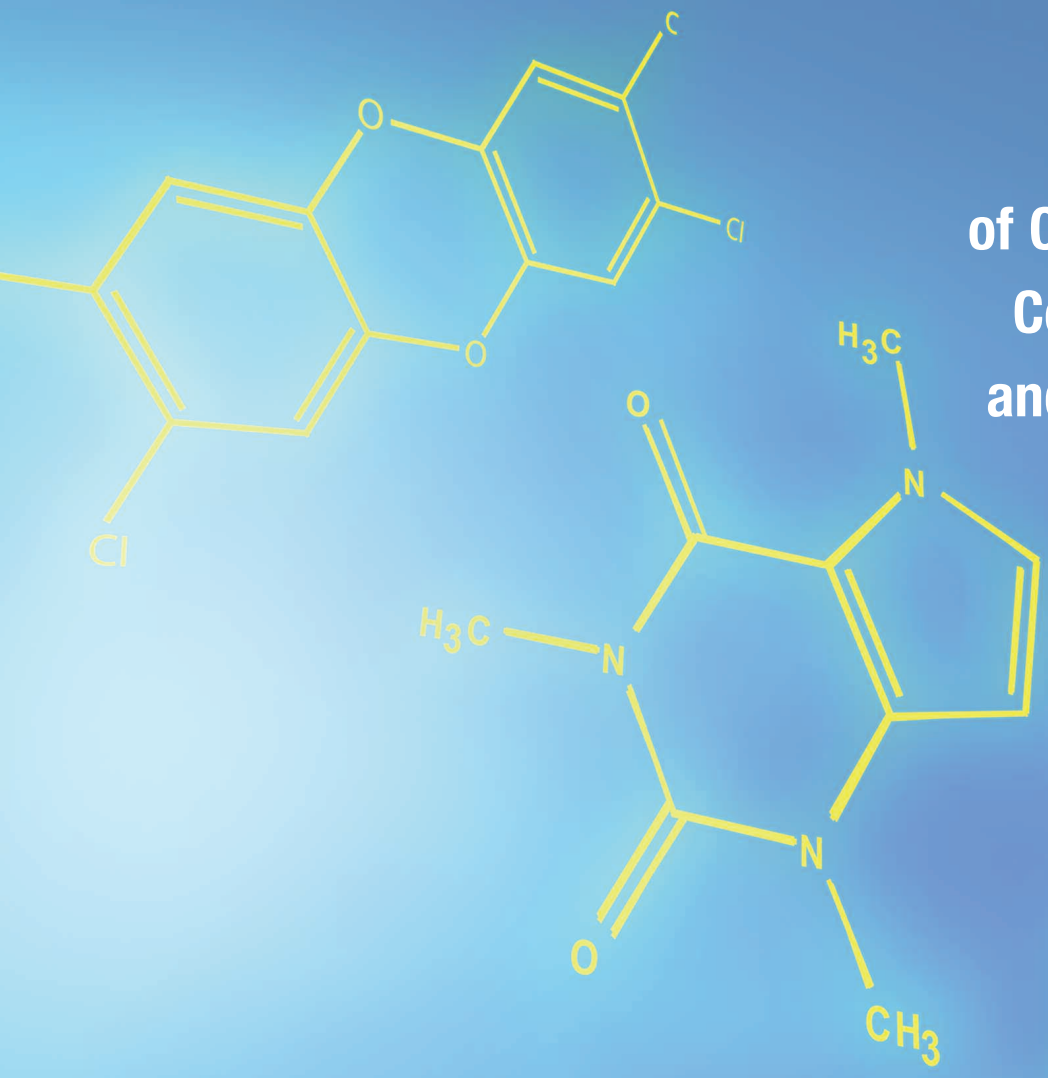
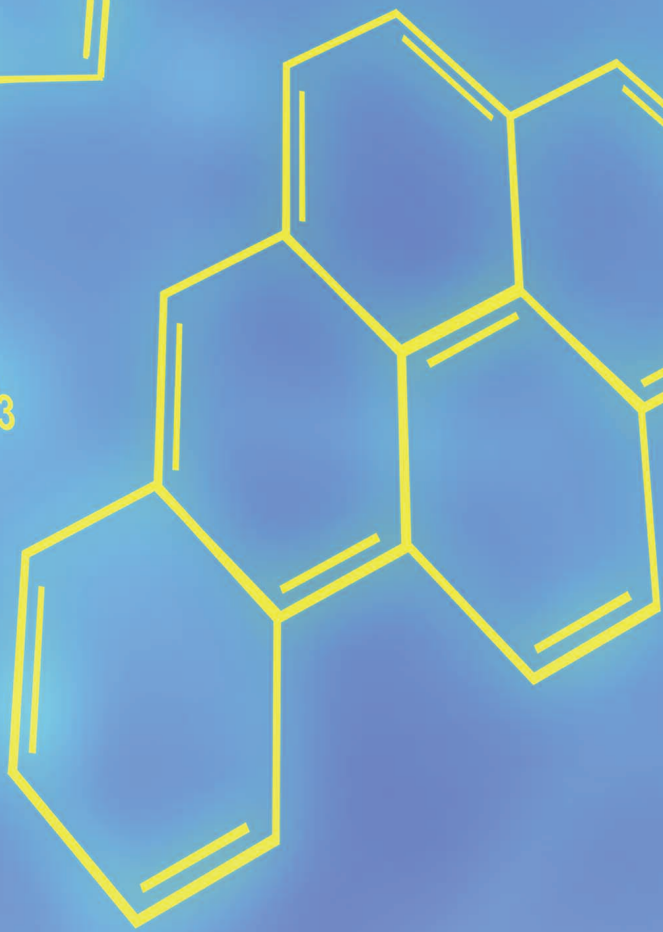
Committees on Toxicity Mutagenicity Carcinogenicity of Chemicals in Food, Consumer Products and the Environment





Committee on TOXICITY

Committee on CARCINOGENICITY

Committee on MUTAGENICITY

Annual Report 2008

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

Annual Report
2008

Contents

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

About the Committees

This is the eighteenth 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 or from the internet sites listed below.

In common with other independent advisory committees, Committee members are required to follow a Code of Conduct which also gives guidance on how 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. Annex 1 contains the terms of reference under which the Committees were set up. The Code of Conduct is at Annex 2 and Annex 3 describes the Committees' policy on openness. Annex 4 has the Good Practice Agreement for Scientific Advisory Committees. Annex 5 contains a glossary of technical terms used in the text. Annex 6 is an alphabetical index to subjects and substances considered in previous reports. Previous publications of the Committees are located at Annex 7.

These three Committees also provide expert advice to other advisory committees, such as the Advisory Committee on Novel Foods and Processes, and there are links with the General Advisory Committee on Science, 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 published on the internet at the following addresses:

COT: http://cot.food.gov.uk
COC: http://www.iacoc.org.uk/index.htm
COM: http://www.iacom.org.uk/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 the Toxicity of Chemicals in Food, Consumer Products and the Environment

Preface

The Committee on Toxicity (COT) evaluates chemicals for their potential to harm human health. Evaluations are carried out at the request of the Food Standards Agency, Department of Health, Health Protection Agency and other Government Departments including the Regulatory Authorities, and are published as statements on the Internet. Details of membership, agendas and minutes are also published on the Internet.

2008 has been another busy year for the Committee with agreement of nine statements. These cover diverse topics, including research on mixtures of certain food additives and on the reproductive effects of caffeine; risks from the presence in food of chemicals such as pyrrolizidine alkaloids and various metals and trace elements; residues in meat and dairy products from animals that have eaten bracken; and chlorinated disinfection by-products in drinking water. We also undertook a review of the evidence relating to the role of exposure to peanut in early life in development of peanut allergy. Advice was given on a number of approaches to toxicity testing and risk assessment, including for nanomaterial toxicology, tobacco products and contaminated land. The Committee held a highly successful scientific workshop on transgenerational epigenetics, which was attended by scientists from academia and industry and other interested individuals. A report of the workshop is included in this report.

I became chair of the COT in April 2008, having previously been a member, and I have found it interesting and rewarding to become more involved in the Committee's varied programme of work, and also to serve as a member of the Food Standards Agency General Advisory Committee on Science. I would like to thank my predecessor, Professor Ieuan Hughes, who completed his term of office at the end of March 2008, and all of the Committee members for their valuable contributions to the work of the COT. I would also like to add my thanks and appreciation for the work carried out by the administrative and scientific secretariats without whom the Committee would not be able to function.

Professor David Coggon (Chairman)
OBE MA PhD DM FRCP FFOM FFPH FMedSci

COT evaluations

Air fresheners

Introduction and background

1.1 During the 2007 horizon-scanning exercise, members asked for a review of exposure data on air fresheners and information on potential respiratory effects. To explore this further, an overview of the published scientific literature was presented to the Committee which focussed on whether the use of air fresheners was associated with adverse respiratory effects.

1.2 Air freshener products can include incense, natural products, scented candles, aerosols, liquid fresheners, electric diffusers and gels. Air fresheners can release volatile organic chemicals (VOCs) which include aromatic hydrocarbons such as xylenes, dichlorobenzenes, terpenes, alcohols, aldehydes and esters, as well as fine and ultrafine particulates. The VOCs released by air fresheners can also react with ozone to produce respiratory irritants such as formaldehyde. The chemistry of such reactions is complex and highly variable depending on the conditions of use. Other products such as cleaning agents may also contain the same VOCs as air fresheners, including those which react with ozone.

Exposed and vulnerable groups

1.3 Adults and children within the home are exposed to substances from air fresheners largely by inhalation, though dermal and ocular exposure to solid or liquid air fresheners may occur. Accidental ingestion by children has also been reported. Potentially susceptible groups might include children and those who are asthmatic, have other respiratory illness or disease or who are otherwise sensitive to chemicals/odorants.

1.4 The data on the pattern and extent of air freshener use and, in particular, the magnitude of exposure to particular VOCs or particulates are limited, though limited evidence suggested that exposure to these substances from cleaning products may be higher than from air fresheners.

Adverse effects

1.5 The data linking exposure to air fresheners to adverse respiratory effects in humans are limited. Descriptive studies have reported adverse health effects in individuals following exposure to air fresheners but these studies have not provided exposure data, excluded effects of cleaning products or provided information on symptoms in control subjects. Data from the Avon Longitudinal Study of Parents and Children (ALSPAC) did not find an association between air freshener use and respiratory effects amongst mothers of infants post-partum. However, data from this study indicated an association between high maternal chemical body burden and persistent wheezing during childhood, though the proportion of the chemical burden attributable to air freshener use, if any, was unknown. Other explanations for this finding are also possible, including the “hygiene hypothesis”. It was noted that exposure patterns are likely to have changed since the children in the ALSPAC cohort were born

and the observed associations with respiratory function might be confounded by differences in cleanliness in the houses of the study group. VOCs from indoor air (all sources combined) are linked to adverse respiratory effects.

1.6 Limited animal data were also available indicating that one solid air freshener product was associated with possible sensory irritation and that high levels of the terpene d-limonene found in household products including air fresheners, resulted in pulmonary irritation.

Conclusion

1.7 It was not possible to reach any specific conclusion on the potential for adverse respiratory effects arising from the use of air fresheners. To assist in the interpretation of future studies, it was important to try to define the nature and extent of VOC exposure attributable to air fresheners and other consumer products, and to try to distinguish between product categories.

Future action

1.8 It was noted that the Committee on the Medical Effects of Air Pollutants (COMEAP) Subgroup on Asthma were reviewing all relevant data on VOCs including air fresheners, and the Committee's comments would be forwarded to them for consideration.

Bracken - Risk to consumers from eating foods derived from animals that had eaten bracken

1.9 Several cases of poisoning of food-producing animals as a result of eating bracken had been reported to the Veterinary Laboratories Agency. These cases had been discussed in July 2007 at the interdepartmental Quarterly Review of Incidents where it was noted that the foods derived from bracken- exposed animals could contain residues of carcinogenic substances from bracken. The Committee was asked to comment on the risk to consumers from eating foods derived from bracken-exposed animals and to advise the FSA on measures that might be taken to protect consumers. The Committee considered the available information on bracken and its constituent chemicals and subsequently prepared a Statement on the Risk to Consumers of Eating Foods Derived from Animals that had Eaten Bracken.

1.10 It was concluded that bracken contains some genotoxic or possibly genotoxic substances, including ptaquiloside, kaempferol and shikimic acid, and that it would be prudent to regard bracken and at least one of its constituents (ptaquiloside) as being potentially carcinogenic to humans at all levels of ingestion. Furthermore it was noted that ptaquiloside from bracken ingested by food-producing animals can be passed into milk or edible tissues that might be consumed by humans. It was recommended that the level of consumer exposure to ptaquiloside and other bracken-derived genotoxic substances should be kept as low as reasonably practicable (ALARP). It was considered that the concentration of ptaquiloside in milk should have depleted to tolerably low levels after a bracken-poisoned cow had been on a bracken-free diet for at least 4 days. However, the available evidence did

not provide a basis for estimating how long it would take for the amounts of ptaquiloside in meat and offal of bracken-exposed animals to decline to tolerable levels.

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

Children's Environment and Health Strategy for the UK Consultation

1.12 Members were invited to comment on the document "A Children's Environment and Health Strategy for the UK" that had been put out for consultation by the Health Protection Agency (HPA). Suggestions for additional areas of relevance included the possible effect of use of biocides in the home on indoor asthma, the possible effects of mobile phone use on intellectual development, the adverse effects of endocrine disrupters, peanut allergy, and nutritional problems in ethnic minority communities. Aspects of toxicology that could be discussed in the document included neurodevelopmental toxicology and other lifelong effects resulting from exposures to chemicals in early life. Members also suggested that the document could indicate the areas where the greatest impact can be made to improve the health of children. These suggestions were forwarded to the consultation.

Chlorination disinfection by-products and risk of congenital anomalies in England and Wales – new SAHSU study

1.13 The COT had previously provided advice in 1998, 2001 and 2004 on the findings of epidemiological studies on chlorinated drinking water and reproductive outcomes.

1.14 In 2008 the Small Area Health Statistics Unit (SAHSU) completed phase 2 of its research – an epidemiological study on chlorinated drinking water and congenital anomalies. The COT considered the findings of this phase 2 study together with additional epidemiological studies relevant to congenital anomalies and other adverse reproductive outcomes.

1.15 The Committee agreed that the phase 2 study showed no association between congenital anomalies and chlorination disinfection by-products. The Committee also agreed that the additional data provided did not show a convincing or consistent relationship between chlorinated drinking water and adverse pregnancy outcomes.

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

Contaminants in soil

1.17 In 2001, the COT provided advice on a framework for assessing the possible risks to human health associated with long-term exposure to chemical contaminants in soil, based upon the Contaminated Land Exposure Assessment (CLEA) model. In 2008, the Environment Agency in conjunction with the HPA and Food Standards Agency (FSA), and at the request of the Department for Environment, Food and Rural Affairs (Defra), updated this framework guidance.

1.18 The Committee evaluated one of the revised contaminated land framework guidance reports, "Human health toxicological assessment of contaminants in soil". The report aimed to provide support to local authorities and others in assessing and managing the risks to public health posed by land contamination. It described approaches to identifying levels of contamination that would be considered tolerable for those contaminants for which a threshold of effect is assumed, based on a Tolerable Daily Intake, or as posing a minimal risk from non-threshold contaminants, based on a Margin of Exposure (MOE) approach.

1.19 The Environment Agency asked for the opinion of the COT on the technical content and toxicological approach proposed within the TOX Guidance Report.

1.20 The COC chair had confirmed that the approach attempted to be as consistent as possible with current COC advice. The Committee agreed that it would be preferable to use the BMD10 and MOE approach to assess compounds with non-threshold toxicity. Despite the report being one of a pair of reports, with the other one describing exposure modelling, Members agreed that there were other routes of exposure that needed to be included. The Committee also highlighted that the use of the As Low As Reasonably Practicable (ALARP) principle needed to be considered earlier in the report; and that clarity was needed regarding non-threshold toxicity as the report assumed that the interaction with DNA but not necessarily the adverse effect was non-threshold. It was agreed that the epidemiologists of the COC would be consulted for further comment.

1.21 The report was published by the Environment Agency in August 2008.

Dioxin research

1.22 Following up on the discussions during 2007 of FSA-funded research on developmental effects of dioxins in rats (page 7, 2007 Annual Report), the Committee received a presentation from Dr D Bell on additional pre-publication results of investigating interspecies differences in the aryl hydrocarbon receptor (AhR).

Mixtures of food additives

1.23 At its meetings in April, May and July 2008, the COT discussed the results of an FSA-funded study on mixtures of food additives. In a report in 2002 the COT made recommendations on approaches to risk assessment of mixtures of pesticides and similar substances and in 2004 it considered whether these could be applied to mixtures of additives and contaminants and outlined other approaches used for assessing mixtures of additives and contaminants. The COT also commented on the Draft guidance document on "Chemical mixtures: a framework for assessing risks" prepared by the Interdepartmental Group on Health Risks from Chemicals (IGHRC).

1.24 The Committee received a presentation by the researchers. Members considered that the study, which was substantial and complex, was carried out to high technical standards. However, at the doses studied, no overt toxicity was observed with the four additives either individually or in combination. Without using dose levels that demonstrate relevant toxicity, or being able to extrapolate to such levels, it was not possible to interpret the results of the transcriptomics studies with respect to implications for risk assessment.

1.25 The Committee concluded that the new research did not raise concerns that combined exposure to the four compounds tested would pose a risk to health at doses individually below Acceptable Daily Intakes.

1.26 The COT recommended further work to determine the applicability of transcriptomics in risk assessment of mixtures.

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

Nanotechnologies in the food and feed area – comments on the European Food Safety Authority opinion on risk assessment

1.28 In 2007 The European Commission asked the European Food Safety Authority (EFSA) to produce a scientific opinion on the need for specific risk assessment approaches for technologies/processes and applications of nanoscience and nanotechnologies in the food and feed area. In doing this EFSA was required to consider existing national and international evaluations of risk assessment nanomaterials. EFSA was also asked to identify the nature of the possible hazards associated with actual and foreseen applications in the food and feed area and to provide general guidance on data needed for the risk assessment of such technologies and applications.

1.29 In October 2008 EFSA published for public consultation a draft opinion on nanotechnology in food and feed that had been compiled by its Scientific Committee. The COT and the Advisory Committee on Novel Foods and Processes (ACNFP) were consulted on the contents of the draft opinion at their November meetings. In addition the COM and COC were consulted by correspondence.

1.30 The draft opinion considered that the traditional risk assessment paradigm is an appropriate starting point to address the additional safety concerns that may arise due to the characteristics of nanomaterials and "it is the view of the Scientific Committee that this is also appropriate in the food and feed area". This risk assessment strategy was similar to those outlined earlier in a joint statement by the COT, COC and COM in 2005 and in opinions by the EU Scientific Committee on Emerging and Newly Identified Health Risks (SCHENIR) in 2006 and 2007.

1.31 The opinion was considered to be a very good summary of available information. There was a lack of data on biological effects of nanoparticles following oral exposure. Members were disappointed that there appeared to have been only limited developments in the available data in the three years since the COT review of this area. The COT noted the regulatory challenge resulting from the mutable surface characteristics of nanoparticles, which might require increased testing of products rather than ingredients. The COT suggested that the opinion should provide more guidance on the use of the different dose metrics described. The COT agreed with the opinion that there were fundamental differences in the biological effects of soluble and insoluble nanoparticles.

1.32 The comments from the COT together with those from the ACNFP provided significant inputs into the FSA response to the public consultation on the opinion.

Nephropathy observed in a 2-year carcinogenicity study

1.33 As part of an update by the Environment Agency of its technical guidance on assessing the human health risks posed by contaminants in soil, a series of reports that review the toxicology of chemicals identified as priority contaminants are being produced. These toxicological reports provide a synopsis of the available expert reviews by national and international organisations, and from these reviews, identify an appropriate health-based guidance value from which a soil guideline value (SGV) may be derived.

1.34 For ethylbenzene, several organisations had differed in their interpretation of data and treatment of uncertainties, and therefore, the EA had been unable to identify an appropriate health-based guidance value for inhalation exposure. The most sensitive effect had been suggested to be nephropathy seen in female rats in a 2-year carcinogenicity study conducted by the US National Toxicology Program (NTP). The Committee was asked to advise on the interpretation of the NTP study results, and on an appropriate uncertainty factor to be used in the derivation of a health-based guidance value.

1.35 The Committee concluded that the NTP rat study indicated a LOAEL of 750 ppm (3225 mg/m^3) and a NOAEL of 250 ppm (1075 mg/m^3) for renal tubule hyperplasia. It was noted that the NTP study also identified pituitary hyperplasia in female mice exposed to 750 ppm (3225 mg/m^3) and to 250 ppm (1075 mg/m^3). However, the data provided were not sufficient for the Committee to be able to make an assessment of this.

1.36 The Committee agreed that adjustment of the NOAEL for continuous exposure would be appropriate in the absence of any toxicokinetic data, as this would be a conservative approach. The standard uncertainty factor of 100 should be used with the adjusted NOAEL to derive a health-based guidance value. Benchmark dose modelling of the data would be an appropriate alternative and the same adjustment and uncertainty factors could be applied to a $BMDL_{10}$[1].

Peanut avoidance - review of the 1998 COT recommendations on

1.37 Peanut allergy is a serious health problem among UK children, with recent estimates of prevalence suggesting that between 0.2 and 1.8% of children may be affected. Unlike certain other food allergies, peanut allergy commonly persists throughout life, and the only means of managing the condition currently is avoidance of peanut and peanut products.

1.38 In view of the particular severity of allergic reactions to peanut (including anaphylaxis) and the possibility that the prevalence of this allergy was increasing, the COT convened a Working Group in 1996. The aim was to review the scientific evidence on peanut allergy and to advise on whether there was an association between early exposure to peanuts/peanut products and the incidence of peanut allergy in later life. The conclusions of that previous review were that there was some support for the suggestion that the development of peanut allergy in infants can result from exposure *in utero* or during lactation, but that the available data were inconclusive. With regard to the mechanism of sensitisation and allergy, however, a link between peanut consumption by pregnant and lactating women and the incidence of peanut allergy in the child was considered possible. On this basis, it was

[1] Lower 95% confidence limit of the benchmark dose for a 10% increase in response

decided that it would be unwise to discount the possibility of sensitisation of offspring resulting from exposure of the mother. Another major factor considered by the Working Group was that many children were reported to display reactions to peanut following their first known dietary exposure.

1.39 On the basis of the available scientific evidence in 1998, the COT Working Group had issued a number of dietary recommendations, on a precautionary basis. These were aimed at pregnant women and mothers of infants considered at higher risk of developing peanut allergy (i.e. those from atopic backgrounds) and advised that mothers of these high-risk infants may wish to avoid peanuts during pregnancy and whilst breastfeeding, and to delay introduction of peanut into their child's diet until 3 years of age. The COT had also made recommendations for further research in a number of areas in order to progress the state of scientific knowledge.

1.40 Since 1998, several studies have been published on the subject of sensitisation and allergy to foods in relation to early life dietary (and non-dietary) exposures, some of these funded by the Food Standards Agency. In addition, several unpublished and preliminary studies have reported results which suggest that exposure to peanut in early life is associated with a lower incidence of peanut allergy. This has led to the emergence of a new hypothesis, that early exposure to peanut might result in tolerance rather than allergy to peanut.

1.41 There was a need to assess the current state of scientific knowledge in this area and, based on the available evidence, to re-consider whether the 1998 COT recommendations remain appropriate.

1.42 To facilitate the COT evaluation, in September 2007 the Food Standards Agency commissioned a literature review of studies that had been published since 1998 on the early life patterns of exposure to, and avoidance of, food allergens and later development of sensitisation and clinical food allergy, with particular reference to peanut. During the COT horizon scanning discussion in February 2008, Members commented on the scope and content of the literature review and discussed suitable scientific experts in the fields of allergy and paediatric immunology who could form part of an advisory group that could help with the review.

1.43 The contractors of the review presented their findings to the COT at the July Meeting, and COT members assisted by 4 invited experts on allergic disease, discussed the review in detail. A number of provisional specific conclusions about the scientific evidence were reached at the July meeting and a COT Statement was agreed at the October 2008 meeting.

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

Pyrrolizidine alkaloids in food

1.45 Pyrrolizidine alkaloids (PAs) are a large group of natural toxins produced by plants, several of which are known to be highly hepatotoxic and have been shown to be carcinogenic in rats. They have been associated with a number of livestock diseases and with cases of human poisoning following consumption of herbal remedies or after contamination of staple foods. There is also potential for PAs to be transferred to other food products such as honey, milk, eggs and offal.

1.46 In 2007, the report of an FSA-funded project on PAs in honey from borage and ragwort was published. This report was provided to the Committee along with a number of risk assessments of PAs from other countries. The COT was asked for its view on the risk assessment of PAs in food and whether it considered potential human exposure, particularly via honey and milk, to be of concern. To support the COT assessment, the COC evaluated the carcinogenicity of PAs.

1.47 The COT derived a guidance value for non-cancer effects of 0.1 µg/kg b.w./day of riddelliine equivalents (with LD50 values used to convert other PAs to riddelliine equivalents). The COC recommended that PAs should be assessed for carcinogenicity as a cumulative assessment group using the BMDL10 derived from a carcinogenicity study on lasiocarpine with an adequate MOE. Allowing for an MOE of at least 10,000, the COT concluded that PA doses of up to 0.007 µg /kg b.w./day are unlikely to be of concern for cancer risk. Such doses would also not be expected to result in non-cancer effects.

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

Reproductive effects of caffeine

1.49 At its meetings in December 2007 and February, April and July 2008, the COT discussed the reproductive health effects of caffeine, including the results of an FSA-funded study on effects of caffeine consumption in pregnancy on fetal growth restriction. The Committee previously considered possible adverse effects of caffeine consumption on reproduction in 2001 and issued a statement at that time (COT 2001/06).

1.50 In light of the Committee's conclusions in 2001, the FSA issued advice that caffeine intake during pregnancy should be limited to not more than 300 mg/day and offered guidance on amounts of caffeine in different foods and drinks. In addition, the Agency commissioned a prospective study, involving around 2500 pregnant women, in order to reduce uncertainties in the risk assessment and provide a more robust basis for the Agency's advice to pregnant women on caffeine consumption.

1.51 The Committee considered the results of this FSA-funded research and a review of the relevant literature on reproductive effects of caffeine published since 2001.

