ETS of 1.29 (95% CI 1.17-1.43).

5. The Committee noted additional evidence of exposure to carcinogenic tobacco-specific nitrosamines derived from ETS[5].

COC conclusion

6. i) The available additional data submitted to the 26 June 2003 COC meeting do not suggest that a full review of the literature since 1997 on this topic is required.

ii) The evidence from a recent systematic review (meta-analysis)[4], and information regarding excretion of tobacco-specific nitrosamines in urine in individuals exposed to ETS[5] support the conclusion reached in 1997.

iii) The evidence from a recent cohort study[2] should be reviewed with caution in view of inadequacies in assessment of exposure to ETS in this investigation.

iv) The COC concluded there was no reason to change the conclusion reached in 1997, namely:

"Taking all the supporting data into consideration we conclude that passive smoking in non-smokers exposed over a substantial part of their life is associated with a 10-30% increase in the risk of lung cancer which could account for several hundred lung cancer deaths per annum in the UK."

COC/03/S2 – July 2003

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Carcinogenic impurities in the pesticide 1-methylcyclopropene (1-MCP)

Introduction

1. 1-methylcyclopropene (1-MCP) is a new pesticide active ingredient (growth regulator) being assessed by the Pesticides Safety Directorate (PSD) under EU Directive 91/414/EEC. 1-MCP blocks the effects of ethylene release in apples thus preventing over ripening and softening. The Committee was asked for advice on the risks posed by the potential for exposure to two impurities present in the active ingredient namely impurities 1 and 2 which were presumed by the Advisory Committee on Pesticides to be genotoxic carcinogens on the basis of published mutagenicity studies and life-time bioassays in rats and mice using oral administration[1,2]. The COC was asked to advice on specific questions given in a referral note from DEFRA which are reproduced below. The COC was not asked to evaluate the active ingredient and data on this compound have not been reviewed. The COC has not been asked to evaluate the other two impurities present in 1-MCP.

2. The COC was provided with published information on the mutagenicity, carcinogenicity and metabolism of impurity 1 and impurity 2 and with details of the manufacture of 1-MCP, information on the formulation and product to be used in the U.K and estimates of potential exposures to impurities present in 1-MCP during use. The data holder is Rohm and Haas company. The data holder provided an estimation of the 5% Bench Mark Dose for the most sensitive carcinogenic response for these two impurities which was for forestomach tumours[3].

3. The Committee considered the questions posed by DEFRA at its 26 June 2003 meeting.

Referral to COC by DEFRA

4. The view of the Committee on Carcinogenicity is sought on whether:

   a. minimum risk levels for the impurities 1 and 2 can be derived

   b. exposures to the impurities 1 and 2 present any significant risks to consumers or operators

   c. maximum levels of the impurities 1 and 2 should be proscribed.

COC discussion

5. The Committee concurred that on the basis of the available evidence submitted it would be prudent to consider impurity 1 and impurity 2 as genotoxic carcinogens[1-7].

6. The Committee considered the available carcinogenicity data and the conditions of use anticipated for 1-MCP as a fumigant for apples, together with potential exposures to impurity 1 and impurity 2 that might arise, and agreed that a pragmatic minimum risk level could be established for these two

impurities. The Committee agreed that it should always be recognised that for impurity 1 and impurity 2 under likely conditions of use that a very small but unquantifiable risk existed and hence the policy of controlling exposures to "as low as reasonably possible" (ALARP) should apply. The 5%BMD for impurity 1 was reported to be 0.6 mg/kg bw/day and for impurity 2 was reported to be 12.8 mg/kg bw/day.

7. The Committee agreed an application of an Uncertainty Factor (UF) of 10,000 to the estimated 5% BMD resulted in estimated minimum risk levels of 0.06 mg/kg bw/day for impurity 1 and 1.28 mg/kg bw/day for impurity 2. The Committee noted that the US EPA and Health Canada had previously stated that a factor of 10,000 represents the highest that could be used in the setting of TDIs. In an analysis of the use of LTD10 (extra life-time cancer risk of 10%) derived from calculations based on TD50s from the Gold database, it was concluded that the reference dose derived from the Gold database and use of a 10,000 UF applied to the LTD10 gave equivalent results to cancer risk at 10-5 derived from LMS modelling and hence represented a fairly conservative approach to setting a minimum risk level[8]. The Committee agreed that this was a conservative approach to estimating minimum risk levels. They had a reasonable degree of confidence that any carcinogenic risk posed at this level would be negligible.