1.51 The Committee concluded that the available evidence did not indicate a threshold level of caffeine intake below which there was no elevation of risk, and that it seemed likely that risk is increased in association with intakes in the order of 200 mg per day and perhaps even lower.

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

Tobacco products

1.53 The Committee considered a discussion paper which presented a preliminary review of published literature on the toxicological evaluation of tobacco, ingredients and additives used in the manufacture of tobacco, and its emission products. The paper was intended to act as a scoping paper for a systematic review of the literature, with a view to updating the 2004 joint COT/COC/COM

statement on the re-assessment of the toxicological testing of tobacco products. The paper discussed a number of modified toxicological tests, and the COT was asked to identify the limitations of the tests reviewed and to identify particular areas or tests for further literature searching and evaluation. Results based on modified tests were submitted by tobacco manufacturers to regulatory agencies in compliance with the European directive on tobacco products (2001/37/EC).

1.55 Currently, there are no adequate and reliable methods by which to assess the contribution of individual ingredients and additives to the toxicity of tobacco smoke or to evaluate the total toxicity of tobacco products. Furthermore, there are no internationally agreed approaches to the hazard assessment of tobacco products but the EU, and consequently the UK, require submission of toxicological data for regulatory purposes if such data are available.

1.56 The Committee commented that the tests may be scientifically valid for product comparison but that extrapolation of the results, particularly of some of the *in vitro* tests, to human exposure was not possible. All tobacco products are harmful and the Committee queried whether there was any evidence that data from toxicological testing of tobacco products could differentiate usefully between different products.

1.57 The Committee advised that smokers be encouraged to quit smoking or not start in the first place. Efforts to make products less harmful might be appropriate but members were concerned that the results of these tests would be used to support claims of reduced health risk, which might be invalid. In addition, the Committee recognised that there might be interest from the tobacco industry in introducing new ways of inducing people to use or keep using tobacco products.

1.58 The Committee confirmed a conclusion from the 2004 joint Committee Statement that it was not possible to compare tobacco products for risk to human health without reliable biomarkers for all chronic diseases associated with tobacco use. There had been a considerable increase in the number of publications from tobacco manufacturers on the development of new toxicological testing methods and adaptation of existing methods, but members considered that there had been an overemphasis on mutagenicity testing. It was also important to derive methods that would permit the comparison of risks related to tumour promotion and diseases of the cardiovascular and pulmonary system. The Committee observed that there had been an increase in the use of *in vitro* cytotoxicity tests and the number of endpoints studied. The 2004 joint statement had indicated that some product comparisons could be undertaken based on *in vitro* cytotoxicity tests but also that the results of such tests could not be extrapolated to *in vivo*.

1.59 Further review work would be valuable to produce a clear rationale for toxicity testing strategies and the limitations of the tests used for tobacco product evaluation. However, the Committee also considered that it would be more appropriate to base product evaluations on epidemiological data where these were available. Members were informed that there was interest in the tobacco industry in developing biomarkers of diseases associated with tobacco product use, which might be used to support produce claims of reduced harm. Challenges in producing a toxicological testing strategy and approach to biomonitoring in volunteers were set out in the 2004 statement, and it was considered unlikely that the data currently available would be adequate to reach meaningful conclusions.

1.60 The Committee was informed that the Department of Health had been presented with data where reduced harm had been claimed but such claims had been refuted on the basis of the 2004 joint Committee statement. The Committee suggested that it would be appropriate for it and its sister committees to be consulted when regulators received a dossier where the claim appeared scientifically plausible based on the 2004 statement. Consideration would be given to updating the 2004 joint COT/COC/COM statement following further review of the literature. Relevant areas on mutagenicity and carcinogenicity would be referred to the COM and COC.

Terephthalic acid

1.61 The COT had previously issued a statement on terephthalic acid (TPA) in 2001. At that time, Members recommended that an appropriate study be conducted to assess the potential for endocrine disruption and asked for the COM to review the genotoxicity of TPA.

1.61 A multigeneration reproductive toxicity study was submitted to the COT in 2003. Histopathological evaluation was subsequently performed on all dose groups from this study along with several other chronic and sub-chronic studies that tested TPA. The Committee assessed these data in 2005 and considered that the data did not indicate a need to reduce the temporary tolerable daily intake set by the Scientific Committee on Food in 1986.

1.63 The COT delayed issuing a statement in order that the conclusions of the COM could be incorporated. The COM considered that the available data indicated that TPA was not an *in vivo* genotoxin and published a statement in 2007.

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

2006 UK Total Diet Study of metals and other elements

1.65 The FSA completed a survey of 24 elements (aluminium, antimony, arsenic, barium, bismuth, cadmium, chromium, copper, germanium, indium, lead, manganese, mercury, molybdenum, nickel, palladium, platinum, rhodium, ruthenium, selenium, strontium, thallium, tin and zinc) in the 2006 total diet study (TDS). The Committee had previously evaluated dietary exposures to the elements in the 2000 TDS in 2003 and published a statement.

1.66 Estimates of dietary exposure were calculated for each of the 24 elements using food consumption data taken from the Expenditure and Food Survey and the National Diet and Nutrition Surveys. The Committee agreed conclusions for each element and recommended priorities for future research and surveys of elements in food.

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

Committee procedures

Horizon Scanning

1.67 At the February 2008 meeting, members were provided with information on planned and possible discussion items for the year, and invited to comment on emerging issues that might also need to be addressed. A number of potential items were discussed in addition to those subsequently discussed as substantive agenda items and described above.

1.68 Developmental neurotoxicity had first been raised as a possible discussion item in the Horizon Scanning exercise in 2007. The Committee noted that the topic was being reviewed by an International Life Sciences Institute (ILSI) Working Group, which had been due to report in early 2007. Members had agreed that as a first step it would be useful to provide the COT with a copy of the ILSI report, which was being published as a series of five papers. To date, only four of the five papers had been published. The fifth paper – *Application of Developmental Neurotoxicity* was still awaiting publication and it was questioned whether the four papers should be presented to the COT before this final paper is available. The Secretariat would follow this up with ILSI.

1.69 Benzimidazole compounds are used as pesticides and/or veterinary medicines. Many benzimidazoles have been demonstrated to be aneugenic *in vitro* and/or *in vivo* and common effects have been identified in the toxicological database for benzimidazoles such as teratogenicity, fetotoxicity, hepatotoxicity, thyroid effects, testicular toxicity/aspermatogenesis and haemotoxicity. It was suggested that the Committee could consider whether some or all of the adverse effects produced by benzimidazoles could be related to a common mode of action such as microtubule disruption. This would aid the FSA and other departments in assigning these compounds to a common mechanism group and taking forward a cumulative risk assessment. The COM had previously provided advice on a strategy assigning these compounds to a common mechanism group for aneugenicity.

1.70 Members observed that the EFSA Panel on Plant Protection Products and their Residues (PPR) was currently preparing an opinion on cumulative risk assessment of pesticides and therefore COT should not follow it up at this time. As with other bodies such as the IGHRC and ongoing work by the International Programme on Chemical Safety (IPCS), this was likely to recommend a framework approach to mixtures risk assessment. Benzimidazoles were already a tentative common mechanism group, based on pesticidal/veterinary mode of action and observed toxicological effects. The first stage would be to perform an initial cumulative risk assessment using the Hazard Index approach. If this indicated no concerns from cumulative exposure, then there would be no need for further work to refine the risk assessment. If it did indicate a potential concern then further work would be warranted, including more detailed consideration of whether all the benzimidazoles should be included in a common mechanism group and consideration of appropriate toxicological equivalency factors (TEFs). It was suggested that other Committees involved in the assessment of pesticides and veterinary medicines should also be included in any further work taken forward by the COT.

1.71 Members noted the following topics as an emerging issue:

1.72 Developments in toxicity testing strategies, as reviewed by the U.S. National Academy of Sciences in its report on toxicity testing for the 21st century which highlighted systems biology as an emerging approach, and the U.S. Environmental Protection Agency which was developing a computational approach for risk assessment. It was suggested that this may be something that the COT could consider, possibly as a topic for a future workshop.

1.73 Members also expressed an interest in keeping informed on nanoparticle toxicology as this is likely to have a large impact on the food industry.

Working Groups and Workshops

Lowermoor Subgroup

1.74 Members were previously informed that the deaths of two individuals who had lived in the area which received contaminated water following the 1988 Lowermoor Water Pollution Incident had been referred to the West Somerset coroner. The two individuals both had a neurodegenerative disease and had been reported to have higher than usual levels of aluminium in the brain. Information on brain neuropathology and aluminium concentrations was available for one of the individuals but not the other.

1.75 The secretariat of the COT Lowermoor Subgroup had written to the coroner to ask whether he would be able to share information on the brain pathology and aluminium measurements of the second case with the Subgroup. The coroner had replied that he had decided that the inquest into both deaths should be brought before a jury and that he considered it imperative that there was no contact between his office and anyone else involved in the Lowermoor investigation in case it compromised his impartiality. Members were advised that there should be no communication between the COT and the coroner's court

1.76 The COT was informed that Department of Health lawyers had advised that publication of the Subgroup's report before the Coroner's proceedings were completed could be seen as an attempt to bias the jury and this had led to a delay in publication.

Workshop on Transgenerational Epigenetics

1.77 In February 2008, a one-day workshop on transgenerational epigenetics was held, to enable the Committee to increase its awareness of current knowledge in this area, and to consider possible implications for chemical risk assessment.

1.78 Invited speakers gave presentations on epigenetics and the epigenetic code, evidence for transgenerational epigenetic inheritance from animal and human studies and implications for risk assessment. Members participated in discussions at the meeting and subsequently while producing a statement containing the speakers' abstracts and summarising the discussion.

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

Ongoing work

Glucosamine and hepatotoxicity

1.80 Glucosamine is a popular food supplement taken alone or in combination with chondroitin sulphate, usually by sufferers of osteoarthritis. In view of a very small number of individual case reports linking glucosamine and hepatitis, the COT was asked to consider whether a causal relation was plausible.

1.81 Glucosamine and/or chondroitin sulphate have been the subject of numerous placebo controlled trials in human volunteers which have not shown any evidence of adverse effects on the liver. More limited data were also available from studies in laboratory animals which did not show any evidence of adverse effects on the liver.

1.82 A COT statement on this issue is in preparation and will be published in 2009.

Potential exposure to substances from landfill sites

1.83 In 1998, the Committee considered an epidemiological study called the EUROHAZCON study which reported that women living near hazardous waste landfill sites had an increased risk of giving birth to a baby with a congenital anomaly. In 2001, it published a statement on a study from the Small Area Health Statistics Unit (SAHSU) which investigated the incidence of birth outcomes and certain cancers around landfill sites in Great Britain. The committee was largely reasssured by the findings but considered that the small raised risk for all congenital anomalies in people living around special waste landfill sites merited further investigation. Further, then unpublished, studies by SAHSU were discussed in 2007. At that time, the committee was reminded of a Government sponsored programme of work on congenital anomalies and landfill sites. At the September 2008 meeting, the committee was presented with an unpublished technical report of a project to measure emissions from landfill sites.

1.84 A statement is being prepared and further discussions will take place in 2009.

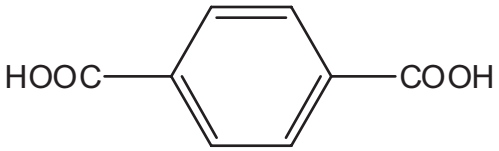
Statements of the COT

Update Statement on the Toxicology of Terephthalic Acid

Background

1. Terephthalic acid (TPA; Figure 1) is used as a starting material in the manufacture of polyethylene terephthalate (PET). PET may be used to coat the internal surface and welded joints (side stripes) of food cans. PET can also be used to manufacture beverage bottles.

Figure 1: Terephthalic acid



2. In research on potential for contamination of food, TPA was found to migrate from can coatings into food at levels between the limit of detection (0.2 mg/kg food) and limit of quantification (0.7 mg/kg food) of the assay employed.[1] Subsequently, TPA was included in a Food Standards Agency (FSA) funded survey of plastic materials and articles in contact with food, which examined compliance with statutory limits on composition and migration[2]. In this survey, fifty foods packaged in PET were tested, with no measurable migration of TPA into the food simulant[a].

3. In law, migration from can coatings is subject to the general requirement that applies to all food contact materials and is laid down by Article 3 of Regulation (EC) 1935/2004. This requires that food contact materials and articles should not transfer their constituents to foodstuffs in quantities that could endanger human health or affect the nature or quality of the food. In addition, migration of TPA is specifically controlled where it is used in food contact plastics. Commission Directive 2002/72/EC lays down a specific migration limit (SML) for TPA of 7.5 mg/kg food or food simulant. This is enacted in England by The Plastic Material and Articles in Contact with Food (England) (No.2) Regulations 2006, with parallel legislation in Scotland, Wales and Northern Ireland.

4. In 1986, the Scientific Committee on Food (SCF) reviewed the toxicology of TPA and established a temporary tolerable daily intake (t-TDI) of 0.125 mg/kg bw/day[3]. Although details of the derivation are not available, it was presumably based on a 90-day oral feeding study in male and female Wistar and CD rats, with application of a 500-fold uncertainty factor to the no observed adverse effect level (NOAEL) of 0.125% TPA in the diet, equivalent to 62.5 mg/kg bw/day (calculated assuming adult rats consume 20 g of food food per day with an average body weight of 400 g). Reduced body weight gain was reported at levels of 0.5, 2 and 5% TPA in the diet in the 90-day oral feeding study. In a separate reproductive toxicity study, formation of renal and bladder calculi was observed at postnatal day 51 in Wistar and CD rats consuming 5% TPA in the diet. The t-TDI was classed as temporary pending the submission of the full report of this study. However, the SCF does not appear to have revisited the risk assessment and re-evaluation of terephthalic acid is included in the current work programme of the European Food Safety Authority (EFSA).

[a] Food simulants are standard test liquids, specified in Directive 85/572/EEC, which are used to simulate real foods in migration studies. The specified simulants, to be used depending on the particular food types being tested, are: distilled water; 3 % acetic acid (w/v in aqueous solution); 15 % ethanol (v/v in aqueous solution); and rectified olive oil. Where there are technical difficulties using rectified olive oil, substitute fatty food simulants such as sunflower oil or synthetic triglycerides may be used.

Previous COT Evaluations

5. In October 2000, the COT considered the possible health effects of TPA in the context of the survey on the migration from can coatings into food[1]. Dietary intakes of TPA from canned foods were estimated for high level (97.5th percentile) consumers. These estimates ranged from 2.5 µg/kg bw/day for adult consumers, to 7.4 µg/kg bw/day for infants. The COT concluded that these exposures were not of concern for public health on the basis of the then available information. However, although the submitted data did not indicate that TPA can modulate the endocrine system, the studies were inadequate to exclude the possibility. This was a concern because of structural similarities to phthalate esters that are reported to have endocrine-disrupting potential. It was therefore recommended that a suitable study be conducted to determine whether TPA has endocrine-disruptor activity. Furthermore, in view of the occurrence of urinary bladder tumours in Fischer F344 rats fed the highest dietary concentration of TPA (2% in the diet) in a 2-year carcinogenicity study, the COT recommended that an opinion be sought from the COM on the potential *in vivo* genotoxicity of the compound[4] in order to gain insights into the likely mechanism of tumour formation.

Reproductive Toxicity

6. In June 2003, a manufacturer submitted the report of a full multi-generation reproductive toxicity study, which concluded that dietary administration of up to 20 g/kg diet TPA for two successive generations did not result in any alterations in reproductive performance.

7. In 2003, Members noted that the bodyweights of the pups in this study were comparable at birth, except for the F_2 generation, where a lower weight was associated with larger litter size. Observed differences in pup bodyweights at later ages were thought likely to result from a direct effect of the TPA on the pups, and were not considered to be developmental effects. Observed changes in developmental endpoints were considered likely to result, in turn, from the reduced bodyweight and size of the pups. It was also noted that the effect of TPA on anogenital distance (AGD) was larger than the effect on bodyweight, although there was no clear dose-response relationship.

8. Overall, Members were satisfied that the information provided in the report was sufficient to demonstrate that terephthalic acid did not have endocrine-disrupting effects at the highest dose tested in this study, resulting from administration of TPA at 20 g/kg diet. Subsequently, Members have noted emerging evidence that this type of study might not be sufficiently sensitive to anti-androgenic activity. In 2003, since histopathological changes in the urinary bladder and the kidney were reported at 20 g/kg, Members considered that further histopathological examination was required.

Histopathology of the kidney and urinary bladder

9. Members noted that reductions in kidney weights occurred at all doses of TPA, making the 1g/kg diet dose level (equivalent to 100 mg/kg bw/day, calculated assuming young rats consume 10 g of food per day with an average body weight of 100 g) the lowest observed effect level (LOEL) for the effect on kidney weight. Histopathological changes in the urinary bladder and the kidney were reported at the high dose (20 g/kg diet), but these organs had not been examined in the mid- and low-dose groups

(1 and 5 g/kg diet). Therefore, the Committee considered it important to receive further information about the effects observed in the kidneys and the urinary bladder.

10. In March 2005, a report describing further histopathological examinations of the kidneys and urinary bladder of animals in the TPA multi-generation study was submitted to the COT. This was accompanied by an expert report discussing the histopathology of the kidneys of animals in this study.

11. A variety of changes were observed in the urinary bladder of rats of both sexes receiving 20 g/kg diet TPA. These changes comprised transitional epithelial hyperplasia, cystitis, inflammatory or mononuclear cell infiltration and haemorrhage. The incidence of observed changes was higher in the F_1 generation than in F_0 animals possibly reflecting the longer period of exposure of the former. The author considered that these changes were related to treatment and indicated an irritant effect of the compound on the bladder mucosa at this dose level. No changes were observed in the bladders of animals receiving 1 or 5 g/kg diet TPA or in controls.

12. Minimal or slight renal papillary necrosis was observed in the kidneys of a few males (2/10 F_0 and 2/11 F_1) receiving 20 g/kg diet TPA but not in any of the control or lower dose group males. Similar changes were observed in 3/10 F0 females receiving 20 g/kg diet. However minimal papillary necrosis was also observed in 1/10 control F_0 females and 1/10 F_0 females receiving 1 g/kg diet TPA. Necrosis, classified as slight, was confined to animals (1 F_0 male and 1 F_0 female) receiving 20 g/kg diet TPA. Although the increase in incidence and severity versus controls was small, the author of the report considered it likely that this was related to treatment with TPA. No other gross or microscopic changes were detected in adults or pups. The NOAEL for pathological changes in this study was 5 g/kg diet TPA.

13. The submitted expert report also considered the observed toxicity of TPA to the urinary system of the rat in a combined 90-day and one-generation reproductive study in Wistar and CD (Sprague Dawley) rats, a two-generation reproductive toxicity study in Alpk: APfSD (Wistar-derived) rats and a chronic/oncogenicity study in Fischer 344 rats. A number of treatment-related histopathological findings were reported in the urinary system but no consistent pattern of renal toxicity was observed across the available studies. In addition there was little evidence to suggest differing susceptibility amongst rat strains with regard to renal toxicity. The author of the report considered that potentially adverse histopathological findings in the two-generation reproduction study were confined to an increase in incidence and severity of papillary necrosis in the F_0 and F_1 parents, which had a clear NOAEL.

14. The physiological response of the rat kidney to TPA has been characterised in multiple studies and includes urinary acidification with increased urinary excretion of calcium and phosphorus. Consistent treatment-related findings in these studies were confined to the bladder and included chronic inflammatory, hyperplastic and neoplastic changes; these occurred both in the presence and absence of calculi. The pattern of treatment-related bladder findings in Alderley Park Wistar-derived rats was consistent with those observed in the bladder of other rat strains. The report noted that kidney weight reduction was observed in F_0 adult rats which had not been exposed *in utero*; without associated adverse pathological changes in the kidney, or urinary system as a whole; and at doses that did not affect the growth and development of treated rats.

15. Members were satisfied that the additional histopathological data indicated a clear no observed adverse effect level (NOAEL) for histopathological changes in the urinary bladder and kidney (renal papillary necrosis) corresponding to administration of TPA at 5 g/kg in the diet in the multi-generation study. Statistically significantly decreased renal weights (adjusted for bodyweight) were present in all generations including the parental generation. However, given that there was no associated histopathology or effect on renal function, it was not clear whether this effect should be considered adverse. It was also noted that this effect was not observed in a chronic toxicity study using a different rat strain.

16. It was agreed that the relevance of the effect should be considered by applying internationally agreed criteria from the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) for distinguishing between adverse and adaptive effects[5]. This would provide a clear description of the rationale for the Committee's conclusions.

Application of ECETOC criteria for distinguishing between adverse and adaptive effects

17. Using this approach, effects are generally considered less likely to be adverse if: there is no alteration in the general function of the organism or organ; it is considered an adaptive response; and if the effect is not a known precursor of an adverse effect. Other considerations in reaching conclusions could include a lack of histopathology associated with an effect and reversibility of the effect if a recovery period was used in the experimental design.

18. As the kidney weight reductions occurred in the F_0 generation, as well as the offspring, this effect was not considered to be specific to development of the kidney. There were no treatment-related findings in the kidneys of F344 rats in a chronic/oncogenicity study. Other 90-day studies carried out using Wistar and Sprague Dawley rats did not find treatment-related effects on kidney weight.

19. The consultant veterinary pathologist reviewing the study for the sponsor considered the kidney weight reduction in the Alderley Park Wistar rats most likely to represent a colony-specific physiological adaptation to exposure to terephthalic acid.

20. The NOAEL from the multi-generation study was therefore the dose resulting from administration of TPA at 5 g/kg in the diet. This was in the region of 425 – 1200 mg/kg bw/day, depending on food consumption of the different treatment groups.

COM Evaluation

21. In November 2001, the COM evaluated a limited package of mutagenicity data. This included *in vitro* bacterial and mammalian mutagenicity assays that, although finding TPA to be negative, were either poorly reported or had inadequate protocols; and a negative *in vivo* mouse micronucleus assay.

22. The COM was provided with additional data in 2006. Although there was some concern over results of the *in vitro* mammalian cytogenetics test, this did not meet the criteria for a positive result. An additional *in vivo* unscheduled DNA synthesis assay (UDS) was also supplied which, together with the mouse micronucleus assay, were considered sufficient to indicate that TPA is not an *in vivo* mutagen.

Therefore, the available evidence was considered to support a non-genotoxic mechanism for the bladder tumours seen in the rat carcinogenicity study[6].

Conclusions

23. We note the conclusions of the COM that terephthalic acid lacks *in vivo* genotoxicity, which supports there being a non-genotoxic mechanism of action for bladder tumour formation.

24. We are satisfied that the submitted reproductive toxicity study demonstrates that terephthalic acid is not an endocrine-disruptor.

25. The decreased kidney weights in the multi-generation study probably constitute an adaptive rather than adverse effect. Therefore, this is unlikely to be a cause for concern with regard to human exposure to teraphthalic acid.

26. Histopathological changes in the kidney represent the most sensitive toxicological endpoint, allowing the identification of a NOAEL at a dose level equivalent to 425 mg/kg bw/day. High level (97.5th percentile) consumer dietary intakes of TPA from canned foods were estimated from the FSA survey[1]. These ranged from 2.5 µg/kg bw/day for adult consumers, to 7.4 µg/kg bw/day for infants; indicating margins of exposure of 170,000 and 57,000 for adults and infants respectively.

27. Therefore, in line with our previous statement[4], we do not consider the concentration of TPA found to migrate from food can coatings in the FSA funded study to be of concern for public health. The new data evaluated do not indicate a need to lower the temporary TDI of 0.125 mg/kg bw/day, proposed by the SCF and scheduled for re-evaluation by the EFSA expert panel on food contact materials, enzymes, flavourings and processing aids.

COT Statement 2008/01
July 2008

References

1 Food Surveillance Information Sheet No. 7/00. (October 2000). Chemical Migration From Can Coatings into Food - Terephthalic and Isophthalic Acids. http://www.food.gov.uk/science/surveillance/fsis2000/7phthal

2 Food Surveillance Information Sheet No. 43/03. (October 2001). Chemicals used in plastic materials and articles in contact with food. http://www.food.gov.uk/multimedia/pdfs/fsis4303.pdf

3 Report of the Scientific Committee for Food on certain monomers and other starting substances to be used in the manufacture of plastic materials and articles intended to come into contact with food (1982) Reports of the Scientific Committee for Food, Seventeenth Series, http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_17.pdf

4 COT Statement on terephthalic and isophthalic acids from can coatings (COT/2000/08) http://www.food.gov.uk/multimedia/pdfs/cotacids.pdf

5 European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Technical report No 85; Recognition of, and Differentiation between, Adverse and Non-adverse Effects in Toxicology Studies

6 COM Statement on the Mutagenicity of Terephthalic Acid (COM/07/S6) http://www.iacom.org.uk/statements/documents/tpa07.pdf

COT statement on a SAHSU Study on Chlorination Disinfection By-Products and Risk of Congenital Anomalies in England and Wales

Introduction

1. The Committee considered the issue of chlorinated drinking water and adverse reproductive outcomes in 1999 and 2001. Our most recent statement on this topic was published in 2004[7]. In light of a new, large study by the Small Area Health Statistics Unit (SAHSU), which investigated potential associations between chlorination disinfection by-products and the risk of congenital anomalies, and additional studies on other reproductive outcomes published since 2004, we were asked to consider whether we wished to revise our earlier advice.

Background

Chlorination and disinfection by-products (DBPS)

2. Disinfection of drinking water is an important public health measure and UK public water suppliers are required to disinfect the water supply. Chlorination is the most commonly used method of disinfection in the UK and is intended to protect human health from microbial contaminants. Disinfection of drinking water is fundamental to preventing the spread of waterborne diseases, such as cholera.

3. In addition to disinfecting drinking water, chlorination can also produce a range of disinfection by-products (DBPs) by reaction between chlorine and natural organic matter (NOM) present in surface waters. In most supplies, the main DBPs are the four chlorinated and brominated trihalomethanes (THMs): chloroform, bromodichloromethane (BDCM), dibromochloromethane (DBCM), and bromoform. Haloacetic acids (HAAs), haloacetonitriles (HANs), halophenols, haloaldehydes and haloketones can also be formed.

4. The sum of the four THMs is termed Total THMs (TTHMs). DBPs are currently regulated in the UK by specifying a maximum concentration of 100 micrograms/litre for TTHMs, measured at the consumers' taps (concentrations of DBPs may increase within the distribution system due to the continued reaction of residual chlorine with NOM). HAAs and other DBPs are not regulated directly. TTHM concentrations are often regarded as a marker for total DBPs. Removal of precursor organic compounds before chlorination is commonly practised in the UK to reduce TTHM concentrations, and this is also considered to reduce the formation of HAAs and other DBPs, which are therefore limited indirectly.

Previous COT advice on chlorinated drinking water and reproductive outcomes

5. During 1998 and 1999, the COT considered the available epidemiological information on the association between chlorination by-products in drinking-water and a range of adverse reproductive outcomes. Animal reproductive toxicity studies with some individual chlorination by-products were also considered. After evaluation of the data, the COT concluded the following:

- "We consider that there is insufficient evidence to conclude that the presence of chlorination by-products in tapwater increases the risk of adverse reproductive outcomes.
- We recommend, however, that the claimed associations between patterns of drinking water-intake and the incidence of adverse reproductive outcomes be investigated further, since any causal association would be of significant public health concern.
- We therefore consider that efforts to minimise exposure to chlorination by-products by individuals and water authorities remain appropriate, providing that they do not compromise the efficiency of disinfection of drinking water" [2].

The COT considered the issue again in 2001 and reaffirmed its 1999 conclusions [3].

SAHSU (first phase) study on adverse reproductive outcomes and chlorination disinfection by-products and 2004 evaluation

6. In 2004, the COT considered a first phase study by SAHSU that investigated chlorinated drinking water and adverse reproductive outcomes using routinely collected THM measurements in drinking water (as an index of exposure to chlorination by-products) and available health statistics on stillbirths and birthweight [4].

7. In the 2004 SAHSU study, modelled estimates of quarterly THM concentrations in water zones from 3 water companies in England (Northumbrian, Severn Trent Water and United Utilities) were linked to about 1 million routine birthweight and stillbirth records based on location of maternal residence at the time of birth. THM estimates corresponding to the final three months of pregnancy were used. Three TTHM exposure categories were defined: low (below 30 micrograms/litre), medium (30 – 60 micrograms/litre) and high (above 60 micrograms/litre). In its evaluation the COT noted that in the North West (United Utilities) THM exposure showed an inverse association with mean birth weight, a direct association with prevalence of low and very low birthweight, and a direct association with the prevalence of stillbirths. However, there was evidence of confounding by social deprivation, adjustment for which may not have been complete. In the Severn Trent region, in contrast, the prevalence of very low birthweight decreased with increasing TTHM exposure, and there was no association with low birthweight or stillbirth rate. In the Northumbrian region, there was no evidence of associations between TTHM levels and any of the pregnancy outcomes, but the number of births included in the study was relatively small.

8. In its 2004 evaluation, the COT also considered data from thirteen other epidemiological studies published after the 1998 evaluation, which investigated associations between chlorinated drinking-water and pregnancy outcomes (other than congenital malformations) [1].

9. Overall, the committee concluded that the data that it had evaluated did not show a causal relationship between chlorinated drinking-water and pregnancy outcomes, namely: low birth weight, very low birthweight, stillbirth, spontaneous abortion, perinatal death, infant death, low Apgar score, infant's head circumference at birth, infant's body length, pre-term delivery, length of gestation, neonatal jaundice and neonatal hypothyroidism [1]. Data on congenital malformations were not

assessed. Further research to reduce the uncertainties in the interpretation of the reported associations between intake of drinking-water and the incidence of adverse reproductive outcomes was recommended. The COT added that while research to determine the effects of chlorinated water continued, efforts by water companies to minimise consumers' exposure to chlorination by-products would remain appropriate provided that the efficiency of disinfection was not compromised.

SAHSU (second phase) study on chlorination disinfection by-products and risk of congenital anomalies in England and Wales

10. SAHSU has now completed phase 2 of its research, which is on congenital anomalies [5]. This study is the largest of its type so far. The study examined the relationship between THM levels in the public water supply and risk of congenital anomalies in England and Wales. The primary analysis focused on TTHM (as a marker for disinfection by-products) and broad categories of congenital anomalies. A secondary analysis focused on restricted subsets of anomalies and specific THM groups including bromoform and brominated THMs.

11. THM data were taken from twelve water companies, where water samples were routinely taken from consumers' taps in each water supply zone (each zone covered a population of up to 50,000). The raw THM data were modelled to give more robust estimates of the mean THM concentration in each zone.

12. Individual records of congenital anomalies and maternal postcode at the time of birth were obtained from the National Congenital Anomalies System (NCAS), the regional congenital anomaly registries via the British Isles Network of Congenital Anomaly Registers (BINOCAR), and the national terminations registry.

13. The broad categories of congenital anomalies in the primary analysis included: cleft lip/palate; diaphragmatic hernia and abdominal defects; major cardiac defects; neural tube defects; urinary tract defects; and respiratory defects.

14. Further analyses were conducted using restricted groups of congenital anomalies with better ascertainment that were considered likely to share the same causes. These included: abdominal wall defects, major cardiac defects, specific urinary tract defects, and respiratory defects. The relevant ICD-10 codes are given in the publication. Additionally, separate analyses were conducted for cleft palate, cleft lip with and without cleft palate, exomphalos, gastroschisis, hypoplastic left heart syndrome, ventricular septal defects, congenital anomalies of the oesophagus and two subsets of urinary tract defects comprising intrinsic kidney disease and urinary obstruction. Further analyses were conducted excluding cases with anomalies that were found to be part of a chromosomal syndrome, as well as examining cases with isolated anomalies only.

15. There were in total 22,828 cases which had at least one congenital anomaly from the broad categories in the primary analysis: 1,641 (7.2 %) of these had a chromosomal defect, 2,249 (9.9%) were classified as having multiple (non-chromosomal) anomalies and 18,938 (83.0%) were classified as having isolated anomalies only.

16. The study period was defined according to the first possible date on which THM data for the first trimester were available (i.e. 15 October 1993 for United Utilities & Severn Trent; 15 October 1997 for Northumbrian; and 15 October 1998 for all other water regions) until 31 December 2001.

17. A postcode to water zone link was created using a Geographical Information System (GIS). The postcode of the maternal residence at the time of birth was used to identify the water zone of interest and the appropriate modelled exposure in the first trimester for each birth record.

18. The weighted average THM estimate associated with each birth record was categorised into one of three pre-defined exposure categories for each of three metrics: concentrations of TTHMs (< 30, 30 - < 60 and 60 + micrograms/litre), total brominated THMs (< 10, 10 - < 20 and 20 + micrograms/litre), and bromoform (< 2, 2 - < 4 and 4 + micrograms/litre).

19. Statistical analysis adjusted for potential confounders including sex, maternal age, and socio-economic status. Also, interactions between THM exposure and potential confounding variables were tested.

20. Mean TTHM concentrations ranged from 16.4 micrograms/litre in the lowest exposure category, to 72.2 micrograms/litre in the highest. The highest correlations were seen between total brominated THMs and dibromochloromethane (0.93), and between TTHM and chloroform (0.90).

21. There was a higher prevalence of each anomaly in the most deprived compared to the most affluent areas. Prevalence of anomalies was similar in males and females, except for cleft lip/palate and urinary defects, where prevalence was 50 – 100% higher in males. There were U-shaped relationships between prevalence of congenital anomalies and maternal age, except for neural tube defects where the prevalence decreased with increasing age. The reported prevalence of each anomaly was substantially higher in the regional registries than in the NCAS reflecting better ascertainment.

22. Unadjusted and adjusted analyses showed similar risk estimates. There were no statistically significant trends across the three exposure categories for TTHMs, total brominated THMs or bromoform, for either the broadly defined or restricted groups of anomalies.

23. The only significant association ($p < 0.05$) within the broadly defined groups of anomalies was an excess risk of major cardiac defects in the medium (but not high) exposure category of total brominated THMs (OR 1.12, 95% CI 1.01 – 1.23). For the restricted set of isolated anomalies, there was a statistically significant excess risk of ventricular septal defects (OR 1.43, 95% CI 1.00 – 2.04) associated with exposure to TTHM in the highest category, and for congenital anomalies of the oesophagus (OR 1.66, 95% CI 1.12 – 2.45) in the medium (but not high) category of TTHM exposure.

24. For bromoform, there was a significant excess of both major cardiac defects and gastroschisis (OR 1.18, 95% CI 1.00 – 1.39 and OR 1.38, 95% CI 1.00 – 1.92, respectively) in the high exposure category.

25. The authors concluded that this large national study found little evidence for a relationship between THM concentrations in drinking water and risk of congenital anomalies. There were no significant interactions between TTHM exposure and any of the potential confounders. The authors noted that the significant positive associations may have been due to chance as there is little toxicological

evidence for reproductive or teratogenic effects for bromoform or other DBPs, and the bromoform concentrations in the study were low. It was also noted that careful selection of subsets of major cardiac defects, ventricular septal defects and gastroschisis as isolated anomalies may have increased accuracy of case definition (and reduced misclassification).

26. We note that the SAHSU study focussed on the main DBPs in chlorinated drinking-water, namely THMs, and did not consider other DBPs such as HAAs.

27. We also note that studies of this type, which use a group level exposure assessment, do not consider variations in individual exposure: for example, movement of women between exposure zones during pregnancy, variation in individual water consumption and additional exposure from other sources such as showering, bathing and swimming were not taken into account. The lack of individual exposure data limits the interpretation of the results and might obscure the detection of an association.

28. Nevertheless, we consider that this was a large and well designed study and that it did not indicate a relationship between THMs and congenital anomalies.

Additional data

29. To date, THMs have been the most widely measured individual DBPs in epidemiological studies. However, THMs may not be a good marker of other DBPs. A preliminary study measuring the next largest group of DBPs, namely HAAs, in the UK drinking water found a high correlation between TTHMs and HAAs in two of three study regions, but no correlation in the third region. A total of five HAAs were detected at relatively high levels, with the means ranging from around 35 to 95 micrograms/L for the three regions investigated [6]. THMs and HAAs were present in by far the greatest concentrations, with other DBPs considered to be present at much smaller concentrations, usually less than 1 microgram/L [7].

Additional epidemiological studies on congenital malformations/birth defects

30. In addition to the SAHSU study, ten other epidemiological studies were identified which investigated associations between drinking water chlorination and congenital anomalies [8,17]. The studies are described in the discussion paper considered at our February 2008 meeting [18]. The search strategy is appended in Annex 1. Subsequently two further studies were published, and reviewed by the Committee [25, 26].

31. No UK-based studies were identified. Most studies were conducted in the USA, Canada, Norway and Sweden. The retrieved studies were smaller in size than the SAHSU study, and most had limitations such as inadequacies in exposure assessment and a main focus on outcomes other than congenital anomalies. The additional studies investigated various categories of anomalies, including all congenital anomalies, cardiac defects, nervous system anomalies, urinary tract defects, respiratory defects and oral clefts. Overall, the additional epidemiological evidence is inconsistent and does not suggest an association between drinking water chlorination DBPs and congenital anomalies.

Additional epidemiological studies on adverse pregnancy outcomes since the 2004 consideration

32. The COT previously reviewed the literature on DBPs in drinking water and other adverse birth outcomes (other than congenital anomalies) when it considered the phase 1 study by SAHSU in 2004. Five additional epidemiological studies were identified for the present review [19, 23]. These studies are described in the Committee discussion paper [18]. The search strategy is appended in Annex A.

33. None of these studies was conducted in the UK. Some evaluated associations with total and individual HAAs in addition to THMs (e.g. monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid), and a number of outcomes were considered including measures of growth retardation (such as intrauterine growth retardation, small for gestational age, and low term birth weight), pregnancy loss or pre-term delivery. These studies do not show a consistent relationship between drinking water DBPs and adverse pregnancy outcomes.

Animal data

34. Animal data on DBPs and adverse birth outcomes were previously considered by the COT in 1998 and 2004. In 1998, the committee concluded that the available reproductive toxicity studies conducted with individual chlorination by-products indicated that the levels of exposure to these substances in drinking water were about 10,000 times lower than levels at which adverse effects occur in animals [1].

35. A recent weight of evidence review [24] considered reproductive and developmental animal studies on a number of individual DBPs, including both THMs and HAAs, and described more recent toxicological studies including studies on congenital anomalies. Studies published between 2001 and 2006 were considered. The updated review found little indication of previously unreported reproductive or developmental toxicity. It concluded that the NOAELs and LOAELs in animals are much higher than known levels of human exposure, and that there are limited data that explore modes of action for reproductive toxicity. The authors noted that in a few instances, mild adverse reactions were reported in fetuses of dams treated at doses that produced maternal toxicity, and were generally considered secondary to maternal toxicity.

36. The review data confirm our previous view that, in animal studies, reproductive/developmental effects have mainly been seen with DBPs at high doses often associated with maternal toxicity. The fact that positive findings are seen in animal studies under these conditions does not provide corroboration for positive associations observed in epidemiological studies.

Overall conclusions

37. We conclude that in human studies there is no consistent relationship between chlorinated drinking-water and adverse pregnancy outcomes, including low birth weight, pregnancy loss, pre-term delivery and congenital malformations. In animal studies, effects have largely been seen at high doses associated with maternal toxicity and these are not considered to be predictive of effects in humans exposed to far lower levels of DBPs.

COT Statement 2008/02
July 2008

References

1 Committee on Toxicity Mutagenicity and Carcinogenicity of Chemicals in Food Consumer Products and the Environment. *Statement on chlorinated drinking water and reproductive outcomes.* 2004. http://cot.food.gov.uk/pdfs/cotstatementchlorwater0408.pdf.

2. Committees on Toxicity Mutagenicity and Carcinogenicity of Chemicals in Food Consumer Products and the Environment. *Annual Report of the Committees on Toxicity Mutagenicity and Carcinogenicity of Chemicals in Food Consumer Products and the Environment. Statement on Chlorinated Drinking Water and Reproductive Outcomes.* 1998 [cited; Available from: http://www.food.gov.uk/multimedia/pdfs/cot_drikwa01.pdf.

3. Committee on Toxicity Mutagenicity and Carcinogenicity of Chemicals in Food Consumer Products and the Environment, *Annual Report 2001.* p. p23.

4. Toledano, M.B., *et al., Relation of trihalomethane concentrations in public water supplies to stillbirth and birth weight in three water regions in England.* Environmental Health Perspectives, 2005. 113(2): p. 225-232.

5. Nieuwenhuijsen, M.J., *et al., Chlorination Disinfection By-Products and Risk of Congenital Anomalies in England and Wales.* Environ Health Perspect, 2008. 116(2): p. 216-222.

6. Malliarou, E., *et al., Haloacetic acids in drinking water in the United Kingdom.* Water Research, 2005. 39(12): p. 2722-2730.

7. World Health Organization, *Disinfectants and disinfection by-products.* Environmental Health Crtiteria 216. International Programme on Chemical Safety, Geneva., 2005.

8. Aschengrau, A., S. Zierler, and A. Cohen, *Quality of community drinking water and the occurrence of late adverse pregnancy outcomes.* Arch Environ Health, 1993. 48(2): p. 105-13.

9. Bove, F.J., *et al., Public drinking water contamination and birth outcomes.* Am J Epidemiol, 1995. 141(9): p. 850-62.

10. Cedergren, M.I., *et al., Chlorination byproducts and nitrate in drinking water and risk for congenital cardiac defects.* Environmental Research, 2002. 89(2): p. 124-130.

11. Dodds, L., *et al., Trihalomethanes in public water supplies and adverse birth outcomes.* Epidemiology, 1999. 10(3): p. 233-7.

12. Dodds, L. and W.D. King, *Relation between trihalomethane compounds and birth defects.* Occupational and Environmental Medicine, 2001. 58(7): p. 443-446.

13. Hwang, B.F., P. Magnus, and J.J.K. Jaakkola, *Risk of specific birth defects in relation to chlorination and the amount of natural organic matter in the water supply.* American Journal of Epidemiology, 2002. 156(4): p. 374-382.

14. Kallen, B.A.J. and E. Robert, *Drinking water chlorination and delivery outcome* - A registry-based study in Sweden. Reproductive Toxicology, 2000. 14(4): p. 303-309.

15. Klotz, J.B. and L.A. Pyrch, *Neural tube defects and drinking water disinfection by-products*. Epidemiology, 1999. 10(4): p. 383-390.

16. Magnus, P., *et al.*, *Water chlorination and birth defects*. Epidemiology, 1999. 10(5): p. 513-517.

17. Shaw, G.M., *et al.*, *Trihalomethane exposures from municipal water supplies and selected congenital malformations*. Epidemiology, 2003. 14(2): p. 191-199.

18. Committee on Toxicity of Chemicals in Food Consumer Products and the Environment. *SAHSU study on chlorination disinfection by-products and risk of congential anomalies in England and Wales*. TOX/2008/06. 2008 [cited; Available from: http://www.food.gov.uk/multimedia/pdfs/committee/tox200806pdf.

19. Hinckley, A.F., A.M. Bachand, and J.S. Reif, *Late pregnancy exposures to disinfection by-products and growth-related birth outcomes*. Environmental Health Perspectives, 2005. 113(12): p. 1808-1813.

20. Infante-Rivard, C., *Drinking water contaminants, gene polymorphisms, and fetal growth*. Environmental Health Perspectives, 2004. 112(11): p. 1213-1216.

21. Porter, C.K., *et al.*, *The effect of trihalomethane and haloacetic acid exposure on fetal growth in a Maryland county*. American Journal of Epidemiology, 2005. 162(4): p. 334-344.

22. Savitz, D.A., *et al.*, *Drinking water disinfection by-products and pregnancy outcome*. AWWA Research Foundation, 2005: p. 1-247.

23. Wright, J.M., J. Schwartz, and D.W. Dockery, *The effect of disinfection by-products and mutagenic activity on birth weight and gestational duration*. Environmental Health Perspectives, 2004. 112(8): p. 920-925.

24. Tardiff, R.G., M.L. Carson, and M.E. Ginevan, *Updated weight of evidence for an association between adverse reproductive and developmental effects and exposure to disinfection by-products*. Regulatory Toxicology and Pharmacology, 2006. 45(2): p. 185-205.

25. Hwang B-F, Jaakkola JJK and Guo H-R (2008). *Water disinfection by-products and the risk of specific birth defects: A population-based cross-sectional study in Taiwan*. Environmental Health 7, 23. doi:10, 1186/1476-069X-7-23

26. Luben T J, Nuckols J R, Mosley B S, Hobbs C and Reif J S (2008). *Maternal exposure to water disinfection by-products during gestation and risk of hypospadias*. Occup. Environ. Med. 65, 420-429.

Annex A

Search strategy for additional epidemiological studies on congenital malformations/birth defects

- The search was carried out on the databases PubMed and Toxline and was limited to papers reporting on studies on humans published between 1 January 2004 and 31 August 2007, inclusive.
- The search terms used were:

  chlorinat* by-product* AND congenital malformation*

  chlorinat* by-product* AND congenital defect*

  drinking water AND congenital malformation*

  drinking water AND congenital defect*

  trihalomethanes AND congenital malformation*

  trihalomethanes AND congenital defect*

  disinfection by-product* AND congenital malformation*

  disinfection by-product* AND congenital defect*

  dbp* AND congenital malformation*

  dbp* AND congenital defect*

Search strategy for additional epidemiological studies on adverse pregnancy outcomes since the 2004 consideration

- The search was carried out on the databases PubMed and Toxline and was limited to papers reporting studies on humans published between 1 January 2004 until 31 August 2007, inclusive.
- The search terms used were:

  chlorinat* by-product* AND pregnancy outcome*

  drinking water AND pregnancy outcome*

  trihalomethanes AND pregnancy outcome*

  disinfection by-product* AND pregnancy outcome*

  dbp* AND pregnancy outcome

  all above terms combined with birth weight (birthweight), preterm delivery, premature*, still birth.

Statement on the COT Workshop on Transgenerational Epigenetics

Introduction

1 As part of the COT's horizon scanning exercise in 2007, the issue of possible transgenerational effects due to epigenetic alterations was raised, a topic which had previously been discussed by the Committee on Mutagenicity in 2006. The COT agreed that transgenerational epigenetic inheritance could potentially mediate a wide range of toxicological effects and was an important area for future research.

2. In February 2008, a one-day workshop on transgenerational epigenetics was held, to enable the Committee to increase its awareness of current knowledge in this area, and to consider possible implications for chemical risk assessment. This report summarises information from the speakers' abstracts, presentations given at the workshop and subsequent discussions.

Epigenetics and the epigenetic code

3. In biology, "epigenetics" is concerned with alterations in phenotype due to changes in cellular properties that may be inherited, but do not represent a change in the DNA base sequence. From a developmental standpoint, it is associated with how a fertilised totipotent zygote progresses, via a series of developmental transformations and inductive processes, into a multicellular embryo and eventually an adult. From a molecular viewpoint it is concerned with how chemical modifications of DNA and histones alter gene function.

4. Epigenetic mechanisms regulate the setting up and maintenance of the two major forms of chromosomal structure; heterochromatin, where the DNA is tightly packed and genes are repressed, and euchromatin where genes are under active transcription. Mechanisms involved in epigenetic regulation of gene expression include DNA methylation and histone tail modifications, while small non-coding RNAs also play a role.

5. DNA methylation at the 5' position of cytosine, typically although not exclusively in CpG DNA sequences within regulatory regions of genes, is associated with gene repression *in vitro* and *in vivo,* while hypomethylation is generally associated with gene expression. Catalysed by DNA methyltransferases (DNMTs), DNA methylation can silence gene expression by directly interfering with the binding of a transcription factor to its recognition element, or indirectly by attracting methyl-CpG-specific binding proteins such as MeCP[2] (reviewed by Ptak and Petronis, 2008[1]). DNMT1 is a 'maintenance' methyltransferase which copies methylation patterns during DNA replication, while DNMT3a and DNMT3b are *de novo* methyltransferases, methylating DNA at previously unmethylated sites[1,2].

6. S-Adenosyl methionine (SAM) provides methyl groups for transfer, and is produced through the folate and methionine cycling pathways, using methionine, choline, folic acid and vitamin B12.

7. Histone modifications such as acetylation, methylation, phosphorylation and ubiquitylation regulate chromatin structure and hence gene expression. Active chromatin is generally characterised by overall hyperacetylation of histones and enrichment of histone H3 trimethylated at lysine 4 (H3K4Me3), di- or tri-methylated at lysine 36 (H3K36Me2/3) and dimethylated at lysine 79 (H3K79Me2), plus DNA hypomethylation. Methylation of Lys 36 and Lys 79 occurs at transcription units whereas Lys 4 is methylated at regulatory regions. Inactive chromatin is characterised by overall histone hypoacetylation, increased levels of H3 trimethylated at Lys 9 (H3K9Me3) and H4 trimethylated at Lys 20 (H4K20Me3), and DNA methylation (reviewed by Turner, 2007[3]). Further, histone modification enzymes can interact with DNMTs and target DNA methylation to chromatin that is already hypo-acetylated, thereby reinforcing gene silencing (reviewed by Meehan *et al.*, 2005[4]).

8. It has been proposed that histone tail modifications, together with DNA methylation, are part of an epigenetic code regulating chromatin structure and gene expression. Semiotics, the study of signs and symbols and their use or meaning, has been used as a guideline for defining the epigenetic code. A semiotic system consists of a sign, its meaning and the code used to interpret the sign. In the case of an epigenetic code the sign would be a combination of histone/DNA modifications and the meaning would be the initiation or termination of transcription at a specific time and cell type. Thus, the code comprises combinations of chromatin modifications that allow the transcriptional status of specific genes to be switched on or off in a particular cell type at a defined stage of development or differentiation[3].

9. The epi-genotype is dynamic and responsive to environmental signals, and it has been proposed that the influence of environment and genotype on the phenotype of an organism may in part be mediated indirectly via the epi-genotype.

10. Epigenetic alterations that arise during the lifetime of an organism are proposed to result from both stochastic processes and systematic environmental influences. Epigenetic marks are in constant flux and the maintenance methyltransferase DNMT1 has been estimated to have a 5% error rate, compounding this flux (reviewed by Whitelaw and Whitelaw, 2006[5]). Environmental influences include dietary factors and maternal behaviour. For example, decreased maternal grooming in rats has been correlated with reduced DNA methylation and histone acetylation within the glucocorticoid receptor gene proximal regulatory unit in the hippocampus, and induction of altered stress responses in later life. Infusion of a histone deacetylase inhibitor reversed these effects[6]. Dietary supplementation with methyl donors has also been shown to induce epigenetic alterations in animal models[7].

11. Epigenetics has been suggested to be involved in a range of complex diseases that arise from a combination of heritable and environmental factors, including cancer, metabolic syndrome, anxiety and depression, schizophrenia and bipolar disorder (reviewed by van Vliet *et al.*, 2007[2]).

Evidence for transgenerational epigenetic inheritance – animal studies

12. While transmission of acquired epigenetic changes to subsequent generations has been well documented in plants[8], in mice epigenetic reprogramming is associated with a global decrease in methylation levels at two developmental periods, during gametogenesis and during early embryogenesis followed by de novo methylation[9], suggesting that acquired epigenetic changes should not be inherited. However, there is robust evidence that epigenetic information can be inherited across generations in mammals (see below and Chong *et al.*, 2007[10]).

13. Epigenetic inheritance has been clearly demonstrated through to the F1 generation in two mouse models; Agouti viable yellow (A^{vy}) and Axin-fused ($Axin^{Fu}$).

14. The A^{vy} allele contains an intracisternal A particle (IAP) retrotransposon upstream of the *agouti* gene, which encodes a signalling protein that causes hair follicle pigment production to switch from eumelanin, which is black, to phaelomelanin which is yellow. When the IAP in the A^{vy} allele is unmethylated, a promoter drives ectopic *agouti* expression resulting in a yellow coat colour, and these mice have a predisposition for development of obesity and diabetes. When the IAP is methylated, agouti expression is not induced, mice have a brown coat and are phenotypically normal. Genetically identical mice heterozygous for the A^{vy} and a (the *nonagouti* allele that does not produce functional *agouti* protein) alleles display a wide range of coat colour phenotypes from yellow to mottled to brown ('pseudoagouti'), depending on the level of methylation at the A^{vy} IAP. The A^{vy} alleles with differing levels of methylation are referred to as 'epialleles'[11,12,13].

15. Dams with a hypomethylated A^{vy} allele (yellow coat phenotype) produce yellow and mottled offspring but no pseudoagouti (hypermethylated) offspring, while pseudoagouti dams produce 20% pseudoagouti offspring[11].