8. The maximum predicted exposures for operators and consumers was reported to be below the proposed minimum risk level by factors varying from 3 to 5311 (as shown below). The risk of carcinogenicity posed by exposure to impurity 1 and impurity 2 following use to fumigate apples is considered to be negligible.

Impurity	Operators	Consumers
1	3	249
2	58	5311

COC response to questions posed by DEFRA

Whether a minimum risk levels for the impurities 1 and 2 can be derived

9. The COC concluded that pragmatic minimum risk levels could be established for impurity 1 and impurity 2. For impurity 1 this was equivalent to a daily oral does of 0.06 mg/kg bw/day. For impurity 2 this was equivalent to 1.28 mg/kg bw/day.

Whether exposures to the impurities 1 and 2 present any significant risks to consumers or operators

10. The maximum predicted exposures for operators and consumers will be below the proposed minimum by factors varying from approximately 3 to 5311. The risk of carcinogenicity posed by exposure to impurity 1 and impurity 2 is considered to be negligible.

Whether maximum levels of the impurities 1 and 2 should be proscribed

11. The COC agreed that maximum levels for these impurities should not result in doses exceeding the minimum risk level and restated that there was always a need to apply "as low as reasonably practical" (ALARP) to genotoxic carcinogens.

COC/03/S3 – September 2003

References

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Risks associated with exposures to low levels of carcinogenic air pollutants

Introduction

1. 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. 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[1,2]. The Expert Panel on Air Quality Standards (EPAQS) provides advice to U.K. Government Departments and Agencies on air quality issues, in particular the levels of pollution at which no or minimal health effects are likely to occur. (http://www.defra.gov.uk/environment/airquality/aqs/index.htm). EPAQS has adopted a pragmatic approach to developing air quality standards for air pollutants which are genotoxic carcinogens[3]. This does not involve the use of mathematical models to estimate cancer risks because of the concerns of COC regarding such approaches[2,3].

2. The Department of Health asked the COC for advice at its November 2002 meeting on whether it was feasible to identify a specific approach to modelling dose-response to carcinogenic air pollutants. The suggested approach would be restricted to modelling of data derived from acceptable epidemiology studies in exposed human populations (predominantly occupationally exposed cohorts). The derived dose-response curves could be then be used following the application of the most conservative model (considered to be the one-hit model)[4] to estimate the maximum upper bound risk of cancer at environmental exposure levels. There was no intention to use data derived from long-term carcinogenicity bioassays in rodents for quantitative estimation of risks to humans. Agreement to the proposed approach would be of value to the Department in formulating advice on prioritising measures for reducing levels of air pollutants.

COC consideration of dose-response modelling for carcinogenic air pollutants

3. The Committee restated its views, published in its guidelines in 1991, that use of mathematical models to evaluate the dose-response for carcinogens, namely that extrapolation of the dose-response curve below the lowest experimental data points, taken from animal bioassay data, gave an impression of precision which cannot be justified from the approximations and assumptions used[2].

4. The Committee considered that it might be acceptable to consider an approach based solely on use of epidemiological data from investigations considered to have been adequately undertaken. The COC cautioned that quantitative risk estimates based on extrapolation to dose levels of one or more orders of magnitude below the observed dose-response in epidemiology studies would involve uncertainties in that the shape of the dose-response curve could not be predicted with any degree of accuracy.

5. However the Committee noted that the one-hit model[4,5] assumed that a single mutation arising from the interaction of one molecule of carcinogen with DNA lead to the development of cancer. This was likely to represent the most conservative approach to risk assessment. Thus the COC agreed that the upper bound (95% Confidence Interval) cancer risk estimate from this approach was likely to overestimate the actual risk associated with exposure to the carcinogen. The Committee agreed to this approach to carcinogenic air pollutants provided the risk estimates were used in the consideration of risk management options rather than being considered as definite values relating to cancer risk levels. The Committee considered it important to restate that any exposure to a genotoxic carcinogen carried a small, but unquantifiable, risk of cancer.