16. Axin regulates embryonic axis formation in vertebrates, and the *axin-fused* ($Axin^{Fu}$) allele is a dominant gain of function allele that has an IAP retrotransposon inserted. The $Axin^{Fu}$ phenotype is a kinked tail, but in some mice the tails appear completely normal. The phenotype has been shown to be correlated with methylation at the IAP long terminal repeat (LTR). The IAP is heavily methylated in mice without the tail kink, while in mice with a kinked tail the region is relatively hypomethylated. $Axin^{Fu}$ transgenerational epigenetic inheritance has been shown to occur with both maternal and paternal transmission[14].

17. Epigenetic inheritance at the A^{vy} and $Axin^{Fu}$ alleles appears to be influenced by the genetic background of the mouse strain in which it is present. Avy in the C57BL/6J strain displays transgenerational epigenetic inheritance after maternal transmission only[11], whereas $Axin^{Fu}$ displays inheritance after maternal and paternal transmission in the 129RrRk/J strain[14]. However, cross-over studies have shown that inheritance of A^{vy} via paternal transmission does occur when C57 males are crossed with 129 females, while paternal $Axin^{Fu}$ transmission does not occur when 129 males are crossed with C57 females[14].

18. This finding suggests that the epigenetic 'mark' is reprogrammed by the C57 egg but not by the 129 egg. The factor that causes this reprogramming is unknown, but could potentially be any protein

that influences reprogramming in early embryogenesis. Genetic variation in such factors would also be expected to influence epigenetic inheritance at other loci. As such events are known to have strain-specific liabilities it is probable that species-specific confounders also exist.

19. In the case of environmental factors that affect the epigenome, confirmation of transgenerational inheritance requires observation of effects in at least the F3 generation. This is because when an F0 pregnant female is exposed to an environmental agent, the F1 generation embryo and the germline of the F2 generation are also directly exposed.

20. Environmental factors have been shown to influence the epigenetic code in the F1 generation in several animal studies. Dietary supplementation of pregnant female A^{vy} mice with methyl donors and co-factors resulted in a darker average coat colour and systemic A^{vy} hypermethylation in their A^{vy}/a offspring[7]. However, a follow-up study indicated that the effect did not accumulate across the F2 and F3 generations, suggesting that the diet-induced A^{vy} hypermethylation is not inherited transgenerationally through the female[15].

21. In sheep, administration of a hypomethylating diet containing low levels of vitamin B12 and methionine from 8 weeks preceding to 6 days following conception resulted in a range of effects in adult offspring. Effects observed included increased weight, altered body composition (increased fat and reduced muscle), altered immune responses to antigenic challenge, immune resistance and elevated blood pressure[16]. Analysis of CpG islands in fetal liver indicated that methyl-deficient animals had alterations in methylation status at a number of loci compared with controls.

22. The only reported example of transgenerational transmission of effects on the epigenetic code elicited by an environmental agent in animals is that of the anti-androgen vinclozolin. Intraperitoneal (i.p.) exposure of pregnant F344 rats to vinclozolin during the time of fetal sex determination (embryonic days 8-14) has been shown to result in a range of adverse effects in male offspring of the F1-F4 generations, transmitted through the male germ line. The effects observed include spermatogenic defects, and in adults, male infertility, prostate disease, kidney disease, immune system abnormalities, hypercholesterolemia and an increased rate of tumour development[17,18,19].

23. The frequencies of these abnormalities ranged from 20-90%, suggesting that mutations in the DNA sequence are not likely to be responsible. The frequency of germ line DNA sequence mutations, even with ionising radiation, has been estimated as being normally less than 0.01% and ranging from only 1-5% for hot-spot mutations in the F0-F2 generations (reviewed by Jirtle and Skinner, 2007[20]). It was therefore suggested that an epigenetic mechanism may be involved, and in support of this several genes and other DNA sequences in the sperm of vinclozolin-treated animals were identified that had altered methylation patterns in the F1-F3 generations[17,21]. In initial reports the anti-androgen methoxychlor was also found to promote male infertility and decreased spermatogenic capacity in the F1 and F2 generations, although effects on DNA methylation with this compound have not been reported.

24. Recently, additional effects have been reported in the offspring of vinclozolin-treated rats. Females showed a mate preference for males from an unexposed lineage over those of the vinclozolin lineage, whereas males exhibited no such preference for female type[22]. These findings led the authors to hypothesise that transgenerational epigenetic inheritance may represent an 'unappreciated force in

sexual selection'. Effects in F1-F3 female progeny have also been reported, including uterine haemorrhage and/or anaemia late in pregnancy, glomerular abnormalities and a statistically non-significant increased incidence of tumours compared with controls[23]. While the effects in males were transmitted through the male germline, both male and female parents had to be of vinclozolin-exposed lines for female offspring to show the pregnancy disease phenotype.

25. The above findings have yet to be reproduced by other laboratories or for other chemical exposures. While the effects described occurred following i.p. administration of high doses (100 mg/kg) of vinclozolin, an abstract presented at the Society of Toxicology annual meeting in 2007 reported no adverse transgenerational effects on sperm number, morphology and motility in F1-F3 generations following oral administration, also at 100 mg/kg b.w., to pregnant rats[24]. However, this study was conducted in a different rat strain (Wistar) from the earlier studies (F344), and the genetic background may have had an influence on the findings. An abstract presented at the 2007 meeting of the International Congress of Toxicology by Kawabe *et al.*[25] reported no effects on spermatogenesis in the F1 generation of Crl:CD(SD) rats, and no alterations in DNA methylation in testes and sperm in the F0, F1 and F2 generations, following i.p. administration of vinclozolin (100 mg/kg b.w.), procymidone (100 mg/kg b.w. i.p.) and flutamide (10 mg/kg b.w. i.p.) on gestational days 8-15.

26. The nature of the inherited epigenetic 'mark' that might mediate transgenerational effects is unclear. There is some evidence that IAPs are largely resistant to demethylation during gametogenesis and early development,[26] but a study in A^{vy} mice showed that methylation at the IAP is cleared during early embyogenesis and then reset[15]. This suggests that histone modifications or RNA-mediated mechanisms may play a role. To date, it is uncertain whether genetic elements other than IAPs, such as promoters, enhancers or other retro-elements may also be sites of epigenetic inheritance. IAPs are transposon units rather than part of the 'normal' genetic code and it is unclear whether effects seen at these sites would also occur in native genomic DNA.

27. RNA may also play a role in mediating transgenerational epigenetic effects. In a recent study, genotypically wild type offspring of heterozygous $Kit^{tm1Alf/+}$ mice were found to display the white-spotted phenotype characteristic of the heterozygous animals[27]. The spermatoza of these paramutated offspring contained unusual amounts of RNA. Furthermore, the white-spotted phenotype could be reproduced in non-paramutated wild type offspring by microinjection of sperm RNA from $Kit^{tm1Alf/+}$ heterozygotes, or microRNAs that target *Kit* mRNA, into fertilised eggs.

Evidence for transgenerational epigenetic inheritance – human studies

28. Conclusive evidence of epigenetic inheritance in humans is currently lacking. A study of a family affected by hereditary nonpolyposis colorectal cancer (HNPCC) reported a germline allele-specific hypermethylation of the DNA mismatch repair (MMR) *MSH2* gene, without evidence of DNA mismatch repair mutation, in three successive generations[28]. Several family members with this methylation developed colorectal cancer or other HNPCC-related cancers. While it has been suggested that this is an example of transgenerational epigenetic inheritance, it has also been argued that the 'epimutation' could be the result of an underlying modifying genetic mutation that causes the

hypermethylation of the *MSH2* gene to be re-established in each generation[10]. It is therefore uncertain whether the epigenetic pattern is causative of the observed predisposition to cancer, or consequential of some other underlying factor.

29. Suter *et al.*[29] reported two individuals with soma-wide, allele specific and mosaic hypermethylation of the MMR gene *MLH1*. Both individuals lacked evidence of mutation in any MMR genes, but had multiple primary tumours showing deficiency in MMR and met the clinical criteria for HNPCC. The epimutation was detected in the spermatozoa of one of the individuals, suggesting a germline defect and potential for transmission to children. However, recent reanalysis of the spermatozoa sample with more sophisticated techniques has indicated that the *MLH1* methylation detected was most likely derived from residual somatic DNA in the sample, rather than present in male germ cells[30].

30. Epigenetic inheritance has also been proposed as a possible mechanism underlying transgenerational responses to smoking and nutrition observed in human populations. Analysis of individuals recruited into the Avon Longitudinal Study of Parents and Children (ALSPAC) indicated that early paternal onset of smoking was associated with greater body mass index at age 9 in sons, but not in daughers[31]. A trend for lower gestation length with earlier paternal smoking was also observed, in boys but not girls.

31. Sex-specific transgenerational effects in a cohort of individuals born in Överkalix, Sweden in 1890, 1905 and 1920 have also been reported. The paternal grandfather's food supply, estimated from historical data on harvests and food prices, was linked to the mortality risk ratio (RR) of grandsons, while the paternal grandmothers' food supply was associated with granddaughters' mortality RR[31,32]. No associations were found between paternal grandfathers' diet and granddaughters' mortality RRs, or between paternal grandmothers' and grandsons' mortality. Analysis suggested that particular periods of exposure were critical. Poor grandfather's or grandmother's food supply during the slow growth period before puberty (8-12 years) was associated with reduced mortality RRs for grandsons and granddaughters, respectively while good food supply at this time was associated with higher mortality RRs. The paternal grandmothers' food supply from when she was a fetus to age 4 years had the opposite effect, with good or poor supply correlating with lower or higher mortality RRs, respectively.

32. Transgenerational effects associated with exposure to betel nuts have been reported in both humans and animals. In CD1 mice, paternal exposure to betel nut was associated with an increased risk of hyperglycaemia in non-betel fed F1 offspring[33], while an epidemiological study in Taiwan reported paternal betel nut chewing was associated with an increased risk of early onset of metabolic syndrome in offspring[34].

33. The mechanisms responsible for these possible sex-line specific transmissions have not yet been identified, but it has been postulated that they may be mediated by a signal, possibly epigenetic, carried on the X and Y chromosomes. It is hypothesised that the non-recombining region of the Y chromosome can transmit environmentally-induced epigenetic states or reversible DNA changes to subsequent generations. Father to son and paternal grandfather to grandson effects could be mediated by the Y chromosome, while the X chromosome passed by a woman to her son can only be passed to her granddaughters, not grandsons[31]. However, evidence demonstrating a role for epigenetics in mediating these effects is currently lacking.

Implications for risk assessment

34. The possibility that environmental exposures during pregnancy or in the neonatal period could result in epigenetic alterations that lead to adverse effects in the F1 generation or even beyond is gaining attention. The hypothesis is being tested by the new, emerging field of investigation known as Environmental Epigenomics.

35. Given that this field is in its infancy and the analytical techniques used to assess epigenetic effects are still evolving, it has been suggested that it is premature to conclude that epigenetic evaluations should be incorporated into chemical risk assessment at this time[35]. There are a number of questions which need to be addressed. Questions raised at the workshop include:

- Do we know enough about the available animal models, such as A^{vy} and $Axin^{Fu}$, in order to properly interpret the data they generate? For example, transposable elements such as IAPs are expected to be methylated, and it is uncertain whether there is something unusual about the Agouti and Axin model IAPs that allows them to be hypomethylated.

- How do we assess whether an epigenetic change is adverse?

- Epigenetic mechanisms include DNA methylation, histone alterations and effects of non-coding RNAs and it is uncertain which of these mechanisms play a role in transgenerational epigenetic inheritance. Is there a need to evaluate all three of these parameters? It is also important to consider what technique(s) would best be employed.

- It is important to consider normal epigenetic variability, from individual to individual and over time. There are also species differences in relative epigenetic stability. For example, stability of methylation in c-myc in the liver and resistance to X chromosome reactivation during aging is greater in humans than in mice[36,37].

- In addition to the agouti and axin models, are there other endpoints, such as imprinted genes, that should be evaluated? What model compounds should be used?

- The finding that maternal grooming behaviour can have epigenetic effects (see para 18) indicates that it is important to take parental behaviour into consideration.

36. There are also questions as to whether current regulatory toxicity testing would be sufficient to detect transgenerational epigenetic effects on phenotype. Comparison of the data generated from regulatory studies for developmental, reproductive and endocrine toxicity of vinclozolin with those generated in the studies on its transgenerational effects suggests that the regulatory tests would adequately detect the effects of vinclozolin on the androgen receptor, fertility, reproductive organ development, male genitalia and anogenital distance. However, multi-generation assays for reproductive toxicity would not predict that treatment of the F0 generation only could produce testicular abnormalities up to the F4 generation, as dosing is continuous across generations in these studies (NB: these observations are being questioned, see para 25 above). In addition, the adult onset increase in prostate, kidney and immune system lesions detected in the transgenerational studies is

unlikely to be picked up by regulatory reproductive studies, as these are generally terminated after weaning or mating. Alternative strategies to detect the potential for such effects without requiring testing up to the F3 or F4 generations may need to be developed.

Discussions

37. Discussion at the workshop predominantly centred on the implications of the data presented for risk assessment. These discussions are summarised below.

38. Opinions vary on the most appropriate approach for assessing the potential for transgenerational epigenetic inheritance following chemical exposures. Focussing on one or two well characterised imprinted genes involved in transcription has been proposed, but there is no evidence that such genes are those most likely to be modified by environmental exposures, or that epigenetic alterations at these genes will have a significant functional impact.

39. Results from the A^{vy} and $Axin^{Fu}$ animal models should be treated with caution, particularly given the reported strain differences in transmission of epigenetic states.

40. An alternative approach may be to use techniques that measure effects on the whole epigenome to search for candidate genes or modifications on which to focus. However, while a broad approach may be useful, it is important to consider the possibility for 'epi-phenomena' – epigenetic changes can occur that have no effect on phenotype. For example, much of the bulk histone that can be analysed will be non-coding and it is possible to get massive changes in bulk histone acetylation without much change in gene expression. It is therefore critical to establish what regions of the epigenome are important. It will also be important to consider the lessons that can be learnt from experience with other 'omic' technologies.

41. Confirming whether transgenerational epigenetic inheritance occurs in humans will be extremely difficult and will require complementary studies in model organisms. A suggested approach is first to identify an environmental insult or influence resulting in an alteration in phenotype; then determine the location and nature of an epigenetic change and establish a link between epigenotype and phenotype. It will also be necessary to rule out other genetic factors or a familial effect such as constant exposure to an environmental factor. The need to study through to the F3 generation was reiterated, as effects in the F1 or F2 generations could be due to *in utero* exposure. A potential problem with this approach is that most differences in phenotype will not be inherited.

42. In addition to identifying critical regions of the epigenome to assess and applying the appropriate technique(s) for analysis, it is important to gain an understanding of the background variation at such regions, both inter- and intra-species. Similarly, there is a need to gain an understanding of background variation that can arise with time; for example, it has been demonstrated that that epigenetic differences between monozygotic twins increase over a lifetime[38].

43. There is some evidence of epigenetic effects in tumour suppressor genes associated with cancer, although it is unclear whether such changes are causal. It was suggested that it may be useful to investigate whether there are environmental causes for these changes.

44. Presuming that transgenerational epigenetic effects can be shown to occur, it was proposed that it will be important to identify whether such effects should be viewed as being thresholded or not. Experiments to define the shape of the dose-response would be necessary in order to determine this.

45. Overall, it is clear that a stronger science base is required before evaluation of epigenetic status can be included in regulatory risk assessment.

COT Conclusions

46. There is reasonable evidence that epigenetic changes associated with environmental exposures during development can result in adverse effects. Such effects might be detected in the F1 and F2 generations by standard regulatory toxicity testing.

47. Transgenerational epigenetic inheritance of effects in the F3 generation and beyond would also be of potential relevance to risk assessment. If epigenetic inheritance does occur, it is possible that this could lead to an accumulation in risk across generations. In addition, such epigenetic changes could be developed as biomarkers of effect.

48. However, the science is not yet developed and therefore assessment of transgenerational epigenetic inheritance cannot be incorporated in regulatory risk assessment at present.

49. It is still unclear whether transmission of environmentally acquired epigenetic changes across generations occurs in humans and if so, what mechanisms of epigenetic modification are important,

50. Priorities for future research include assessment of whether important examples of epigenetic inheritance seen in animals also occur in humans. In addition, it may be useful to investigate aberrant phenotypes in humans which might possibly have a transgenerational, epigenetic basis. It is feasible to undertake genome wide profiling to ascertain if changes in DNA methylation patterns underlie environmentally acquired epigenetic changes that occur in experimental models and perhaps human populations.

Acknowledgements

The Committee would like to thank the attendees of the workshop for their contributions, particularly the invited speakers:

Dr Richard R Meehan – MRC Human Genetics Unit, Edinburgh

Professor Lorraine Young – Wolfson Centre for Stem Cells, Tissue Engineering and Modelling, University of Nottingham

Professor Bryan M Turner – Institute of Biomedical Research, University of Birmingham Medical School

Professor Marcus Pembrey – Institute of Child Health, University College London and Avon Longitudinal Study of Parents and Children, Bristol University

Dr Jenny Odum – Syngenta Central Toxicology Laboratory

Dr Vardhman Rakyan – Centre for Diabetes and Metabolic Medicine, Barts and the London School of Medicine and Dentistry

Professor Jay I Goodman – Department of Pharmacology and Toxicology, Michigan State University

COT Statement 2008/03
July 2008

References

1 Ptak, C. and Petronis, A. (2008). Epigenetics and complex disease: from etiology to new therapeutics. *Annu Rev Pharmacol Toxicol* 48: 257-276.

2 van Vliet, J., Oates, N.A., Whitelaw, E. (2007). Epigenetic mechanisms in the context of complex diseases. *Cell Mol Life Sci* 64: 1531-1538.

3 Turner, B.M.(2007). Defining an epigenetic code. *Nat Cell Biol* 9: 2-6.

4 Meehan, R.R., Dunican, D.S., Ruzov, A., Pennings, S. (2005). Epigenetic silencing in embryogenesis. *Exp Cell Res* 309: 241-249.

5 Whitelaw, N.C. and Whitelaw, E. (2006). How lifetimes shape epigenotype within and across generations. *Hum Mol Genet* 15 Spec No 2: R131-R137.

6 Weaver, I.C., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., Meaney, M.J. (2004). Epigenetic programming by maternal behavior. *Nat Neurosci* 7: 847-854.

7 Waterland, R.A. and Jirtle, R.L. (2003). Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 23: 5293-5300.

8 Takeda, S. and Paszkowski, J. (2006). DNA methylation and epigenetic inheritance during plant gametogenesis. *Chromosoma* 115: 27-35.

9 Reik, W., Dean, W., Walter, J. (2001). Epigenetic reprogramming in mammalian development. *Science* 293: 1089-1093.

10 Chong, S., Youngson, N.A., Whitelaw, E. (2007). Heritable germline epimutation is not the same as transgenerational epigenetic inheritance. *Nat Genet* 39: 574-575.

11 Morgan, H.D., Sutherland, H.G., Martin, D.I., Whitelaw, E. (1999). Epigenetic inheritance at the agouti locus in the mouse. *Nat Genet* 23: 314-318.

12 Rakyan, V.K., Blewitt, M.E., Druker, R., Preis, J.I., Whitelaw, E. (2002). Metastable epialleles in mammals. *Trends Genet* 18: 348-351.

13 Rakyan, V.K. and Beck, S. (2006). Epigenetic variation and inheritance in mammals. *Curr Opin Genet Dev* 16: 573-577.

14 Rakyan, V.K., Chong, S., Champ, M.E., Cuthbert, P.C., Morgan, H.D., Luu, K.V., Whitelaw, E. (2003). Transgenerational inheritance of epigenetic states at the murine Axin(Fu) allele occurs after maternal and paternal transmission. *Proc Natl Acad Sci U S A* 100: 2538-2543.

15 Blewitt, M.E., Vickaryous, N.K., Paldi, A., Koseki, H., Whitelaw, E. (2006). Dynamic reprogramming of DNA methylation at an epigenetically sensitive allele in mice. *PLoS Genet* 2: e49-

16 Sinclair, K.D., Allegrucci, C., Singh, R., Gardner, D.S., Sebastian, S., Bispham, J., Thurston, A., Huntley, J.F., Rees, W.D., Maloney, C.A., Lea, R.G., Craigon, J., McEvoy, T.G., Young, L.E. (2007). DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. *Proc Natl Acad Sci U S A* 104: 19351-19356.

17 Anway, M.D., Cupp, A.S., Uzumcu, M., Skinner, M.K. (2005). Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 308: 1466-1469.

18 Anway, M.D., Leathers, C., Skinner, M.K. (2006). Endocrine disruptor vinclozolin induced epigenetic transgenerational adult-onset disease. *Endocrinology* 147: 5515-5523.

19 Skinner, M.K. and Anway, M.D. (2007). Epigenetic transgenerational actions of vinclozolin on the development of disease and cancer. *Crit Rev Oncog* 13: 75-82.

20 Jirtle, R.L. and Skinner, M.K. (2007). Environmental epigenomics and disease susceptibility. *Nat Rev Genet* 8: 253-262.

21 Chang, H.S., Anway, M.D., Rekow, S.S., Skinner, M.K. (2006). Transgenerational epigenetic imprinting of the male germline by endocrine disruptor exposure during gonadal sex determination. *Endocrinology* 147: 5524-5541.

22 Crews, D., Gore, A.C., Hsu, T.S., Dangleben, N.L., Spinetta, M., Schallert, T., Anway, M.D., Skinner, M.K. (2007). Transgenerational epigenetic imprints on mate preference. *Proc Natl Acad Sci U S A* 104: 5942-5946.

23 Nilsson, E.E., Anway, M.D., Stanfield, J., Skinner, M.K. (2008). Transgenerational epigenetic effects of the endocrine disruptor vinclozolin on pregnancies and female adult onset disease. *Reproduction* 135: 713-721.

24 Schneider, S., Hellwig, J., Kaufmann, W., van Ravenswaay, B. (2007). The lack of a transgenerational effect of vinclozolin following oral prenatal exposure to 100 mg/kg bw. *The Toxicologist* 96[1], 116.

25 Kawabe, M., Takahashi, S., Doi, Y., Inawaka, K., Tomigahara, Y., Shirai, T. (2007). Maternal exposure to anti-androgenic compounds has no effects on spermatogenesis and DNA methylation in male rats of subsequent generation. *XI International Congress on Toxicology*. 16-7-2007.

26 Lane, N., Dean, W., Erhardt, S., Hajkova, P., Surani, A., Walter, J., Reik, W. (2003). Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. *Genesis* 35: 88-93.

27 Rassoulzadegan, M., Grandjean, V., Gounon, P., Cuzin, F. (2007). Inheritance of an epigenetic change in the mouse: a new role for RNA. *Biochem Soc Trans* 35: 623-625.

28 Chan, T.L., Yuen, S.T., Kong, C.K., Chan, Y.W., Chan, A.S., Ng, W.F., Tsui, W.Y., Lo, M.W., Tam, W.Y., Li, V.S., Leung, S.Y. (2006). Heritable germline epimutation of MSH2 in a family with hereditary nonpolyposis colorectal cancer. *Nat Genet* 38: 1178-1183.

29 Suter, C.M., Martin, D.I., Ward, R.L. (2004). Germline epimutation of MLH1 in individuals with multiple cancers. *Nat Genet* 36: 497-501.

30 Hitchins, M.P. and Ward, R.L. (2007). Erasure of MLH1 methylation in spermatozoa-implications for epigenetic inheritance. *Nat Genet* 39: 1289-

31 Pembrey, M.E., Bygren, L.O., Kaati, G., Edvinsson, S., Northstone, K., Sjostrom, M., Golding, J. (2006). Sex-specific, male-line transgenerational responses in humans. *Eur J Hum Genet* 14: 159-166.

32 Kaati, G., Bygren, L.O., Pembrey, M., Sjostrom, M. (2007). Transgenerational response to nutrition, early life circumstances and longevity. *Eur J Hum Genet* 15: 784-790.

33 Boucher, B.J., Ewen, S.W., Stowers, J.M. (1994). Betel nut (Areca catechu) consumption and the induction of glucose intolerance in adult CD1 mice and in their F1 and F2 offspring. *Diabetologia* 37: 49-55.

34 Chen, T.H., Chiu, Y.H., Boucher, B.J. (2006). Transgenerational effects of betel-quid chewing on the development of the metabolic syndrome in the Keelung Community-based Integrated Screening Program. *Am J Clin Nutr* 83: 688-692.

35 Moggs, J.G., Goodman, J.I., Trosko, J.E., Roberts, R.A. (2004). Epigenetics and cancer: implications for drug discovery and safety assessment. *Toxicol Appl Pharmacol* 196: 422-430.

36 Ono, T., Yamamoto, S., Kurishita, A., Yamamoto, K., Yamamoto, Y., Ujeno, Y., Sagisaka, K., Fukui, Y., Miyamoto, M., Tawa, R. (1990). Comparison of age-associated changes of c-myc gene methylation in liver between man and mouse. *Mutat Res* 237: 239-246.

37 Holliday, R.(1989). X-chromosome reactivation and ageing. *Nature* 337: 311-

38 Fraga, M.F., Ballestar, E., Paz, M.F., Ropero, S., Setien, F., Ballestar, M.L., Heine-Suner, D., Cigudosa, J.C., Urioste, M., Benitez, J., Boix-Chornet, M., Sanchez-Aguilera, A., Ling, C., Carlsson, E., Poulsen, P., Vaag, A., Stephan, Z., Spector, T.D., Wu, Y.Z., Plass, C., Esteller, M. (2005). Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci U S A* 102: 10604-10609.

Statement on reproductive effects of caffeine

Background

1. Caffeine is present in coffee, tea, chocolate, cocoa, cola drinks, many of the increasingly popular 'energy drinks', and in over-the-counter and prescription medications including many cold and 'flu remedies, headache treatments, diet pills, diuretics and stimulants. Most pregnant women in the UK consume caffeine from one or more sources.

2001 COT Evaluation

2. The Committee last considered possible adverse effects of caffeine consumption on reproduction in 2001 and issued a statement at that time with the following conclusions. [1]

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

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

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

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

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

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

The FSA funded research projects (T01032 & T01033)

9. In light of the Committee's conclusions in 2001, the Food Standards Agency issued advice that caffeine intake during pregnancy should be limited to not more than 300 mg/day and offered guidance on amounts of caffeine in different foods and drinks. In addition, the Agency commissioned a prospective study, involving around 2500 pregnant women, in order to reduce uncertainties in the risk assessment and provide a more robust basis for the Agency's advice to pregnant women on caffeine consumption.

10. This research was funded as two linked projects, 'Determination of maternal caffeine intakes associated with increased risk to the fetus' (FSA project code T01032, University of Leicester) and 'Assessment of caffeine consumption, altered caffeine metabolism and pregnancy outcome' (T01033, University of Leeds).

11. The FSA-funded research was designed to overcome some of the limitations of earlier studies. It was prospective in design, recruiting women at approximately 12 weeks of gestation, and ascertained caffeine consumption and other relevant exposures through a structured questionnaire. The questionnaire, which was completed on three occasions (once in each trimester of pregnancy), detailed all sources of caffeine, as well as gathering information about other aspects of diet (including alcohol consumption), smoking habits, drug use (medicinal and recreational), work, physical activity and symptoms. The information was recorded for each 4 week period of pregnancy. The main outcome measure was fetal growth restriction (FGR) defined as failure of the baby to attain its growth potential as determined by genetic and environmental factors. A weakness of many of the previous epidemiological studies had been their reliance on birth weight as the endpoint for assessing fetal growth. It is well recognised that low birth weight does not necessarily indicate poor growth, and depends also on gestational age at birth and on other factors such as maternal height, ethnicity and parity. Given that approximately 10% of babies were expected to have FGR, each of the two study sites recruited in the region of 1,250 women in order to ensure sufficient statistical power to detect small differences in the prevalence of FGR births according to caffeine intake.

12. FGR is an important outcome because it is associated with an increased risk of perinatal mortality and morbidity, including perinatal asphyxia. Moreover, there is epidemiological evidence that FGR correlates with adverse effects in adult life[2,3]. For example, affected individuals have an increased incidence of metabolic syndrome, manifesting as obesity, hypertension, hypercholesterolemia, cardiovascular disease, and type 2 diabetes[4,5,6].

13. Of the four primary routes of caffeine metabolism in humans, 3-demethylation is quantitatively the most important, the caffeine being converted to paraxanthine by CYP1A2. Studies have shown there to be varying levels of CYP1A2 activity in humans. Women recruited to the study were asked to participate in a "caffeine challenge" at approximately 14 and 28 weeks of gestation in order to assess metabolic phenotype for caffeine metabolism. Participants drank a defined volume of caffeine-containing cola and provided saliva samples, which allowed the half-life of caffeine and the ratio of its metabolites to be measured. Cotinine was also measured in these samples to verify reported smoking habits.

14. The Committee was presented with a pre-publication draft of the primary manuscript from these studies. The subjects' mean caffeine consumption was reported to decrease from 238 mg/day to 139 mg/day during the first trimester of pregnancy, and then increased to 153 mg/day by the third trimester. The major contributions to caffeine consumption in pregnancy were from tea (62%), coffee (14%) and cola drinks (12%), whilst chocolate contributed 8%. After adjustment for various potential confounders, caffeine consumption was associated with an increased risk of FGR which was statistically significant at intakes of 200-299 mg/day and above (Table 1).

Table 1

Odds ratios for FGR from a logistic regression analysis that adjusted for smoking status, amount smoked (cotinine concentration), and alcohol intake.

	Caffeine (mg/day)	OR	(95% CI)	ptrend
Average intake over pregnancy	<100	1	-	
	100-199	1.2	(0.9, 1.6)	
	200-299	1.5	(1.1, 2.1)	
	300+	1.4	(1.0, 2.0)	P=0.02

15. The relation between FGR and caffeine intake during pregnancy was modelled using the best-fitting second-order fractional polynomial (Figure 1). The curve in Figure 1 was derived from a model that took into account other risk factors such as salivary cotinine levels, self-reported alcohol consumption, maternal height, weight, ethnicity, parity, gestation at delivery and gender of the neonate. The results were robust to exclusion of those women with high risk pregnancies, multiparity, and extremely high or low caffeine intakes. For all levels of caffeine intake, lower intakes of caffeine were associated with lower risk of fetal growth restriction. It is possible that the steep decline in risk associated with caffeine intakes of less than 30 mg/day may be attributable to residual confounding. This analysis suggested a continuously increasing risk across the exposure range, and gave no indication of a threshold level of exposure, below which risk was not elevated. The Committee requested a repeat of this analysis, excluding those women who consumed more than 300 mg caffeine per day. This confirmed that the high level consumers did not materially alter the shape of the exposure-response curve.

16. Further statistical analysis with regression models (logistic regression for binary outcomes, e.g. FGR vs no FGR, and linear regression for continuous outcomes, e.g. birth weight centile) gave no indication of important residual confounding by smoking.

17. Analysis of the data on half-lives of caffeine (as a proxy for metabolism) in saliva suggested an increased risk of FGR in fast metabolisers (shorter half life) as compared with slow metabolisers (longer half life), although the difference was not statistically significant (P=0.06).

18. It is interesting that among the women with caffeine intakes > 300 mg/day prior to pregnancy, a subset had chosen to reduce their caffeine intake to <50 mg/day by weeks 5-12 of pregnancy (n=109). The mean birth weight of infants in this subset was higher than that in women who maintained their caffeine intake above 300 mg/day (n=193) (difference in birth weight=161g, 95% CI: 24 to 297g, p=0.02). However, these two groups of women may have differed in other ways apart from their caffeine intakes.

19. The Committee noted that energy intake needed to be considered as a potential confounder of effects on fetal growth rate. Energy intake had been recorded in the Leeds arm of the study, but not in Leicester. An analysis of data from Leeds that adjusted for energy intake indicated that energy intake did not importantly confound the risk estimates for caffeine in this study.

Literature review (post-2001 COT statement)

20. In addition to being presented with the results from the FSA-funded research, the Committee was provided with an update on relevant research on reproductive effects of caffeine in humans published since the previous COT review. Table 2 summarises the key data provided to the Committee in tabular form. The references detailed in Table 2 were sourced through a systematic search of key scientific databases, details of which are given in Annex A.

21. It was noted that published studies differed substantially in their design, which may account for some of the variation in the estimated risks of adverse reproductive outcomes reported for specified levels of caffeine intake.

22. Most studies assessed caffeine intake at various stages of pregnancy, generally by use of dietary questionnaires. In most reports, caffeine intakes were assessed by multiplying the number of servings of a beverage or food by an estimated mean caffeine content, and different studies assumed different caffeine contents for beverages and foods. Further variation may have been introduced according to whether participants were asked to estimate serving size or the researcher assumed a default serving size.

23. Errors in recall would be expected to affect the accuracy of information provided on caffeine intake and on potential confounders, particularly in studies where information was gathered retrospectively. In case-control studies that ascertained caffeine intake after the outcome of pregnancy was known, differential errors may have spuriously exaggerated risk estimates. It should also be noted that many of the studies did not assess caffeine intake from all sources.

24. There is considerable inter-individual variation in caffeine metabolism, and measures of caffeine consumption do not necessarily indicate the levels of caffeine and caffeine metabolites in the maternal or fetal circulation. A small number of studies therefore measured levels of caffeine and its metabolites in maternal or umbilical cord blood rather than assessing caffeine consumption.

25. Further variation in estimates of caffeine effect may have occurred because the range of confounding factors that was taken into account differed between studies. Notably, several studies did not adjust for smoking or nausea during pregnancy.

26. Caffeine consumption greater than or equal to 300 mg/day was reported in several studies[7-14] to be associated with FGR, decreased mean birth weight, miscarriage, or increased risk of still birth, with one study finding a doubled risk of miscarriage for caffeine intakes above 200 mg/day[15]. Another study in pregnant women with Type 1 diabetes suggested an increased risk of miscarriage for a caffeine intake of just 1-2 caffeine-containing beverages per day in the first trimester, compared to non-consumers, although the elevation of risk only reached statistical significance for daily intakes of three or more drinks16. On the other hand, there were well-conducted studies that reported no statistically significant association between maternal caffeine intake and miscarriage, FGR, still birth or prematurity, after adjustment for potential confounders[17-21]. Overall, the findings were consistent with an increased risk of FGR and miscarriage from higher consumption of caffeine, but because of limitations in study designs (e.g. inaccurate assessment of caffeine exposures, potential for recall bias in case-control studies, and possible residual confounding), they do not allow firm conclusions about the relation of risk to levels of exposure.

27. Fewer studies looked at CYP1A2 activity and pregnancy outcomes, due to substantial confounding by smoking status, which is hard to correct for. One investigation suggested an increased risk of FGR in women with fast metabolic phenotype[22], while another found an association of caffeine intake with miscarriage only in women with low CYP1A2 activity[23].

Related observations from studies using experimental animals

28. The potential reproductive effects of caffeine have been studied in a wide range of species and strains of animals. In studies administering repeat doses of caffeine (12.5 mg/kg body weight per day and higher) to rats throughout pregnancy, significantly decreased birth weights have been noted[24]. It is not possible to determine whether this was due to a direct effect of caffeine on the fetus or secondary to decreased maternal body weight gain since it was observed only when there was a decrease in maternal body weight gain. In mice administered caffeine in drinking water at levels equating to consumption of 22, 44 and 88 mg/kg/day a reduction in number of live pups/litter of 15 and 20% was observed in the medium and high dose level group, respectively. For the F0 animals there were no effects on body weight, but alopecia occurred in 55% of the medium dose and 50% of the high dose animals[25].

29. Studies in the Cynomolgus monkey, *Macaca fascicularis*, have shown a high rate of still births and miscarriage with maternal caffeine intakes of 10-15 mg/kg body weight per day, given via drinking water[26]. In 2001 the COT noted *"that the main serum metabolite of caffeine in monkeys is theophylline, whereas in humans it is paraxanthine and that information on the comparative toxicities of these metabolites is not available."* It should be noted that the Cynomolgus monkey does not constitutively express CYP1A2 (which is the main enzyme responsible for caffeine metabolism) [27].. Thus, for a given dose of caffeine, the monkeys' systemic exposure is likely to be higher. Furthermore, there were limitations in the study design and therefore this study is not informative for assessing the risks of caffeine intake in humans.

Committee discussion

30. With regard to the new FSA-funded research, FGR was considered to be a relatively robust endpoint, unlike miscarriage, which is difficult to ascertain reliably as it often occurs before women know they are pregnant, or before they have been recruited to a study. Members noted that decreases in birth weight of as little as 10-15 g can have implications for future health outcomes, particularly in pre-term babies.

31. Caffeine consumption was assessed retrospectively by means of a questionnaire completed at interview at the end of each of the three trimesters of pregnancy, women being asked to recall their caffeine consumption during 4 week periods. Data on caffeine intake from all sources were recorded (tea, coffee, hot chocolate, soft drinks, chocolate) including information on brand, serving size and preparation of product. Caffeine consumption is likely to have been estimated with reasonable accuracy as, because of the repeated administration of the questionnaire, recall was recent and for most before pregnancy outcome or birth weight were known. Salivary cotinine measurement confirmed the accuracy with which the women reported their smoking habits.

32. The half-life of caffeine was measured in women in a "caffeine challenge" the *a priori* hypothesis being that an increased half-life would be associated with an increased risk of FGR, based upon the assumption that clearance would remove the potential hazard. However, this was not found. Rather, the analysis suggested that if anything, risk of FGR was higher in faster metabolisers than in slower metabolisers. This result is consistent with the findings of Grosso *et al*[22], who reported increased risk of intrauterine growth retardation (IUGR) in association with serum paraxanthine levels >149 ng/ml and higher paraxanthine/caffeine ratios. There are no reports of animal studies investigating reproductive effects of paraxanthine.

33. It was noted that adjustment for various potential confounding factors had little impact on risk estimates.

34. The fact that repeat modelling of the risk of FGR according to caffeine intake showed a similar dose-response relation after exclusion of women with caffeine intakes in excess of 300 mg per day suggested that the modelled relationship was not unduly influenced by findings for women with the highest caffeine intakes. The Committee noted the possibility that fitting a different mathematical model to the data may have importantly influenced the dose-response relationship observed in Figure 1, and that there was considerable uncertainty about the shape of the dose-response relationship at lower intakes. They noted that the model used placed weight on those with intakes below 50 mg/day (who may have differed in their exposure to confounding lifestyle factors) and questioned whether it was the most appropriate choice. Hence it would be inappropriate to attempt to determine a threshold dose from this figure.

Committee conclusions

35. We consider that the FSA-funded research contributes usefully to the body of evidence on the relation between caffeine intake and adverse birth outcomes.

36. From this work and from the other studies that have been published, we conclude that caffeine intake during pregnancy is associated with an increased risk of FGR. It is still not possible to be confident that the association is causal rather than a consequence of residual confounding, but it would be prudent to assume causation.

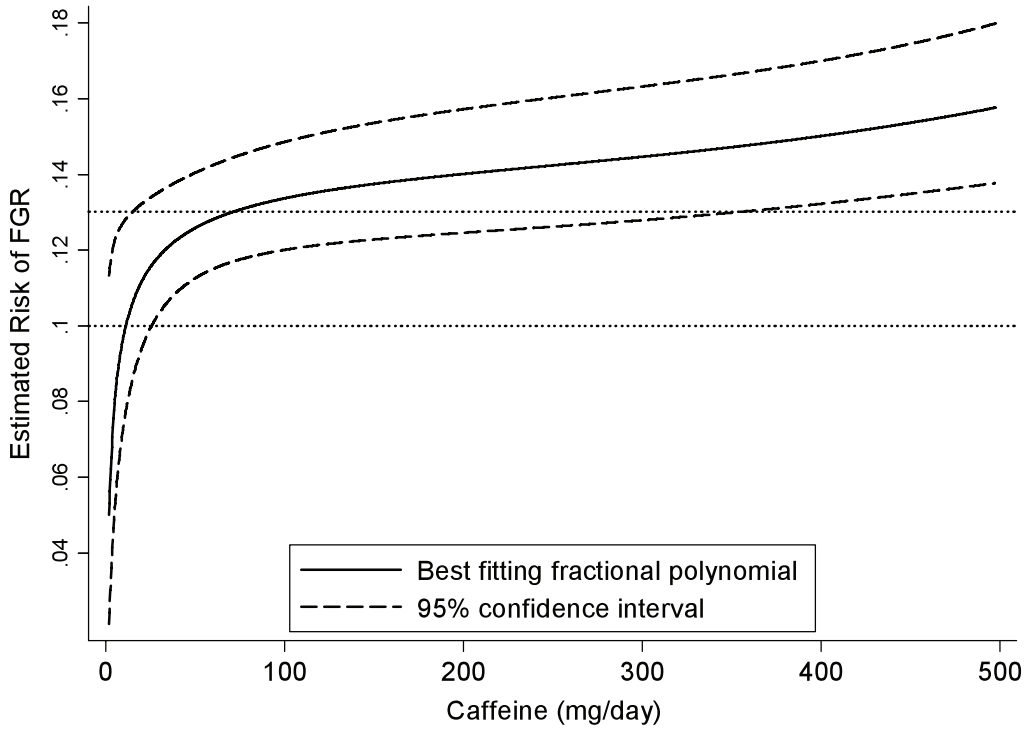
37. The evidence that is now available does not make it possible to identify a threshold level of caffeine intake below which there is no elevation of risk, and it seems likely that risk is increased in association with intakes in the order of 200 mg per day and perhaps even lower. However, if the relation is indeed causal, then the absolute increase in incidence of FGR from intakes less than 200 mg per day is likely to be less than 2% of infants.

38. The literature suggests a positive association of caffeine intake with miscarriage, but there are uncertainties relating to possible recall bias and residual confounding.

39. Data on maternal caffeine consumption during pregnancy and associations with adverse effects other than FGR and spontaneous miscarriage, such as pre-term birth and congenital malformations, are inconclusive.

COT Statement 2008/04
September 2008

Figure 1 Modelled relation between risk of fetal growth restriction (FGR) and caffeine intake (mg/day) during pregnancy.



The relation is modelled by the best-fitting second-order fractional polynomial, with 95% confidence intervals. For clarity, the graph is restricted to caffeine intakes <500mg/day. Horizontal dotted lines mark national average risk of FGR (10%) and the average risk in the cohort (13%).

Table 2. Key data from relevant human studies published since the previous COT review

Author + location	Study period	Outcome variables	Study sample	Measure of caffeine exposure	Study authors description of results	OR/RR (95% CI)	Comments
Studies on Birth Weight							
Balat *et al* (2003)[7] Turkey	Not stated	BW, length and head circumference, weight and diameter of placenta.	63 pregnant non-smokers + 60 pregnant smokers with spontaneous vaginal deliveries in gestational wks 37-41	Daily consumption of tea and coffee (#cups). Participants grouped as daily caffeine intake < or > 300 mg/day.	Non-smokers and smokers consuming >300 mg/day had significantly lower newborn and placental weights than those consuming <300 mg/day. No differences in other parameters.	Not reported.	No adjustment reported.
Bech *et al* (2007)[26] Denmark	1998-2002	Birth weight and length of gestation.	1207 pregnant women drinking at least 3 cups of coffee/day, recruited before 20 wks gestation.	Randomised to drink caffeinated (n=568) or decaffeinated (n=629) instant coffee at usual consumption levels. Interviewed throughout pregnancy on daily consumption coffee, tea, cola and cocoa.	No significant differences in mean bw or mean length of gestation between caffeinated and decaffeinated groups. Mean bw of babies of women in the decaffeinated group was 16 g (95% CI: -40, +73) higher than those from the caffeinated group.	Not reported.	Adjustment for length of gestation, parity, prepregnancy BMI and smoking at entry to study. Women were not asked to avoid intake of other caffeinated beverages.

Table 2. Key data from relevant human studies published since the previous COT review *continued*

Author + location	Study period	Outcome variables	Study sample	Measure of caffeine exposure	Study authors description of results	OR/RR (95% CI)	Comments
Bicalho and Barros Filho (2002)[27] Brazil	1994-1995	LBW, prematurity and IUGR.	354 newborns with bw <2,500 g (cases) 354 newborns ≥3,000 g (controls).	Daily consumption of coffee, tea and soft drinks.	No association between caffeine consumption during pregnancy and low birthweight, prematurity and intrauterine growth restriction.	Caffeine (mg/day) LBW <300 0.72(0.45,1.25) ≥300 0.47(0.24,0.92) IUGR <300 1.16(0.45,3.01) ≥300 0.64(0.20,1.98)	Abstract only in English. Adjustment for age, schooling, income, marital status, skin colour, parity, smoking, previous lbw child, pre-pregnancy weight, employment status, interval between pregnancies, prenatal care and high blood pressure.
Bracken *et al* (2003)[29] USA	1996-2000	IUGR LBW	2,291 women with singleton live births.	Interviews on coffee, tea and soda consumption - Interview on trimester 1 intake conducted before gestation wk 25 - Post natal interview on trimester 3 intake. Urine analysis at interview 1.	No significant association of caffeine consumption in trimesters 1 or 3 or urinary caffeine with the various endpoints. Mean bw reduced by 28 g per 100 mg caffeine consumed daily in trimester 1 [vs 178g reduction for smoking 10 cigarettes daily in trimester 3].	Trimester 1 Caffeine (mg/Day) IUGR 1-149 1.35(0.95,1.92) 150-299 1.05(0.53,2.09) ≥300 1.75(0.81,3.76) LBW 1-149 1.45(0.89,2.35) 150-299 1.59(0.70,3.60) ≥300 1.32(0.46,3.78) Reference: average 0 mg/day	Adjustment for age, parity, # prior pregnancies, marital status, race, education, height, smoking in 3rd trimester, weight. Similar ORs reported for caffeine consumption in trimester 3.

Author + location	Study period	Outcome variables	Study sample	Measure of caffeine exposure	Study authors description of results	OR/RR (95% CI)	Comments
Clausson *et al* (2002)[32] Sweden	1996-1998	BW, gestational age, BW standardised for gestational age (BW ratio).	873 women with singleton live births.	Interviews in gestational wks 6-12 and 32-34 on intake of coffee, tea, soft drinks, cocoa, chocolate and caffeine containing medication.	No associations between caffeine consumption and the endpoints assessed, neither when caffeine exposure averaged from conception to gestational wks 32-34, nor when stratified by trimester.	Not reported	Adjustment for age, height, BMI, country of birth, parity, previous LBW infant, education, work, nausea, vomiting, fatigue diabetes and hypertensive disorders.
Grosso *et al* (2006)[22] USA	1996-2000	IUGR	Pregnant women at ≤24 gestational wks: 718 consuming ≥150 mg/day caffeine in previous wk. 2,915 consuming < 150 mg/day in previous wk.	Caffeine and primary metabolites measured in umbilical cord blood.	Higher serum caffeine levels associated with reduced risk IUGR. Paraxanthine levels ≥149 ng/ml associated with increased risk. Increase in paraxanthine:caffeine ratio increased likelihood of IUGR.	Standard deviation increase in paraxanthine: caffeine ratio. 1.21(1.07,1.37) No statement of baseline.	Adjustment for smoking in trimester 3, parity, pre-pregnancy wt, maternal race and maternal age at delivery.
Infante-Rivard, (2007)[17] Canada	1998-2000	SGA	493 SGA cases, 480 controls.	Interview within 2 days of delivery on number of cups of coffee, tea and cans of cola daily for each trimester, and month before pregnancy.	No association caffeine consumption and SGA overall (smokers and non-smokers combined). ORs for caffeine intake in trimester 1 statistically heterogeneous between smokers and non, authors suggest an increased risk for non-smokers.	Caffeine (mg/day) ≥300 in trimester 1 vs <300 Reference category: <300 mg/day Smokers 0.43(0.18,1.03) Non smokers 2.13(0.82,1.03) Heterogeneity: p=0.01	Adjustment for, nausea, race, pre-pregnancy BMI, parity. Smoking also adjusted for when analysis not stratifying smokers and non-smokers.

Table 2. Key data from relevant human studies published since the previous COT review *continued*

Author + location	Study period	Outcome variables	Study sample	Measure of caffeine exposure	Study authors description of results	OR/RR (95% CI)	Comments
Klebanoff *et al* (2002)[34] USA	1959-1966	SGA	2,515 women.	Paraxanthine levels in serum collected in trimester 3 (>26 wks of gestation).	Mean levels higher in women with SGA babies (754 ng/ml) vs women with NGA babies (653 ng/ml) Significant linear trend for smokers but not non-smokers before adjustment.	Not reported	Adjustment for maternal age, pre-pregnancy weight, education, parity, ethnicity and no. cigarettes smoked per day.
Ørskou *et al* (2003)[36] Denmark	1990-1999	High birth weight (>4,000 g)	24,093 non-diabetic pregnant women.	Questionnaire at approx. 16 wks gestation on average daily coffee intake.	Women with a caffeine intake of >200 mg/day had a statistically reduced 'risk' of giving birth to an infant weighing > 4,000 g compared to women with an intake of <200 mg/day.	OR for high BW Caffeine (mg/day) 300-399 0.88(0.81,0.96) ≥400 0.87(0.79,0.95) Reference: <200 mg/day	Adjustment for pre-pregnancy weight and height, parity, smoking, alcohol, marital status, education level, gestational age and infant gender.
Parazzini *et al* (2005)[19] Italy	Not stated	SGA	555 women with SGA babies [<10th percentile based on Italian standard] (cases) 1966 women with term babies of normal weight (controls).	Interviews on tea, cola and coffee intake prior to pregnancy and in each trimester.	No significant associations between tea, cola, caffeinated coffee or decaffeinated coffee consumption and SGA.	Coffee (≥3 cups/day) Reference category: 0 cups/day Trimester 1 1.2(0.8,1.8) Trimester 2 1.2(0.8-1.8) Trimester 3 0.9(0.6,1.4)	Adjustment for age, education, parity, smoking in trimester 3, gestational hypertension and history of SGA birth.

Author + location	Study period	Outcome variables	Study sample	Measure of caffeine exposure	Study authors description of results	OR/RR (95% CI)	Comments
Vik *et al* (2003)[14] Sweden	1986-1988	SGA	111 mothers of small for gestational age (SGA) babies. 747 mothers of non-SGA babies.	3-day food records collected in Trimesters 2 and 3. Caffeine intake calculated from tea, coffee, soft drinks and chocolate, classed as high or low based on median.	Mean caffeine intake higher in SGA mothers than controls in trimester 3 (281 vs 212 mg/day) but not in trimester 1.	Caffeine intake classed as high/low based on median High intake week 17 1.1(0.6,2.1) High intake week 33 1.6(1.0,2.5) High avge intake over pregnancy 1.5(1.0,2.4) No statement of baseline.	Adjustment for smoking at conception, pre-pregnancy wt, low education, previous SGA birth High mean caffeine intake in trimester 3 or in trimester 1 and 3 combined associated with increased risk for SGA birth of male but not female babies.
Xue *et al* (2007)[43] USA	Inform-ation collected from mothers in 2001-2002	BW, IUGR	34,063 women in Nurse's Mother Cohort.	Interviews conducted on coffee intake when pregnant with their nurse daughters.	Daily consumption of each additional cup of coffee associated with a 10g decrease in bw	Coffee (cups/day) IUGR <1 1.00(0.82,1.21) 1-2 1.28(1.12,1.47) 3-4 1.30(1.10,1.55) >5 1.63(1.25,2.12) Trend test (cup/day) 1.09(1.05,1.13) Baseline: Never drank coffee	Interviews with mothers conducted a long time after pregnancy - when their offspring were adults. Adjustment for maternal BW, height, BMI, birth order, maternal weight gain, diabetes in pregnancy, smoking, gestational age, occupation, maternal milk consumption, paternal BMI, maternal infertility.

Table 2. Key data from relevant human studies published since the previous COT review *continued*

Author + location	Study period	Outcome variables	Study sample	Measure of caffeine exposure	Study authors description of results	OR/RR (95% CI)	Comments
Studies on miscarriage, stillbirth and infant death							
Bech *et al* (2005)[8] Denmark	1996-2002	Fetal death (miscarriage or stillbirth).	88,482 pregnant women recruited into Danish National Birth Cohort by GPs.	Telephone interview at approx gestational wk 16 on daily coffee consumption.	High levels of coffee consumption associated with an increased risk of fetal death.	Cups coffee/day: 0.5-3 1.03(0.89,1.19) 4-7 1.33(1.08,1.63) ≥8 1.59(1.19,2.13) Reference category: 0 cups coffee/day	Adjustment for age, parity, smoking, pre-pregnancy BMI, alcohol consumption, socio-occupational status.
George *et al* (2006)[10] Sweden	1996-1998	Repeated miscarriage	108 women with ≥2 consecutive miscarriages (cases). 953 control women matched by wks of gestation.	Interviews within 2-6 wks of miscarriage on intake of coffee, tea, cocoa, chocolate, soft drinks and caffeine-containing medication.	Mean caffeine intake 311 mg/day in cases, 240mg/day for controls.	Mean caffeine intake in pregnancy (mg/day) Smokers 100-299 0.5 (0.04,6.9) ≥300 0.4 (0.05,4.1 Non smokers 100-299 1.9 (0.8,4.3 ≥300 2.7 (1.1,6.2) Reference: 0-99 mg/day	Adjustment for age, previous pregnancy history, induced abortions, myoma, time to conceive, alcohol intake and folate levels.
Giannelli *et al* (2003)[11] UK	1987-1989	Miscarriage	160 nulliparous women with miscarriage (cases). 314 nulliparous pregnant women attending for antenatal care in trimester 3.	Interview 3 wks after miscarriage or at antenatal appointment on coffee, tea and cola consumption.	Caffeine consumption >300 mg/day during pregnancy associated with an increased risk of miscarriage.	Caffeine intake in pregnancy (mg/day) 151-300 1.19(0.67,2.12) 301-500 1.94(1.04,3.63) >500 2.18(1.08,4.40) Reference: ≤ 150 mg/day	Adjustment for maternal age, severity of nausea and gestational age.