Conclusion

6. The COC agreed to a proposal from the Department of Health, that the upper bound (95% Confidence Interval) cancer risk estimate at environmental exposure levels to air pollutants, which are genotoxic carcinogens, based on data from adequately performed cancer epidemiology studies, using the one-hit model for dose-response extrapolation, could be used as an aid in deriving risk management strategies. The COC agreed it important to restate that exposure to any level of a genotoxic carcinogen carried a small, but unquantifiable, risk of cancer.

COC/03/S4 – September 2003

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2. Department of Health (1991). Report on health and Social Subjects. No 42. Guidelines for the Evaluation of Chemicals for Carcinogenicity. Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment, London HMSO.

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2003 Membership of the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

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Declaration of COC members' interests during the period of this report

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Professor P G Blain CBE (Chairman)	NONE	NONE	Unilever plc	Research Studentship
Prof A Boobis OBE	Abbey Life	Shareholder		
	Abbey National	Shareholder		
	Barclays Bank	Shareholder		
	BG Group	Shareholder		
	BT Group	Shareholder		
	Centrica	Shareholder		
	HBOS	Shareholder	Servier	Research support
	Marks and Spencers	Shareholder		
	National Grid Transco	Shareholder		
	Scottish Power	Shareholder		
	OSI Pharmaceuticals	Consultancy		
Dr P Carthew	Provalis	Share Holder	NONE	NONE
	Unilever	Salary		
Prof P B Farmer	Abbey National	Share Holder	NONE	NONE
	Bradford & Bingley	Share Holder		
	Celltech	Share Holder		
	Friends Provident	Share Holder		
	Torotrak	Share Holder		
Prof D Forman	Barclays	Share Holder	NONE	NONE
	Friends Provident	Share Holder		
	HBOS	Share Holder		
	Woolwich	Share Holder		
Prof D Harrison	Barclays	Share Holder	NONE	NONE
	Friends Provident	Share Holder		
	HBOS	Share Holder		
	Woolwich	Share Holder		
Ms D Howel	NONE	NONE	NONE	NONE

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Dr S J Kennedy	Unilever	Share Holder	NONE	NONE
MS M Langley	Business Consolidating Services	Share Holder	NONE	NONE
	Ciebel	Director		
	CREE Research	Share Holder		
	Cyber Care	Share Holder		
	Eshelon	Share Holder		
	HBOS	Share Holder		
	MMO2	Employee		
	Quelcom	Share Holder		
	Wibex	Share Holder		
Prof D Phillips	Abbey National	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		
	Servier	Consultant		
D R Roberts	AstraZeneca	Share Holder	NONE	NONE
	Aventis	Slary		
	P & O	Share Holder		
Prof D Shuker	NONE	NONE	NONE	NONE
Dr N Wallis	AstraZeneca	Share Holder	NONE	NONE
	Syngenta	Slary		
		Share Holder		

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

Medical Health products Regulatory 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 223);

- 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 224 to 225.

(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.

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

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.

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.

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

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

3. In discussing these proposals (during the course of 1999) the Committees were aware that the disclosure of information which is of a confidential nature and was communicated in circumstances importing an obligation of confidence is subject to the common law of confidentiality. Guidance is set out in the Code of Practice on Access to Government Information (second edition, 1997). Thus an important aspect of implementing initiatives for greater openness of Committee business concerns setting out clear guidelines for the handling of information submitted on a confidential basis.

General procedures for openness

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

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

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

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

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

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

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

Summary of proposals for committee openness

Issue	Proposals	Comment
Open meetings on specified topics (eg invited audience, interest groups, consumer organisations, professional socieities).	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	Published in accordance with procedures for previous years.
(*Procedures for handlng 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

Subject	Year	Page
Accelerator Mass Spectrometry – An aid to carcinogen risk assessment	2000	103
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.

2001 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Food Standards Agency/Department of Health, FSA/0681/0802.

2002 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Food Standards Agency/Department of Health, FSA/0838/0803.

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).

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment: Risk Assessment of Mixtures of Pesticides and Similar Substances, Food Standards Agency, FSA/0691/0902 (2002).

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment: Phytoestrogens and Health, Food Standards Agency, FSA/0826/0503 (2003).

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