Author + location	Study period	Outcome variables	Study sample	Measure of caffeine exposure	Study authors description of results	OR/RR (95% CI)		Comments
Karypidis *et al* (2006)[12] Sweden	1996-1998	First trimester miscarriage.	507 women with miscarriage in trimester 1 (cases). 908 women with a normal trimester 1 pregnancy (controls).	Interview on intake of coffee, tea, caffeine-containing soda and hot chocolate. Consumption based on women's estimate of cup size.	Significant association between caffeine intake of 100-299 and >500 mg/day and miscarriage in women with CYP1B1 Val/Val genotype.	Caffeine (mg/day) 100-299 300-499 >500 Reference: Leu/Leu genotype & <100 mg/day intake.	Val/Val genotype 2.63(1.39,4.98) 1.82(0.84,3.93) 3.61(1.36,9.61)	Adjustment for age, smoking, alcohol, previous miscarriage, parity, pregnancy symptoms. Unclear when interviews took place.
Khoury *et al* (2004)[16] USA	1978-1993	Wide range including, miscarriage, congenital malformation, pre-eclampsia, delivery at <37wks.	191 pregnant women with type 1 diabetes.	Monthly interviews; caffeine consumption based on number cups caffeinated beverages/day.	Significant associations observed for spontaneous miscarriage (+ve), pre-eclampsia and infant hypoglycaemia (-ve).	Drinks/day 1-2 ≥3 Reference: 0 drinks/day Caffeine intake at >20 wks gestation.	Spont. miscarriage 3.8 (0.8, 16.9) 5.2 (1.2, 22.0) Pre-eclampsia 0.3 (0.1, 1.0) Infant hypoglycaemia 0.2 (0.1, 1.0)	Adjustment for age, yrs since diagnosis of diabetes, nephropathy, retinopathy, glycaemic control, cigarette smoking.
Macono-chie *et al* (2007)[18] UK	1980-2002	Trimester 1 miscarriage.	603 women with most recent pregnancy ending in trimester 1 miscarriage (cases). 6116 women with most recent pregnancy progressing beyond 12 wks (controls).	Questionnaire on reproductive history sent to UK women in 2001. Caffeine intake determined by tea, coffee and caffeinated drink consumption.	Apparent association between caffeine intake and risk of miscarriage not significant after adjustment for nausea.	Caffeine (mg/day) <151 151-300 301-500 >500 Baseline: 0 mg/day.	 1.03(0.71,1.49) 0.93(0.64,1.33) 1.04(0.72,1.50) 1.14(0.79,1.66)	Adjustment for year of conception, maternal age, previous miscarriage, previous live birth and nausea.

Table 2. Key data from relevant human studies published since the previous COT review *continued*

Author + location	Study period	Outcome variables	Study sample	Measure of caffeine exposure	Study authors description of results	OR/RR (95% CI)	Comments
Matijas-evich *et al* (2006)[35] Uruguay	2002-2003	Fetal death.	382 women with fetal death ≥20wks gestational age or weighing >350g (cases). 792 women with live term NGA births (controls).	Questionnaire on coffee and mate consumption.	Mean caffeine intake significantly higher in cases than controls (156.5 mg/day vs 113.6).	Mean caffeine (mg/day) 1-59 0.74(0.42,1.31) 60-149 0.93(0.51,1.67) 150-299 1.22(0.69,2.17) ≥300 2.33(1.23,4.41) Reference: 0 mg/day	Adjustment for maternal and partner's education, history of miscarriages + fetal deaths, vomiting/ nausea in trimester 1 and attendance for prenatal care.
Rasch *et al* (2003)[13] Denmark	1994	Miscarriage.	330 women with miscarriage in gestational wks 6-16 (cases). 1168 women with live fetuses in gestational wks 6-16 (controls).	Questionnaire on daily tea, coffee, cola and chocolate bar consumption during pregnancy.	Consumption of ≥ 375 mg caffeine /day associated with increased risk of miscarriage.	Caffeine (mg/day) 200-374 1.31(0.92,1.86) ≥375 2.21(1.53,3.18) Baseline: 0-199 mg/day.	Adjustment for age, parity, occupation, smoking and alcohol.
Sata *et al* (2005)[38] Japan	2003-2004	Recurrent pregnancy loss.	58 women with two or more miscarriages (cases). 147 women with live births (controls).	Questionnaire on coffee, tea and cola consumption during pregnancy.	CYP1A2*1F (AA vs CA CC) genotype found to influence risk.	Caffeine (mg/day) All women 100-299 1.29(0.66,2.50) ≥300 1.82(0.72,4.58 CYP1A2 CC+CA 100-299 1.03(0.42,2.52) ≥300 1.03(0.29,3.70) CYP1A2 AA 100-299 1.94(0.57-6.66) ≥300 5.23(1.05-25.9) Reference: 0-99 mg/day for each grouping.	Adjustment for age and smoking status in pregnancy.

Author + location	Study period	Outcome variables	Study sample	Measure of caffeine exposure	Study authors description of results	OR/RR (95% CI)		Comments
Savitz *et al* (2008)[20] USA	2000-2004	Miscarriage at <20 gestational wks.	2407 women recruited at <12 gestational wks.	Interview before 16 weeks on caffeine-containing coffee, tea and soda consumption pre-pregnancy, 4 wks after last menstrual period (LMP), + at time of interview or when still pregnant.	Coffee and caffeine consumption at all 3 timepoints were unrelated to overall risk of miscarriage.	Caffeine (mg/day) >0-<348 ≥348-695 >696 >0-<348 ≥348-695 >696 >0-<348 ≥348-695 >696 Reference category: 0 mg/day	Pre-preg 1.6 (0.7,3.4) 1.2 (0.6,2.6) 1.1 (0.4,2.6) 4wks post LMP 1.2 (0.6,2.2) 1.0 (0.5,2.0) 0.5 (0.2,1.4) Time of interview 1.1 (0.6,2.2) 1.9 (1.1,3.5) 2.3 (1.2,4.5)	Median levels caffeine consumption modest relative to previous studies + authors suggest this restricted the ability to examine effects above 300-400 mg/day. Adjustment for maternal age, race ethnicity, maternal education, marital status, alcohol consumption + vitamin use.
Signorello *et al* (2001)[23] Sweden	1996-1998	Miscarriage.	101 women with normal karyotype miscarriages (cases). 953 pregnant women at 6-12 gestational wks (controls).	Interviews within 2 wks of miscarriage or 6 days of enrolment (controls). Coffee, tea, cocoa, chocolate, soft drinks + caffeine-containing medication.	Caffeine found to be a significant risk factor among women with low, but not high, CYP1A2 activity. Association with NAT2 genotype less clear.	Caffeine (mg/day) 100-299 ≥300 100-299 ≥300 100-299 ≥300 100-299 ≥300 Reference: 0-99 mg/day for each group.	Low CYP1A2 activity 0.32(0.08,1.23) 0.46(0.12,1.73) High CYP1A2 activity 2.42(1.01,5.80) 3.17(1.22,8.22) Slow acetylators 2.38(1.04,5.49) 1.65(0.67,4.06) Fast acetylators 1.07(0.39,2.95) 1.93(0.67,5.51)	Adjustment for age, gestational week, smoking + nausea.

Table 2. Key data from relevant human studies published since the previous COT review *continued*

Author + location	Study period	Outcome variables	Study sample	Measure of caffeine exposure	Study authors description of results	OR/RR (95% CI)	Comments
Tolstrup *et al* (2003)[40] Denmark	1991-1995	Miscarriage.	303 women from a population-based cohort with miscarriage (cases). 1381 women in cohort who gave birth (controls).	Interview on tea and coffee intake at enrolment into cohort and again 2 yrs later.	High pre-pregnancy caffeine intake (>900 mg/day) associated with an increased risk of miscarriage.	Caffeine intake pre-pregnancy (mg/day) 75-300 1.26(0.77-2.06) 301-500 1.45(0.87,2.41) 501-900 1.44(0.87,2.37) >900 1.72(1.00,2.96) Reference category: <75 mg/day	Only considers pre-pregnancy caffeine intake Adjustment for age, marital status, smoking and alcohol intake.
Weng *et al* (2008)[15] USA	1996-1998	Miscarriage at <20 gestational wks.	1063 pregnant women recruited at ≤15 gestational wks.	Interview on intake of coffee, tea, caffeine-containing soda and hot chocolate.	Increasing caffeine consumption associated with an increased risk of miscarriage.	Caffeine mg/day <200 1.42(0.93,2.15) ≥200 2.23(1.34,3.69) Baseline: Non-user	Adjustment for maternal age, race, education, family income, marital status, previous miscarriage, nausea and vomiting since LMP, smoking status, alcohol consumption, Jacuzzi use and magnetic field exposure.
Wisborg *et al* (2003)[42] Denmark	1989-1996	Stillbirth and infant death in 1st yr of life.	18,478 singleton pregnancies.	Questionnaire at approx 16 wks of gestation on coffee intake.	Drinking coffee during pregnancy associated with an increased risk of stillbirth but not infant death.	Coffee (cups/day) Stillbirth 1-3 0.6 (0.3, 1.1) 4-7 1.4 (0.8, 2.5) ≥8 2.2 (1.0, 4.7) Infant death 1-3 0.9 (0.6, 1.6) 4-7 0.2 (0.1, 0.7) ≥8 1.6 (0.7, 3.6) Baseline: 0 cups/day	Caffeine exposure from tea, cola or drinking chocolate considered insignificant so not included in analysis. Adjustment for smoking and alcohol, parity, age, marital status, BMI, yrs education and employment status in pregnancy.

Author + location	Study period	Outcome variables	Study sample	Measure of caffeine exposure	Study authors description of results	OR/RR (95% CI)	Comments
Studies on pre-term birth							
Bech *et al* (2007)[26] Denmark	1998-2002	Birth weight and length of gestation.	1207 pregnant women drinking at least 3 cups of coffee/day, recruited before 20 wks gestation.	Randomised to drink caffeinated (n=568) or decaffeinated (n=629) instant coffee at usual consumption levels Interviewed throughout pregnancy on daily consumption coffee, tea, cola and cocoa.	No significant differences in mean bw or mean length of gestation between caffeinated and decaffeinated groups.	Not reported	Adjustment for length of gestation, parity, prepregnancy BMI and smoking at entry to study. Women were not asked to avoid intake of other caffeinated beverages.
Bicalho and Barros Filho (2002)[27] Brazil	1994-1995	LBW, prematurity and IUGR.	354 newborns with bw <2,500 g (cases). 354 newborns ≥3,000 g (controls).	Daily consumption of coffee, tea and soft drinks.	No association between caffeine consumption during pregnancy and low birthweight, prematurity and intrauterine growth restriction.	Caffeine (mg/day) — Prematurity <300 — 0.59(0.32,1.09 ≥300 — 0.32(0.15,0.72)	Abstract only in English. Adjustment for age, schooling, income, marital status, skin colour, parity, smoking, previous lbw child, pre-pregnancy weight, employment status, interval between pregnancies, prenatal care and high blood pressure.

Table 2. Key data from relevant human studies published since the previous COT review *continued*

Author + location	Study period	Outcome variables	Study sample	Measure of caffeine exposure	Study authors description of results	OR/RR (95% CI)	Comments
Bracken *et al* (2003)[29] USA	1996-2000	Preterm delivery.	2,291 women with singleton live births.	Interviews on coffee, tea and soda consumption - Interview on trimester 1 intake conducted before gestation wk 25 - Post natal interview on trimester 3 intake. Urine analysis at interview 1.	No significant association of caffeine consumption in trimesters 1 or 3 or urinary caffeine with preterm delivery.	Trimester 1 Caffeine (mg/Day) 1-149 Preterm del 1.20(0.80,1.76) 150-299 1.74(0.93,3.27) ≥300 1.67(0.76,3.81) Reference: average 0 mg/day	Adjustment for age, parity, # prior pregnancies, marital status, race, education, height, smoking in 3rd trimester, weight. Similar ORs reported for caffeine consumption in trimester 3.
Chiaffarino *et al* (2006)[31] Italy	1989-1999	Preterm birth of SGA or normal for gestational age babies.	502 women who delivered at <37 wks (cases). 1966 women who gave birth at ≥37 wks.	Post pregnancy interview on coffee, tea and cola consumption.	No significant association with overall intake of caffeine Inverse association coffee consumption (≥2 servings/day) disregarding caffeine from other sources and risk of SGA preterm babies (OR= 0.5 [0.3,0.8])	Caffeine servings/day Preterm SGA 1 1.1 (0.7,1.8) ≥2 1.0 (0.6,1.7) Preterm NGA 1 0.9 (0.6,1.2) ≥2 0.9 (0.7,1.3) Reference category: 0 servings/day	Adjustment for age, education, gestational hypertension and history of preterm birth.
de Souza + Sichieri (2005)[21] Brazil	Not stated	Prematurity.	140 newborns with gestational age <37wks (cases). 162 newborns with gestational age ≥37 wks (controls).	'Semi-quantitative' food frequency questionnaire on coffee, tea + powdered chocolate.	Total caffeine consumption during pregnancy not associated with prematurity.	Caffeine (mg/day) 50-99 1.58(0.32,2.84) ≥100 1.35(0.48-3.80) Baseline: Below 50 mg/day	Abstract only in English. Most caffeine intakes were less than 300 mg/day.

Author + location	Study period	Outcome variables	Study sample	Measure of caffeine exposure	Study authors description of results	OR/RR (95% CI)	Comments
Studies on teratogenicity							
Browne *et al* (2007)[30] USA	1997-2002	Cardio-vascular malformations (CVMs).	4,196 mothers of infants with cardio-vascular malformation 3,957 controls.	Telephone interviews on consumption of caffeinated coffee, tea, soda or soft drinks and chocolate in year prior to pregnancy.	No significant positive associations between maternal caffeine consumption and CVMs.	Caffeine (mg/day) All CVM 10-<100 1.17(0.91,1.50) 100-<200 1.05(0.80,1.38) 200-<300 1.23(0.91,1.66) ≥300 1.24(0.91,1.68) Baseline: <10 mg caffeine/day	Adjustment for mother's state of residence at time of birth.

References

1 Annual report of Committees on Toxicity, Mutagenicity, Carcinogenicity of Chemicals in Food, Consumer Products and the Environment, 2001. COT Statement 2001

2. Barker DJ. Childhood causes of adult diseases. *Arch Dis Child* 1988;63:867–69.

3. Nathanielsz PW. Fetal programming: How the quality of fetal life alters biology for a lifetime. *NeoReviews* 2000;1:126–31.

4. Eriksson M, Wallander MA, Krakau I, Wedel H, Svardsudd K. The impact of birth weight on coronary heart disease morbidity and mortality in a birth cohort followed up for 85 years: a population-based study of men born in 1913. *J Intern Med* 2004;256:472–81.

5. Ong KK, Dunger DB. Birth weight, infant growth and insulin resistance. *Eur J Endocrinol* 2004;151 (Suppl 3):U131–39.

6. Levy-Marchal C, Jaquet D. Long-term metabolic consequences of being born small for gestational age. *Pediatr Diabetes* 2004;5:147–53.

7. Balat, O., Balat, A., Ugur, M. G., and Pence, S. 2003 The effect of smoking and caffeine on the fetus and placenta in pregnancy. Clin Exp Obstet.Gynecol. 30[1], 57-59.

8. Bech, B. H., Nohr, E. A., Vaeth, M., Henriksen, T. B., and Olsen, J. 15-11-2005 Coffee and fetal death: a cohort study with prospective data. Am J Epidemiol 162[10], 983-990.

9. Bracken, M. B., Triche, E. W., Belanger, K., Hellenbrand, K., and Leaderer, B. P. 1-3-2003 Association of maternal caffeine consumption with decrements in fetal growth. Am J Epidemiol 157[5], 456-466.

10. George, L., Granath, F., Johansson, A. L., Olander, B., and Cnattingius, S. 2006 Risks of repeated miscarriage. Paediatr.Perinat.Epidemiol 20[2], 119-126.

11. Giannelli, M., Doyle, P., Roman, E., Pelerin, M., and Hermon, C. 2003 The effect of caffeine consumption and nausea on the risk of miscarriage. Paediatr.Perinat.Epidemiol 17[4], 316-323.

12. Karypidis, A. H., Soderstrom, T., Nordmark, A., Granath, F., Cnattingius, S., and Rane, A. 2006 Association of cytochrome P450 1B1 polymorphism with first-trimester miscarriage. Fertil.Steril. 86[5], 1498-1503.

13. Rasch, V. 2003 Cigarette, alcohol, and caffeine consumption: risk factors for spontaneous abortion. Acta Obstet.Gynecol.Scand. 82[2], 182-188.

14. Vik, T., Bakketeig, L. S., Trygg, K. U., Lund-Larsen, K., and Jacobsen, G. 2003 High caffeine consumption in the third trimester of pregnancy: gender-specific effects on fetal growth. Paediatr.Perinat.Epidemiol 17[4], 324-331.

15. Weng, X., Odouli, R. and Li, D.E. 2008 Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. American J Obstet. Gynecol.198(3):279.e1-8

16. Khoury, J. C., Miodovnik, M., Buncher, C. R., Kalkwarf, H., McElvy, S., Khoury, P. R., and Sibai, B. 2004 Consequences of smoking and caffeine consumption during pregnancy in women with type 1 diabetes. J Matern.Fetal Neonatal Med 15[1], 44-50.

17. Infante-Rivard, C. 2007 Caffeine intake and small-for-gestational-age birth: modifying effects of xenobiotic-metabolising genes and smoking. Paediatr.Perinat.Epidemiol 21[4], 300-309.

18. Maconochie, N., Doyle, P., Prior, S., and Simmons, R. 2007 Risk factors for first trimester miscarriage--results from a UK-population-based case-control study. BJOG 114[2], 170-186.

19. Parazzini, F., Chiaffarino, F., Chatenoud, L., Tozzi, L., Cipriani, S., Chiantera, V., and Fedele, L. 2005 Maternal coffee drinking in pregnancy and risk of small for gestational age birth. Eur J Clin Nutr 59[2], 299-301.

20. Savitz, D.A., Chan, R.L., Herring, A.H., Howards, P.P. and Hartmann, K.E. 2008 Caffeine and miscarriage risk. Epidemiology19[1], 55-62.

21. de Souza, R. A. and Sichieri, R. 2005 [Caffeine intake and food sources of caffeine and prematurity: a case-control study]. Cad.Saude Publica 21[6], 1919-1928.

22. Grosso, L. M., Triche, E. W., Belanger, K., Benowitz, N. L., Holford, T. R., and Bracken, M. B. 1-6-2006 Caffeine metabolites in umbilical cord blood, cytochrome P-450 1A2 activity, and intrauterine growth restriction. Am J Epidemiol 163[11], 1035-1041.

23. Signorello, L. B., Nordmark, A., Granath, F., Blot, W. J., McLaughlin, J. K., Anneren, G., Lundgren, S., Ekbom, A., Rane, A., and Cnattingius, S. 2001 Caffeine metabolism and the risk of spontaneous abortion of normal karyotype fetuses. Obstet.Gynecol. 98[6], 1059-1066.

24. National Institutes of Health. Final Report on the reproductive toxicity of caffeine (CAS #508-2) administered by gavage to Sprague-Dawley rats. National Toxicology Program. National Institute of Environmental Health Services. July 1996

25. Gilbert, S. G., Rice, D. C., Reulhl, K. R., Starvric, B. 1988 Adverse pregnancy outcome in the monkey (Macaca fascicularis) after chronic caffeine exposure. J Pharmacol Exp Ther 245, 1048-1053

26. Bech, B. H., Obel, C., Henriksen, T. B., and Olsen, J. 24-2-2007 Effect of reducing caffeine intake on birth weight and length of gestation: randomised controlled trial. BMJ 334[7590], 409.

27. Bicalho, G. G. and Barros Filho, Ade A. 2002 [Birthweight and caffeine consumption]. Rev Saude Publica 36[2], 180-187.

28. Bille, C., Olsen, J., Vach, W., Knudsen, V. K., Olsen, S. F., Rasmussen, K., Murray, J. C., Andersen, A. M., and Christensen, K. 2007 Oral clefts and life style factors--a case-cohort study based on prospective Danish data. Eur J Epidemiol 22[3], 173-181.

29. Bracken, M. B., Triche, E., Grosso, L., Hellenbrand, K., Belanger, K., and Leaderer, B. P. 2002 Heterogeneity in assessing self-reports of caffeine exposure: implications for studies of health effects. Epidemiology 13[2], 165-171.

30. Browne, M. L., Bell, E. M., Druschel, C. M., Gensburg, L. J., Mitchell, A. A., Lin, A. E., Romitti, P. A., and Correa, A. 2007 Maternal caffeine consumption and risk of cardiovascular malformations. Birth Defects Res A Clin Mol.Teratol. 79[7], 533-543.

31. Chiaffarino, F., Parazzini, F., Chatenoud, L., Ricci, E., Tozzi, L., Chiantera, V., Maffioletti, C., and Fedele, L. 2006 Coffee drinking and risk of preterm birth. Eur J Clin Nutr 60[5], 610-613.

32. Clausson, B., Granath, F., Ekbom, A., Lundgren, S., Nordmark, A., Signorello, L. B., and Cnattingius, S. 1-3-2002 Effect of caffeine exposure during pregnancy on birth weight and gestational age. Am J Epidemiol 155[5], 429-436.

33. Cnattingius, S., Signorello, L. B., Anneren, G., Clausson, B., Ekbom, A., Ljunger, E., Blot, W. J., McLaughlin, J. K., Petersson, G., Rane, A., and Granath, F. 21-12-2000 Caffeine intake and the risk of first-trimester spontaneous abortion. N.Engl.J Med 343[25], 1839-1845.

34. Klebanoff, M. A., Levine, R. J., Clemens, J. D., and Wilkins, D. G. 1-1-2002 Maternal serum caffeine metabolites and small-for-gestational age birth. Am J Epidemiol 155[1], 32-37.

35. Matijasevich, A., Barros, F. C., Santos, I. S., and Yemini, A. 2006 Maternal caffeine consumption and fetal death: a case-control study in Uruguay. Paediatr.Perinat.Epidemiol 20[2], 100-109.

36. Orskou, J., Henriksen, T. B., Kesmodel, U., and Secher, N. J. 2003 Maternal characteristics and lifestyle factors and the risk of delivering high birth weight infants. Obstet.Gynecol. 102[1], 115-120.

37. Parazzini, F., Cortinovis, I., Bortolus, R., Fedele, L., and Decarli, A. 1995 Weight at birth by gestational age in Italy. Hum Reprod. 10[7], 1862-1863.

38. Sata, F., Yamada, H., Suzuki, K., Saijo, Y., Kato, E. H., Morikawa, M., Minakami, H., and Kishi, R. 2005 Caffeine intake, CYP1A2 polymorphism and the risk of recurrent pregnancy loss. Mol.Hum Reprod. 11[5], 357-360.

39. Schmidt, R. J., Romitti, P. A., Burns, T. L, Druschel, C. M., Browne, M., and Olney, R. S. 2007 Maternal caffeine intake, selected metabolic gene variants, and neural tube defects. Ann Epidemiol 17[9], 750.

40. Tolstrup, J. S., Kjaer, S. K., Munk, C., Madsen, L. B., Ottesen, B., Bergholt, T., and Gronbaek, M. 2003 Does caffeine and alcohol intake before pregnancy predict the occurrence of spontaneous abortion? Hum Reprod. 18[12], 2704-2710.

41. Tsubouchi, H., Shimoya, K., Hayashi, S., Toda, M., Morimoto, K., and Murata, Y. 2006 Effect of coffee intake on blood flow and maternal stress during the third trimester of pregnancy. Int J Gynaecol.Obstet. 92[1], 19-22.

42. Wisborg, K., Kesmodel, U., Bech, B. H., Hedegaard, M., and Henriksen, T. B. 22-2-2003 Maternal consumption of coffee during pregnancy and stillbirth and infant death in first year of life: prospective study. BMJ 326[7386], 420.

43. Xue, F., Willett, W. C., Rosner, B. A., Forman, M. R., and Michels, K. B. 12-10-2007 Parental characteristics as predictors of birthweight. Hum Reprod.

Annex A

Search strategy for review of research on reproductive effects of caffeine

Pubmed

Colleagues at the Food Standards Agency's Information Centre searched using the following search terms:

(caffeine OR coffee) AND [("adverse effects" AND "pregnancy") OR "fetal growth restriction" OR "fetal growth retardation" OR "FGR" OR "fetal growth" OR "miscarriage" or "outcomes" OR "birth weight" OR "intrauterine growth retardation" OR "IUGR" OR "small for gestational age" OR "SGA" OR "fetus" OR "preterm birth")]

Limits imposed on search: Published between 2001-2008, limited to 'humans'

Total number of papers: **32**

Of these, 2 papers were not ordered as they were review articles, 2 reported studies performed in rodents, 1 described a study of factors affecting IVF fertility and 6 referred to caffeine only as a confounder in irrelevant studies.

Search conducted on **PubMed** using the following search string:

(caffeine OR coffee) AND ("adverse effects" AND "pregnancy")

Limits imposed on search: Published between 2001-2008, limited to 'humans'

Total number of papers: **88**

This search yielded 24 potentially useful references that were not identified in the previous search. All of the other references in this search were duplicates of those already obtained, or were disregarded primarily as they described studies where pregnancy outcome was not the main focus of the study, or focussed on different species such as primates, rats or mice, or for one of the reasons stated previously.

British Library Inside

Search conducted on **British Library Inside** using the following search string:

(caffeine OR coffee) AND pregnancy

Limits imposed on search: Published ≥2001 only.

Total number of papers: **72**

Of these 18 had not been previously identified. All of the other references in this search were either duplicates of papers already obtained, duplicates within the search, or were disregarded for the reasons outlined above.

Current Contents

Colleagues at the Information Centre searched using the following search terms:

(caffeine OR coffee) AND [("adverse effects" AND "pregnancy") OR "fetal growth restriction" OR "fetal growth retardation" OR "FGR" OR "fetal growth" OR "miscarriage" or "outcomes" OR "birth weight" OR "intrauterine growth retardation" OR "IUGR" OR "small for gestational age" OR "SGA" OR "fetus" OR "preterm birth")]

Limits imposed on search: Published ≥2001 only.

Total number of papers: **175**

From the results of this search only 2 had not been previously identified. Most references were duplicates of those already found and some related to studies conducted in rodents and primates.

From the references (n = 75) obtained, 42 were excluded once the full paper was retrieved: as they were reviews (9), letters in response to papers (7), included in the 2001 COT review (3), studies reporting on maternal health outcomes/fertility (5), reported health issues in young children (6), reporting intake estimates (4), described the use of caffeine for apnoea of prematurity (2), in a foreign language (2), or duplicates (4).

Statement on the Risk to Consumers of Eating Foods Derived from animals that had eaten bracken

Background

1. Several cases of bracken poisoning in farm animals have been reported. The Committee was asked to consider the hazards to the health of consumers eating foods derived from bracken-poisoned animals, and whether there were sufficient data to establish how long poisoned animals should be left before they may safely be milked or slaughtered for human consumption. It was noted that bracken is eaten as a vegetable in some parts of the world, but the Committee was not aware of it being eaten in the UK.

2. Bracken (*Pteridium aquilinum*) is an invasive fern that is common throughout the world with several different sub-species (formerly referred to as varieties). The sub-species that are found in the UK are *aquilinum* (the common form), *latiusculum* (found in Scottish pine forests) and *atlanticum* (found mostly in limestone areas of Wales and Scotland)[1]. Bracken is found in all parts of the country and dense growths cover large areas of land in Wales, Scotland and northern England[2].

3. Eating bracken can be harmful to farm animals and there is some evidence that it might also be harmful to humans. The sub-species found in the UK are toxic and potentially fatal to farm animals if eaten. However, there is great variation in the amount of the bracken toxin ptaquiloside, and possibly of other bracken toxins, in the different sub-species of bracken that are found throughout the world[3]. There is also variation between strains within particular sub-species of bracken[4] and at different times of the year[5]. All parts of the bracken plant contain potentially harmful chemicals, some of which can be excreted in milk and may leave residues in meat and offal derived from animals that have eaten the plant. Thus there is a potential hazard to consumers.

4. The COT and its sister committees the COM and the COC last advised on the safety of foods derived from animals reared on bracken-infested land in the Annual Reports of the COM in 1993[6] and of the COT in 1996[7]. The Committees had considered the available information on carcinogenicity and mutagenicity of bracken, along with the results of a government-sponsored study of the transfer of bracken mutagens into milk from goats fed on bracken. The COT Annual Report for 1996[7] reported that the COC concluded in 1988 that *"There were...few data available at that time about the extent to which farm animals grazed on bracken and the occurrence of bracken constituents in dairy products."*

5. It was also stated in the COT Annual Report[7] that *"Human epidemiology data [were] limited and the COC concluded that evidence of carcinogenicity was inconclusive. Carcinogenicity studies in laboratory animals in which whole bracken had been administered in the diet, in some cases accounting for up to one third of the total diet, were flawed in their experimental design, execution and interpretation. However, despite these limitations, the COC concluded that these studies had demonstrated a clear trend for increased benign and malignant tumours of the small and/or large intestine and/or urinary bladder and that there was a need for properly conducted carcinogenicity bioassays in rats and mice. The COC also considered the active constituents of bracken and concluded that ptaquiloside, an inherent constituent of bracken, had been shown to be capable [of]*

reproducing some [of] the carcinogenic effects of whole bracken." In addition, the International Agency for Research on Cancer (IARC) classified bracken in Group 2B: possibly carcinogenic to humans[125].

6. It was reported in the COM Annual Report for 19936 that:

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

   - *"Work carried out by MAFF[50], [156] [the Ministry of Agriculture, Fisheries and Food] on the possibility of bracken mutagens being transmitted to the milk of bracken-fed goats suggested that very little, if any, mutagenic activity is present in the milk of the goats which were exposed to UK bracken for short time periods of time (1 month)."*

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

7. It was reported in the COT Annual Report for 1996[7] that the COT *"accepted the advice of the COC and COM, and agreed that the risk to the population was very low and that further research need not be undertaken on bracken fern mutagens".*

8. Since 1996, there have been several reports of farm animals eating bracken. As a consequence, the expectation that farm animals would not readily eat bracken needs to be reconsidered. The Veterinary Laboratory Agency (VLA) has identified several cases of suspected bracken poisoning in farm animals. There were 22 cases of bracken poisoning in cattle reported to the VLA between 1999 and 2007[8]. In addition, there was a report11 in 2007 of two pigs that were suspected of having been poisoned by bracken. It is likely that many more cases of bracken poisoning would have gone unreported, as there is no requirement to report bracken poisoning. It is also likely that many other animals would have eaten bracken without showing clinical signs of poisoning. Several food-producing species, including pigs and sheep, readily eat bracken and are used to clear bracken from pastures[8]. Furthermore, cattle have been observed to eat hay containing up to 30% bracken[9]. It has been reported that some horses and sheep eat bracken in preference to their normal pasture[10].

9. In addition, several reports of new studies of the safety of bracken have been published since the last consideration by the COT, the COM and the COC. The areas of interest covered by the new studies include human epidemiology and possible modes of action for the carcinogenicity of ptaquiloside. Both new and old studies are summarised in this Statement. The criteria by which relevant research was identified are set out in the Appendix to the Statement.

Constituents of Bracken

10. Bracken contains a large number of potentially harmful substances, including illudane and protoilludane glycosides (such as ptaquiloside[12, 13], ptaquiloside Z[14], isoptaquiloside[15], pteroside A2[16], pteridanoside[16] and caudatoside[15], terpenic indanones (pterosins)[15, 16], *p*-hydroxystyrene glycosides (ptelatosides A and B)[13], the cyanogenic glycoside prunacin[17], braxin glycosides[18], the flavinoid quercetin and its glycoside rutin[19, 20], kaempferol[19, 20], shikimic acid[21], thiaminases[22], ecdysteroids[22] and tannins[22]. Other substances detected in bracken include dihydrocinnamic acids, phloretic acid, dihydroferulic acid, 2,3-butanediol, 3-methylbutan-2-ol, monomethylsuccinate, methyl-5-oxoproline, 2(3H)-dihydrofuranone and *t*-2-methylcyclohexanol[13, 23, 24, 25, 26]. Little is known of the toxicology of many of these substances and information on the amounts in bracken is often lacking.

Toxicity of Bracken

Experimental Studies in Laboratory Animals and *in vitro*

11. Most of the available studies are investigations of the carcinogenicity and mutagenicity of bracken. There is no available carcinogenicity bioassay of bracken that has been performed to modern standards, but the carcinogenicity of bracken has been investigated in numerous more limited studies of several species of bracken. Feeding of bracken to several strains of mice at 25% or more in the diet for 6 weeks or longer[27, 28, 29, 30] produced neoplasms, including leukaemia, lung adenomas, intestinal tumours, bladder tumours and liver nodules. In rats[19, 31, 32, 33, 34, 149], 1% or more dietary bracken caused tumours including gastrointestinal adenocarcinomas and sarcomas, mainly in the ileum; transitional cell carcinomas of the urinary bladder; and pre-neoplastic nodules in the liver. In female Sprague Dawley rats, 1% or 2% dietary bracken caused mammary adenomas and fibroadenomas, but these were not seen in female F344 rats given the same treatment[149]. In guinea-pigs[35, 36, 37], 30% dietary bracken caused intestinal adenomas and adenocarcinomas and transitional cell carcinomas of the bladder, and it also caused panmyelopathy of the bone marrow and haematuria. In toads[38], dietary bracken caused ileal adenocarcinomas and malignant liver tumours. In 1988, the COC concluded[7] that the carcinogenicity studies of bracken in laboratory animals had demonstrated a clear trend towards increased incidence of benign and malignant tumours of the small and/or large intestine and/or urinary bladder but that there was a need for properly-conducted carcinogenicity studies in rats and mice.

12. Mice given Welsh bracken spores by stomach tube were found to have DNA-adducts in their stomach and small intestines, but not in the liver[39]. In contrast, rats fed Brazilian bracken (a sub-species not found in the UK) did not have DNA-adducts in their stomach or ileum[160]. Cytogenetic analysis of blood taken from cows[40, 41, 42] or people[43, 44] who had eaten bracken showed increased numbers of chromosomal aberrations.

13. Processed bracken and various extracts from bracken have been tested for carcinogenicity[13] or mutagenicity[12]. All parts of the bracken plant were carcinogenic in rats but the tips of young fronds (parts of the plant that are eaten by humans in some parts of the world) were the most potent[45]. Traditional methods of preparation of bracken for human consumption (boiled, treated with wood ash

or sodium bicarbonate, pickled in salt) reduced its carcinogenic potency in rats[28]. However, drying or freezing preserved the carcinogenic/mutagenic potency of bracken and the carcinogenic/mutagenic component(s) was extractable in aqueous media and in several organic solvents[12, 13]. Various extracts of bracken (including boiling water, acetone, methanol and ethanol extracts) were mutagenic to Ames strains of *Salmonella typhimurium*[152, 153, 154, 155]. There is some evidence that bracken and/or its extracts may be mutagenic *in vivo*, as indicated by the results of cytogenetics assays on tissues from intraperitoneally-dosed mice[157], bracken-fed cattle[40, 41, 42] and bracken-consuming humans[43, 44]; a dominant lethal assay in *Drosophila*[46, 47]; an unspecified test in mice[46] and 32P-postlabelling to detect DNA adducts in tissues of exposed mice[39] and rats[160]. Milk from bracken-fed cows[48, 49] was mutagenic to Ames strains of *Salmonella typhimurium*, and milk from cows fed on substantial amounts of bracken was carcinogenic in mice[51] and rats[48]. In 1993, when the COM considered[7] the available mutagenicity studies on bracken extracts, it concluded that there was evidence that mutagenic activity was expressed in mammalian cells *in vitro* and there was limited evidence (from observations of effects in exposed farm animals) that this activity might also be expressed *in vivo*. Positive results in several *in vivo* studies[41, 42, 43, 44, 157, 160] that have been performed since 1993 strengthen the evidence that bracken is an *in vivo* mutagen.

14. Repeat-dose toxicity studies have been performed in rats, guinea-pigs, rabbits and cats but a NOAEL was not identified in any of the studies. In rats[52] and rabbits53, 25% dietary incorporation of dried bracken, containing 4.6 to 20.7 ppm ptaquiloside, for 30-90 days caused various adverse effects, including reduced bodyweight gain, leucopaenia, oedema of the brain and degenerative changes in the liver and testes. In the rats there were also sub-epicardial haemorrhages in the heart and hypersecretion into the intestines. In the rabbit study, there was also haemorrhaging in various organs and depletion of lymphoid follicles in the spleen and mesenteric lymph nodes. In guinea-pigs[143] given 30% dried bracken in the diet, there was decreased feed intake and decreased bodyweight gain. Cats given 10 g of dried bracken every 48 hrs died at 9-10 days after the start of treatment[54]. All of the cats suffered hepatotoxicity.

15. The results of a developmental toxicity study in mice[55] showed that bracken in the diet at a maternally toxic dose caused low fetal weights and skeletal abnormalities (extra cervical or lumbar ribs, incomplete fusion of sternebrae, retarded ossification) in the offspring. No studies have been performed in laboratory animals to investigate reproductive toxicity over several generations.

Clinical and Epidemiological Findings in Farm Animals

16. Acute poisoning of farm animals fed on bracken can be fatal[56, 57]. Prolonged ingestion of sub-lethal amounts of bracken can lead to toxic effects that differ between species. Consumption of bracken is also associated with the development of tumours. The sites where tumours develop vary between species.

17. Sudden death with signs of toxicity similar to cyanide poisoning has been reported in animals fed on young fronds of bracken[58]. This is thought to be related to the presence in bracken of the cyanogenic glycoside, prunacin.

18. In non-ruminant species, the principal adverse effect of dietary bracken is to cause a deficiency of thiamine (vitamin B1) through the action of thiaminases in the bracken[56]. Bracken has not been seen to cause thiamine deficiency in ruminants, whose gut microflora can synthesise this vitamin, or in humans, perhaps because their level of exposure to bracken is lower and they have a more varied diet.

19. In adult cattle, dietary exposure to bracken can cause a severe panmyeloid depression of bone marrow activity, which is expressed clinically as an acute haemorrhagic syndrome[57]. Calves show a different acute clinical syndrome involving bradycardia, laryngeal oedema and death from heart failure[57]. Prolonged dietary exposure of cattle can result in chronic depression of bone marrow activity, which can cause leucopaenia and thrombocytopaenia, which in turn leads to widespread petechial haemorrhages[59]. It has been proposed[121] that immunosuppression resulting from the effects of bracken on the bone marrow might make animals more prone to infections. Chromosomal instability has been reported in lymphocytes taken from cattle that had been fed bracken[145]. Prolonged exposure of adult cattle can result in a chronic disease called bovine enzootic haematuria (BEH) that involves changes to the blood vessels of the urinary bladder and the later development of benign and malignant bladder tumours[60]. Syndromes similar to BEH have also been described in buffalo, sheep and deer[61]. Bracken feeding of cattle has also been associated with a slow-developing epidermoid carcinoma of the upper digestive tract and a progressive retinal degeneration[56]. Sheep are more prone to progressive retinal degeneration (called bright blindness or PRD in sheep) than cattle[58].

20. In quail, the feeding of bracken caused reduced testis weight and reduced male fertility, and feeding of a solvent extract of bracken caused adenocarcinomas of the caecum, colon and distal ileum[35].

Clinical and Epidemiological Findings in Humans

21. When COC reviewed bracken in 1988[7], it concluded that human epidemiological data were limited and that evidence of carcinogenicity was inconclusive. Since then, several new observations have been reported[54, 62, 67, 68, 70, 71], but the totality of evidence remains sparse. The human populations that have been investigated include people in Japan and Brazil who had been directly exposed by eating bracken and people in Wales and Costa Rica who may have been indirectly exposed as a result of living in bracken-infested areas (eg. by consuming residues of chemicals from bracken in drinking water or animal-derived foods such as milk).

22. Ecological studies have indicated higher rates of stomach cancer (both sexes) and oesophageal cancer (men) in rural districts of Gwynedd, North Wales where a larger proportion of land area was covered by bracken[70], of stomach and oesophageal cancer in a bracken-infested as compared with a bracken-free region of Costa Rica[54], and of stomach cancer in highland areas of Venezuela where pastures were infested by bracken[68]. A large cohort study found elevated risk of oesophageal cancer in relation to combined intake of bracken fern and hot tea gruel among inhabitants of mountainous districts of Japan[66], and a case-control investigation, also in Japan, showed a similar association[65], although it is unclear from the brief descriptions available whether this was an independent investigation or part of the same study. A case-control study of stomach cancer in Gwynedd found a statistically significant association ($p < 0.001$) with bracken in the vicinity of the childhood home with an estimated relative

risk (RR) of 2.34 (confidence intervals not reported)[71]. A small case-control study of upper gastrointestinal (stomach and oesophageal) cancer in Brazil gave an odds ratio of 3.63 (95%CI 1.24-10.63) for bracken consumption, but the choice of controls was not ideal, and the statistical analysis failed to account for the matching in the study design[67]. More recently, a large, prospective cohort study in Japan found a significantly increased risk of oesophageal cancer in men who reported frequent consumption of wild edible plants (mainly bracken) in a questionnaire at baseline (RR 2.98, 95%CI 1.46-6.07)[62]. However, the corresponding risk estimate in women was unremarkable (RR 1.39, 95%CI 0.56-3.47)[62].

23. Few conclusions can be drawn from these epidemiological findings. Many of the studies have been inadequately reported or were methodologically unsatisfactory. The three ecological investigations were limited by their inability to adjust for potential confounding factors, and the case-control study in Gwynedd[71] did not take into account the possibility of confounding by Helicobacter pylori infection, which has been linked to domestic crowding in childhood[150]. In the past, domestic crowding was common in rural North Wales.[151] In the strongest and best reported investigation[62], the association of oesophageal cancer with bracken consumption was found only in men and not in women.

Toxicity of the Main Constituents of Bracken

Ptaquiloside and activated ptaquiloside (APT)

24. In 1983, two separate groups of workers in Japan[13] and the Netherlands12 independently identified ptaquiloside (a norsesquiterpene glycoside of the illudane type) as the principal substance responsible for the carcinogenicity and mutagenicity of bracken. They had used extraction and separation techniques to obtain various fractions, which they had then tested with mutagenicity assays[12] or short-term carcinogenicity assays in rats[13] to find those with the highest mutagenic/carcinogenic potency. The chemical structure of the principal compound (ptaquiloside) in the most potent fraction was then determined.

25. Ptaquiloside is a water-soluble substance that has been detected in all parts of the bracken plant[18]. It is stable in dried bracken[18]. In mildly alkaline conditions, isolated ptaquiloside readily breaks down to its carcinogenically-active form, activated ptaquiloside (APT)22. Both ptaquiloside and APT slowly break down in acidic conditions to form pterosin B18, which is not carcinogenic. Composting bracken destroys the ptaquiloside[5].

26. Some of the observed clinical effects of dietary bracken in farm animals have been reproduced in experiments in which animals were dosed with ptaquiloside. Progressive retinal degeneration (PRD or bright blindness) has been reproduced in sheep given ptaquiloside intravenously[73]. Haemorrhagic cystitis and haematuria have been produced in guinea-pigs (but not rats or mice) given subcutaneous ptaquiloside[74].

27. It has been suggested that ptaquiloside is responsible for more than half of the mutagenic potency of bracken[12], and the COM agreed[7] that most of the mutagenic activity of bracken appeared to be due

to ptaquiloside. No carcinogenicity bioassay of ptaquiloside has been performed to modern standards, but its carcinogenicity has been investigated in more limited studies in rats. Ptaquiloside was administered as either an initial oral dose of 780 mg/kg bw followed by 8 weekly doses of 100-200 mg/kg bw, or as twice weekly doses of 100-150 mg/kg bw for 8½ weeks, after which the rats received no further treatment for the rest of their lives[75]. Rats given the initial high dose developed haematuria and a loss of bodyweight, and both treatments produced tumours of the mammary gland (adenocarcinomas, papillary carcinomas and anaplastic carcinomas) and ileum (adenocarcinomas). "Conspicuous preneoplastic hyperplasia of the mucous membrane of the urinary bladder" was seen in all of the treated rats that survived 40 days or more. In another study, rats given a diet containing 0.027 to 0.080% ptaquiloside in their diet (equivalent to 27 - 80 mg/kg bw/day) developed cancers of the ileum and/or bladder within 15 to 60 days[76]. In a parenteral-dosing study, no tumours were seen in rats that had been given weekly intravenous doses of 20.7 mg/kg bw of ptaquiloside (equivalent to 3 mg/kg bw/day) for 10 weeks, followed by 30 weeks without further treatment, but these rats developed monocytosis and focal renal tubular necrosis[77]. The COC noted[7] that ptaquiloside had been shown to be capable of reproducing some of the carcinogenic effects of bracken.

28. Oral dosing of rats with APT at 10 weekly doses of 20.7 mg/kg bw (equivalent to an average dose of 3 mg/kg bw/day) or at 3 weekly doses of 41.4 mg/kg bw (equivalent to 6 mg/kg bw/day) did not produce any tumours detectable when the animals were killed 30 weeks later[78]. Nor did administration of APT as 10 weekly intravenous injections of 20.7 mg/kg bw. However, other adverse effects were seen in all groups: tubular necrosis of the kidneys, monocytosis and elevated plasma tumour necrosis factor TNFα. In addition, the orally-treated rats showed necrosis of blood cell precursors in the bone marrow and had apoptotic bodies in their livers.

29. Ptaquiloside was tested for mutagenicity at different pHs. It was not mutagenic in either TA98 or TA100 strain of *Salmonella typhimurium* when tested at pH 7.4 in the absence of metabolic activation, but was mutagenic in both strains if it was pre-incubated at pH 8.5[79, 80, 81]. It caused chromatid exchange type aberrations in CHL-cells in the presence and absence of S9 at pHs 5.3, 7.4 and 8.3, but the genotoxic potency was greater at the higher pHs[82]. Ptaquiloside also produced DNA-adducts *in vitro*[83] and caused *in vitro* unscheduled DNA synthesis in a rat hepatocyte culture at pH 7.2[84]. Ptaquiloside and APT were shown to be alkylating agents, with APT being the more potent[60]. No *in vivo* mutagenicity assays of ptaquiloside were found.

30. It seems likely that ptaquiloside is responsible for at least some of the carcinogenicity and toxicity of bracken. The carcinogenicity appears to involve a genotoxic mode of action.

Illudane Substances Other than Ptaquiloside & APT

31. No toxicological data are available for the illudane and protoilludane glycosides other than ptaquiloside that have been identified in bracken: ptaquiloside Z, isoptaquiloside, pteroside A2, pteridanoside and caudatoside. However, their chemical similarity to ptaquiloside raises the concern that some of them might be similarly carcinogenic by a genotoxic mode of action.

Terpenic Indanones

32. A large number of terpenic indanones have been isolated from bracken, including pterosins A, A2, B, C, D, E, F, G, J, K , l, N, O V and Z and pterosides A, B, C and M[16, 56]. Indanones are found in high concentrations (up to approximately 24 ppm) in young fronds[22], but they do not act as alkylating agents[22]. Pterosin B is much less electrophilic than ptaquiloside[84]. A range of different indanones (pterosins A, B, C, D, E, F, G, K, L, N and Z; acetyl pterosin C; benzoylpterosin B; and palmitylpterosins A and B) were shown to be non-mutagenic at pH 7.4, when tested in *Salmonella typhimurium* strains TA98 and TA100 in the presence and absence of S9[79, 80, 85, 86]. A selection of indanones (pterosins A, B, C, F and L; and pterosides A, B and C) were also non-clastogenic when tested in CHL cells in the absence of metabolic activation[87] (not tested in the presence of metabolic activation). No studies of the mutagenicity or carcinogenicity of pteroside A2 were available. Extracts of fronds and of rhizomes of Welsh bracken, containing high levels[a] of pterosins, pterosides and other non-specified indanones, did not cause leucopaenia or thrombocytopaenia in calves[88].

33. There is insufficient evidence to establish whether or not terpenic indanones are likely to be responsible for any of the toxicity of bracken.

p-Hydrostyrene Glycosides

34. The *p*-hydrostyrene glycoside, ptelatoside A (ρ-β-primerverosyloxystyrene) was tested in rats at a concentration of 1.3 ppm in the diet (equivalent to 0.065 mg/kg bw/day) for 109 or 125 days[13]. At this dose, there was no evidence of any carcinogenicity when the animals were killed at 520 days after the start of the experiment. There was insufficient ptelatoside A available to test higher concentrations.

35. Another *p*-hydrostyrene glycoside, ptelatoside B (ρ-β-neohesperidosyl oxystyrene), has also been isolated from bracken, but it has not been toxicologically tested. There is no evidence to indicate whether or not any of the toxicity of bracken is due to the presence of *p*-hydrostyrene glycosides.

Prunacin

36. Prunacin is a cyanogenic glycoside that is present in some sub-species of bracken. Cyanogenic glycosides can become toxic by releasing hydrocyanic acid (HCN) when hydrolysed by enzymes that may be released when tissues are damaged. A polymorphism exists in bracken: not all plants are cyanogenic as some lack either prunacin or the enzymes needed to liberate hydrocyanic acid from it. Farm animals seem to avoid eating the cyanogenic sub-species. Prunacin is usually present in bracken at harmless quantities, but there have been fatal cases of cyanide poisoning in animals that have been fed on young fronds of bracken[22, 56].

[a] Pterosins A, B, C and others were isolated from the fronds with yields of 40, 190, 40 and 20 ppm, respectively, and pterosins A, B and others and pterosides A, B, C and others were isolated from rhizomes with yields of 20, 20, 20, 320, 570, 370 and 90 ppm, respectively, and the concentrated extracts that were tested contained the equivalent of 3.6 and 1.8 kg of the dried frond and rhizome, respectively, in each litre. Each morning for 30 days, a calf was fed an amount of extract of frond or rhizome that was equivalent to 1 kg of dried bracken material. Thus, one calf received daily doses of frond extract supplying pterosins A, B, C and others at respective concentrations of 11, 52, 11 and 5.6 ppm; whereas another calf received daily doses of rhizome extract supplying pterosins A, B and others and pterosides A, B, C and others at respective concentrations of 11, 11, 11, 178, 317, 206 and 50 ppm.

37. Up to 61 mg/g of prunacin has been detected in fresh plant material from a Venezuelan tropical sub-species of bracken (*Pteridium aquilinum* var. *arachnoideum*), with the highest concentrations being found in young fronds[17]. It was noted in Venezuela that the *arachnoideum* sub-species of bracken contained more prunacin than the caudatum sub-species[22]. No quantitative information is available on the amount of prunacin in British sub-species of bracken, but it has been reported that the highest concentrations occur in early to mid spring[22]. One gram of prunacin has the potential to release up to 96 µg of HCN[17]. Thus up to 5.9 µg of HCN might be released from 1 gram of the fresh Venezuelan bracken.

38. In its 2006 Statement on Cyanogenic Glycosides in Bitter Apricot Kernals[89], the COT concluded that the limited chronic toxicity data available were not sufficient to propose a tolerable daily intake (TDI) for cyanide, but it noted that the World Health Organisation (WHO) and the Council of Europe (CoE) had established TDIs of 12 and 20 µg/kg bw, respectively. The COT proposed a nominal acute reference dose (ARfD) of 5 µg/kg bw, by applying a 100-fold uncertainty factor to the lowest reliably observed acute lethal dose in humans of 0.5 mg/kg bw. A person of 60 kg bodyweight would need to eat more than 50 g of the Venezuelan bracken to exceed this ARfD, and to regularly eat about 125 g per day of this bracken to exceed the TDI that was set by WHO. It is conceivable that an extreme consumer of bracken might experience acute cyanide toxicity as a result of eating a large portion of a sub-species of bracken that is high in prunacin. However, consumption of bracken by humans is not known to occur in the UK. No data are available on residues of prunacin or cyanide in foods derived from animals that have eaten bracken, so it is unclear whether UK foods derived from bracken-exposed animals would contain sufficient prunacin or cyanide to cause toxicity in human consumers without causing serious toxicity in the animals. As the level of exposure of humans to prunacin and cyanide from foods derived from animals that have consumed bracken is likely to be less than that of the directly-exposed farm animals and as humans are not markedly more susceptible to cyanide than other species[158], it is considered likely that animals would show clear signs of toxicity if they had consumed sufficient bracken to leave toxic levels of cyanide or prunacin in foods derived from them. Furthermore, the Scientific Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) has advised[159] that the carry-over of cyanide and cyanogenic residues into milk, meat and eggs derived from animals intoxicated with cyanogenic glycosides is likely to be "very low" in all food-producing species.

Braxin Glycosides

39. Braxins A1, A2 and B have been detected in rhizomes of bracken[90]. Braxins A1 and A2 were present in rhizomes at a combined concentration of up to 600 ppm, but were not detected in fronds[90]. Braxins A1 and A2 are aromatic β-glucopyranosides[90], but their precise chemical structure has not been established. The chemical structure of braxin B is not known. ("Braxin C" is ptaquiloside.)

40. Subcutaneous injections of braxins A1, A2 and B induced haemorrhagic cystitis in guinea-pigs (as did ptaquiloside)[91]. Braxins A1 and A2 also caused a dose-related release of histamine from rat peritoneal cells *in vitro*, with swelling of the cells[90]. The *in vitro* histamine-releasing activity of glycosides extracted from rhizomes (which include braxins A1 and A2) was about ten times greater than that of glycosides from the fronds (braxins A1 and A2 not present) [90].

41. It is possible that braxin glycosides play a part in the aetiology of the haemorrhagic cystitis that is seen in cattle and some other species.

Quercetin

42. Quercetin was found in bracken at concentrations of up to 860 ppm (dry weight)[20], but is also found in many fruits and vegetables, often at higher concentrations (eg. up to 65,000 ppm in onions)[24]. It has been estimated[146] from national dietary records in Australia, the Netherlands, Finland, Italy, Croatia, Japan, and the USA that the habitual diets of consumers gave them mean intakes of quercetin from <5 to approximately 40 mg/person/day, although intakes as high as 200-500 mg/person/day could be achieved by high consumers of fruit and vegetables.

43. Orally administered quercetin was not very well absorbed[92]. It is either converted to phenolic acids by the gut flora or voided unchanged in the faeces.

44. Quercetin was of low cytotoxicity when tested *in vitro* using CHO cells, 3T3 mouse fibroblasts and normal rat kidney (NRK) cells[93].

45. In calves, oral doses of up to 20 g/calf/day for several months had no effect on incidences of BEH or papillomavirus-induced cancer of the urinary bladder[94]. It has been claimed that exposure to quercetin is associated with bovine cancers of the upper alimentary tract[22], but no evidence has been found to support this claim.

46. There is limited evidence to suggest that quercetin could be carcinogenic. In a two-year feeding study performed in F344 rats given 1000, 10000 or 40000 ppm quercetin in their diet (equal to approximately 40, 400 and 1900 mg/kg bw/day), there were increased incidences of hyperplasia and adenomas of renal tubules at all doses in males with adenocarcinomas also being seen in the top-dose males (no adverse effects in females)[95]. In another study in F344 rats[96], 40000 ppm in the diet (1900 mg/kg bw/day) caused benign tumours in the renal tubules of males, but not in females, although no adverse effects were seen at dietary concentrations of 100 or 1000 ppm. It is noted that renal tumours were not produced when rats were fed bracken. In a third study in F344 rats[149], females were given dietary levels of 10000 or 20000 ppm of quercetin (500 and 1000 mg/kg bw/day) throughout their lives (approximately 750 days) and at both dose levels there were increased incidences of liver pre-neoplastic foci, hepatomas and bile duct tumours. In a study in Norwegian albino rats, administration of 10000 ppm quercetin in the diet (equivalent to 400 mg/kg bw/day) for 406 days caused transitional cell carcinomas of the bladder[19]. It is noted that bladder transitional cell carcinomas were also produced in rats fed bracken[31, 34, 35, 51, 62, 73]. The results of other carcinogenicity studies gave no evidence to suggest that quercetin was carcinogenic when given in the diet: to ACI rats at up to 100 000 ppm (4000 mg/kg bw/day) for 850 days[97]; to F344 rats at 10000 ppm (400 mg/kg bw/day) for 540 days[98]; to F344 rats at up to 50000 ppm (2000 mg/kg bw/day) for 728 days[99]; to F344 rats at up to 2000 ppm (76 mg/kg bw/day for males and 58 mg/kg bw/day for females) for 448 days[147]; to ddY mice at 20000 ppm (equivalent to 3000 mg/kg bw/day) for a lifetime (about 842 days)[100]; to strain A mice at 50000 ppm (7500 mg/kg bw/day) for 161 days[148]; or to golden hamsters at up to 100 000 ppm (equivalent to 12000 mg/kg bw/day) for up to 735 days followed by treatment with 1% croton oil for a further 350 days[101].

47. There is also some evidence that quercetin has anti-cancer properties. It has been suggested that it causes inhibition and induction of different phase I and phase II metabolism enzymes, that it has antioxidant effects, that it can induce apoptosis and that it can down-regulate oncogenes[102]. Oral doses of quercetin given to rats caused a decrease in the ability of benzo(a)pyrene metabolites to bind to DNA, and *in vitro* it inhibited the growth of cells from various human cancers[92].

48. There is some evidence that quercetin is genotoxic. It can bind to DNA and cause single-strand breaks[103, 146]. It gave positive results in several mutagenicity assays, including the *Salmonella*/microsome reverse mutation assay[95, 104, 105, 106], tests of SOS repair and reverse mutations in *Escherichia coli*[146], gene mutation (tk locus) assays in mammalian cells[24, 107, 108], cytogenetics tests in mammalian cells[24, 87, 95, 107, 109], and a sex-linked recessive lethal mutation test in *Drosophila*. On the other hand, quercetin gave negative results in a forward mutation assay in Bacillus subtilis and in mammalian cell gene mutation assays that used loci other than tk (hprt, aprt, ATPase)[146]. Quercetin gave inconsistent results in the mouse bone marrow micronucleus test: with one experiment finding it mutagenic[110] whilst others did not[111, 112]. Quercetin did not cause unscheduled DNA synthesis in gastric mucosal cells of rats that had been given oral doses[111]. Thus, although quercetin is mutagenic in several *in vitro* tests, the balance of evidence suggests that it does not express its mutagenicity in mammalian systems *in vivo*.

49. In a recent review[146] that covered the carcinogenicity and genotoxicity data on quercetin, it was concluded that quercetin at dietary levels of up to 50 mg/person/day "would not produce adverse health effects". Given that many commonly eaten fruits and vegetables contain higher concentrations of quercetin than are found in bracken, it is considered unlikely that quercetin is responsible for the adverse effects caused by eating bracken.

Kaempferol

50. Kaempferol is chemically similar to quercetin, from which it differs by lacking one hydroxyl group. It was found in bracken at a concentration of 1100 ppm (dry weight)[19]. It is also commonly found in other plants[24]. Tea can contain up to 10,000 ppm of quercetin plus kaempferol, combined[24].

51. A limited study in ACI rats (400 ppm feed given to 6 males and 6 females for 540 days) showed no evidence to suggest that kaempferol was carcinogenic[98]. Mutagenicity tests suggest that kaempferol is an *in vivo* mutagen. It gave a positive result for mutagenicity in a bone marrow micronucleus assay in which mice were dosed intraperitoneally[110]. It also gave positive results in a sex-linked recessive lethal assay in Drosophila[113] and in several *in vitro* mutagenicity tests: the *Salmonella*/microsome assay[86, 104, 105, 106, 114], gene mutation tests in mammalian cells (mutation at the tk locus of CHO cells[107] and development of resistance to 8-azoguanine in V79 cells[108]) and a cytogenetics assay in mammalian cells[107]. Negative results were obtained for induction of gene mutations at the *aprt, hgpt* and ATPase loci of CHO cells[107].

52. It is possible that the presence of kaempferol contributes to the overall carcinogenicity of bracken. However, given that many commonly eaten fruits and vegetables contain higher concentrations of kaempferol, it is considered unlikely that kaempferol is responsible for other features of the toxicity of bracken.

Shikimic Acid

53. Shikimic acid was found at 1440 ppm (dry weight) in Welsh bracken[21]. It is also present in several edible plants, including soybeans, star anise and green tea. Shikimic acid was destroyed in alkaline conditions[115].

54. Shikimate was of low cytotoxicity when tested *in vitro* in CHO cells, 3T3 mouse fibroblasts and normal rat kidney (NRK) cells, with the concentrations inhibiting cell growth by 50% after 48h of incubation being respectively 0.8, 0.7 and 1.0 millimolar (139, 122 and 174 ppm)[93].

55. Intraperitoneal injection of 10 mg/mouse of shikimic acid caused death in mice within a few hours of dosing, with haemorrhaging and "denudation" of the intestinal mucosa[115].

56. Developmental toxicity was tested in pregnant CD-1 mice given 17 daily oral gavage doses of 250 or 1000 mg/kg bw shikimic acid[116]. There was a reduced number of implantations at both dose levels, as compared with untreated controls. The results showed no evidence of fetotoxicity or teratogenicity.

57. TF1 mice given single intraperitoneal doses of 1 to 30 mg of shikimic acid (equivalent to 50 to 1500 mg/kg bw) had increased incidences of cancer of the glandular stomach and of leukaemia when observed for up to 70 weeks[117]. The only mouse tested with a single oral gavage dose of 100 mg (5000 mg/kg bw/day) died after 34 weeks, having developed stomach cancer and leukaemia[117]. These lesions were consistent with the sites of tumours seen in mice fed bracken. In ACI rats, however, the feeding of shikimic acid at a dietary concentration of 1000 ppm (equivalent to 150 mg/kg bw/day) for 142 days had no effect on tumour incidences, after an observation period of a further 70 days [21].

58. Shikimic acid was not genotoxic *in vitro* in bacterial[118] or *in vivo* mammalian assays (mouse bone marrow assay and unscheduled DNA synthesis in rat gastric mucosa)[111]. However, the results of a dominant lethal assay[117] indicated that shikimic acid could cause mutations *in vivo*. Male TF1 mice were each treated either with a single intraperitoneal injection of 25 mg of shikimic acid or an oral dose of 80 mg of shikimic acid by stomach tube prior to mating with untreated virgin females. The proportions of embryos having dominant lethal mutations in the control, intraperitoneally-treated and orally-treated groups were 4.4, 22.1 and 13.6%, respectively.

59. It cannot be excluded that shikimic acid might make some contribution towards the overall carcinogenicity of bracken. There was limited evidence to suggest that it might be mutagenic and carcinogenic. Although a single intraperitoneal dose of 0.15 mg/kg bw/day or more produced tumours in mice, a dietary dose of 50 mg/kg bw/day did not appear to be carcinogenic in rats. Nevertheless, it is unlikely that shikimic acid makes a major contribution to the overall carcinogenicity of bracken as it was destroyed by alkaline conditions whereas the mutagenicity and carcinogenicity of bracken appears to be increased under such conditions.

Thiaminases

60. Anti-thiamine enzymes, thiaminases, seem to cause most of the short- to medium-term symptoms of bracken poisoning in monogastric animals. Thiaminase activity is highest in rhizomes and very young fronds. Thiaminases types 1 and 2 have been found in bracken at activities of 3.1 and 3.5 µg thiamine

destroyed/g plant material/hour, respectively[119]. A third, more heat-stable, thiaminase (possibly caffeic acid, a substance that also has both pro- and anti-cancer properties) might also play a role[10].

61. Rats fed on bracken that had thiaminase activity developed lesions of the nervous system that were considered by the authors of the study[13] to be typical of antivitaminosis-B1. The lesions could be cured by thiamine (vitamin B1). Similar effects have been reported in monogastric farm animals, including horses and pigs[22].

62. In order to test whether the anti-thiamine activity of bracken contributed to its carcinogenicity, a 52 week feeding study was performed in groups of rats fed either control diet, a diet containing bracken or a diet containing bracken supplemented with a subcutaneous injection of 2 mg/rat/week of thiamine[120]. No tumours were found in controls. All survivors in the two treatment groups developed multiple intestinal tumours. Bladder tumours were found in 11% of males and 7% of females in the group given bracken alone and in 53% of males and 67% of females in the group given bracken plus thiamine. It was noted that as thiamine supplements did not reduce the incidence of tumours (if anything, the incidence increased), it seemed unlikely that thiaminase caused the carcinogenicity of bracken.

63. No reports of thiamine deficiency in bracken-consuming human populations have been found. It is possible that humans are less prone to thiamine deficiency than farm animals because they have a more varied diet. It is also likely that humans who eat bracken would eat much less bracken (per kg body weight) than animals fed on bracken.

Ecdysteroids

64. Bracken contains several ecdysteroids which can prevent insects from moulting and developing into adults[22]. This mode of action is not relevant to mammals.

65. It has been claimed that α-ecdysone induced neoplastic lesions in Egyptian toads (*Bufo regularis*)[121], but no details or evidence were presented to back-up this claim.

66. There is no reliable evidence to suggest that ecdysteroids are a toxic hazard to humans.

Tanins

67. The tannins in bracken are mainly condensed tannins derived from procyanidin and prodelphinidin. Fronds of tropical bracken can contain more than 120,000 ppm of condensed tannins[22]. No information was available on the amount in sub-species of bracken that are found in the UK. Tannins are present in many foods (including legumes, chocolate, fruits and smoked foods) and drinks (including tea, wine and beer). For instance, wine contains 570 to 2.470 ppm[122], red kidney beans (*Phaseolus vulgaris*) contain 6300 to 9100 ppm raw and 3100 to 5500 ppm cooked[123], raw soyabeans[123] contain 500 ppm and "corn"[b] contains 100 ppm[123].

[b] It was not clear from the report whether "corn" referred to maize, wheat or some other cereal.

68. There is limited evidence suggesting that parenteral administration of some tannins might be carcinogenic. Subcutaneous injections caused liver tumours[124] and fibrous histiocytomas at the injection site[126] in rats, and caused local sarcomas and liver tumours in mice[124]. Although liver nodules were noted in one of the mouse carcinogenicity studies of bracken[33], the liver was not the major site for neoplasia in mice. More usual were leukaemias, lung tumours and gastrointestinal cancers[28, 29, 30, 33]. The feeding of bracken tannins at a dietary concentration of 4000 ppm (equivalent to 100 mg/kg bw/day) for up to 72 weeks did not cause any cancer in F344 or Sprague-Dawley rats[126]. IARC has classified tannic acid and tannins in Group 3: "The agent (mixture or exposure circumstance) is not classifiable as to its carcinogenicity to humans"[125]. Bracken tannins were not mutagenic to strains TA98 and TA100 of *Salmonella typhimurium* when tested in the absence of metabolic activation[126].

69. Although bracken can contain higher concentrations of tannins than commonly consumed foods, there is no evidence that ingestion of these tannins is harmful.

Summary of Toxicity

70. In monogastric animals the main effect of eating bracken is often a deficiency of thiamine as a result of the action of thiaminases in the bracken. However, this effect is not known to occur in humans. The more varied human diet and the lower dietary consumption of bracken are factors that make it less likely that bracken will cause thiamine deficiency in human consumers.

71. Ruminants do not experience thiamine deficiency as a result of eating bracken as their gut flora can make thiamine from other substances in the diet. Instead they develop several other non-neoplastic diseases as a result of eating bracken, including panmyelopathy of the bone marrow, haemorrhagic cystitis and progressive retinal degeneration. It is likely that ptaquiloside in bracken is at least partly responsible for causing these effects. Braxins might also play a role. These non-neoplastic effects of bracken have not been reported in humans, probably because levels of dietary exposure are lower than in animals.

72. Animals have died, showing signs of toxicity consistent with cyanide poisoning, following ingestion of large amounts of bracken containing the cyanogenic glycoside, prunacin. The amount of prunacin in bracken is almost certainly too low to cause cyanide poisoning in humans exposed either by direct consumption of bracken as a vegetable or by eating foods derived from animals that have eaten bracken.

73. Bracken also seems to cause cancer in a wide variety of species. The site and type of cancer can differ between species. Evidence from epidemiological studies for a carcinogenic effect of bracken in humans is inconclusive. It is likely that the presence in bracken of the genotoxic carcinogen, ptaquiloside, contributes towards its carcinogenicity. Other components of bracken, such as kaempferol and shikimic acid, might make a more minor contribution towards the overall genotoxicity and carcinogenicity of bracken.

Modes of Action of Relevance to Humans

74. Carcinogenicity is the toxic effect of bracken that is of most relevance to humans exposed by eating bracken or foods derived from animals that have eaten bracken. The non-neoplastic effects that have been seen in heavily exposed laboratory animals and farm animals have not been reported as occurring in exposed humans.

75. Bracken and various extracts of bracken were mutagenic in a range of *in vitro* and *in vivo* tests. As bracken is both carcinogenic and mutagenic, it is reasonable to assume that it can cause cancers by a genotoxic mode of action, although it is possible that non-genotoxic modes of action could also be involved.

Contributions of Constituent Chemicals to the Carcinogenicity of Bracken

76. Bracken contains a large number of component chemicals, some of which have been shown to be mutagenic and/or carcinogenic: ptaquiloside, quercetin, kaempferol, shikimic acid and tannins. Illudanes other than ptaquiloside have not been tested, but their chemical similarity to ptaquiloside raises suspicions about their possible carcinogenicity and mutagenicity.

77. There is evidence that ptaquiloside is responsible for at least some of the carcinogenicity of bracken. In addition it produced DNA adducts in rat ileum that gave a spot in thin-layer chromatography in an identical position to the adducts that had been found when rats were treated with bracken[127, 128]. Ptaquiloside has been shown to be an *in vivo* mutagen and it produces similar types of tumours to bracken in the various species that have been tested. It can be present in bracken in appreciable amounts: between 447 and 1211 ppm were detected in sub-species that are found in the UK, but higher amounts have been detected in tropical sub-species.

78. Quercetin appears not to be genotoxic *in vivo*, but has been shown to cause tumours in experimental animals at sites that are consistent with the sites of tumours formed when these animals were fed bracken. However, it seems unlikely that quercetin contributes to the carcinogenicity of bracken, as the concentration in bracken is considerably lower than in other innocuous foods, such as red onions.

79. The available mutagenicity test results indicate that kaempferol is genotoxic *in vivo*, but it has not been tested for carcinogenicity. It is possible that kaempferol contributes to the carcinogenicity of bracken.

80. Shikimic acid was not genotoxic in a limited range of *in vitro* tests, but *in vivo* it caused dominant lethal mutations in mice. Although subcutaneous doses caused tumours in mice, a large oral dose was not carcinogenic in rats. Furthermore, it is destroyed in alkaline conditions, whereas the genotoxicity of bracken increased in such conditions. It seems unlikely that the shikimic acid in bracken makes a major contribution to the overall carcinogenicity of bracken.

81. The evidence for bracken tannins being a cause of the carcinogenicity of bracken is weak because the only bioassay of the carcinogenicity of oral doses of bracken tannins gave a negative result, and there is no evidence that they are genotoxic.

Mode of Action of Ptaquiloside

82. The genotoxic potency of ptaquiloside was found to be dependent upon the pH of the medium. It was not mutagenic to *Salmonella* strains TA98 or TA100 when tested at pH 7.4 but was mutagenic without metabolic activation when it was first preincubated at pH 8.5[80]. It was also discovered that ptaquiloside was less potent at causing chromosomal aberrations when tested at pH 5.3[82]. The higher genotoxic potency of ptaquiloside at higher pH is probably because, under mildly alkaline conditions, ptaquiloside is converted by β-elimination into the illudane-dienone compound, APT (activated ptaquiloside)[82]. APT possesses a highly reactive cyclopropyl ring. It is electrophilic with a greater capacity to alkylate DNA than ptaquiloside[60]. It is stable in mildly alkaline conditions, but under acidic conditions it is converted to a less reactive non-genotoxic substance, pterosin B. Pterosin B is also formed from the breakdown of ptaquiloside under acidic conditions[12, 129].

83. Compounds that are chemically similar to APT but lack an activated cyclopropane moiety (hypoloside B, hypoloside C, illudin M and illudin S) were not mutagenic to *Salmonella typhimurium* TA98 or TA100 strains[82]. It is not known whether the other illudanes that have been found in bracken form an activated cyclopropane moiety in a similar way to that in which ptaquiloside forms APT.

84. APT binds covalently to purine bases on DNA130, opening the cyclopropyl ring of the molecule and forming adducts with the N-7 of guanine and the N-3 of adenine. Alkylation of adenine (but not that of guanine) caused cleavage of the N-glycosidic linkage of the modified adenines to produce abasic sites on the DNA molecule[131]. The abasic sites were unstable and breakage of the phosphodiesterpentose backbone of the DNA molecule occurred. Investigations were made[132] of the H-*ras* and *p53* genes in cells from the mammary glands of rats that had received weekly intravenous doses of 20.7 mg/kg bw and had been killed immediately after dosing. No mutations were found in *p53*[31], but there were double mutations at codons 58 and 59 of the H-*ras* gene[78]. Mutations of the H-*ras* proto-oncogene also occurred in cells from the ileums of cattle fed bracken in their diet[132, 134, 135].

85. It has been shown that infections of cattle by papillomaviruses increase the chances of them developing benign papillomas of the upper gastrointestinal tract (associated with BPV-4 infection) or the urinary bladder (associated with BPV-2 infection) and repeated dietary exposure to bracken can further increase the chances of developing these tumours and of them progressing to malignancy[56, 94, 121, 133, 134, 135, 136, 137, 138, 139]. It has been proposed[121] that immunosuppression caused by bracken's suppression of the bone marrow makes the animals more prone to viral infection and that this makes them more likely to develop cancers of the upper gut and bladder when exposed to bracken. Such a mode of action might be relevant to humans.

Exposure

86. It is rare for people in the UK to eat bracken. However, in some parts of the world including Japan, Brazil, New Zealand, Canada and the USA, the young curled bracken fronds (called crosiers or fiddleheads) are eaten as a vegetable. Analysis of fronds and rhizomes of the bracken sub-species that is most common in the UK (*Pteridium aquilinum* var. *aquilinum*) found them to contain ptaquiloside concentrations of 213 to 2145 ppm and 11 to 902 ppm, respectively.

87. The Committee is not aware of any UK commercial sources of bracken for human consumption. In August 2007, the FSA received a single hearsay report of vacuum-packed bracken shoots being on sale in the UK, but could find no evidence to substantiate this. Although it is possible that bracken could be imported from abroad or harvested on a small scale locally, the Committee is not aware of any instances of this happening. As bracken does not appear to have been marketed as a food in the EU, it is likely that it would require a pre-market safety assessment in accordance with the Novel Foods Regulation (EC) 258/97 before it could be sold for human consumption. The Food Standards Agency obtains expert advice on the risk assessment of all novel foods from the Advisory Committee on Novel Foods and Processes (ACNFP).

88. The most likely food-related route by which UK consumers could be exposed to bracken-derived substances is by eating foods derived from animals that have eaten bracken. Some food-producing animals eat bracken. There have been 22 recent (1999-2007) documented cases of UK cattle being poisoned by bracken[8], although the total number of poisonings is likely to be greater than this. Intensive grazing, usually by sheep or pigs, is used in the UK to clear bracken and to reduce invasion of pastures. Exposure might occur at a lower level as a result of animals eating bracken that is growing as a weed in their enclosures. There might also be some exposure to bracken as a result of the traditional use of bracken as bedding for animals.

89. It is theoretically possible that animals that are exposed to bracken could have residues of harmful bracken-derived chemicals in their tissues, which could be eaten by human consumers. No information is available on the concentrations in meat and offal of residues of any toxic substances derived from bracken or on their rates of depletion from edible tissues.

90. Substances from bracken can be excreted into the milk. The milk from bracken-fed cows caused leucopaenia in calves, produced bladder cancer in mice, and produced cancers of the intestines, bladder and kidneys in rats. Various solvent extracts from the milk were mutagenic to *Salmonella typhimurium* strains TA98 and TA100, and caused pulmonary adenomas in the offspring of mice that had been exposed during pregnancy. Thus it seems that toxic agents in bracken can be passed into the milk and can cross the placental barrier. There is a potential hazard from toxic components of bracken being passed into milk intended for human consumption. People who consume local unbulked milk or dairy products from bracken infested areas would be expected to be at greater risk than those drinking only bulked milk from commercial dairies.

91. Ptaquiloside has been detected in milk from bracken-fed cows[140]. A concentration of 0.11 mg/L of ptaquiloside was found in milk from a cow that had been fed for 7 days on 6 kg/day of fresh bracken fronds that contained 250 ± 50 ppm of ptaquiloside[141, 142]. The total amount of ptaquiloside that was excreted into the milk of this cow was equal to 1.2% of the amount of ptaquiloside that it ingested. In another study[143], two cows transferred into their milk 8.6 ± 1.2% of the ptaquiloside they ingested from 6 kg/cow/day of fresh fronds of bracken that provided doses of 2400 to 10000 mg/cow/day of ptaquiloside. Doses of 2400, 4500, 8100 and 10000 mg/cow/day of ptaquiloside in bracken produced peak concentrations of 10, 27, 38 and 55 mg/L of ptaquiloside in the milk. After a few days the cows refused to eat the feed containing the highest concentration of ptaquiloside. Ptaquiloside was first detected in milk at 38 h after the start of dosing and peaked at 86 h. After feeding of bracken was

stopped, the concentrations gradually dropped off until none was detectable at 86 h after the end of the dosing period. The limit of quantitation of the analytical method was approximately 0.5 ppm of pterosin, which is equivalent to about 1 mg/L of ptaquiloside. It may be concluded that concentrations of ptaquiloside fell off rapidly following withdrawal of oral exposure and minimal amounts would be present in milk at 4 days after ingestion of bracken ceased.

92. Data from the most recent UK food surveys[c] indicate that the sector of the UK population with the highest chronic intake of milk is infants aged 6-12 months (1992-1993 survey). It has been estimated by extrapolation from the results of the study[143] described in the preceding paragraph that a UK infant having the upper 97.5th percentile chronic intake of milk (851 g/person/day for milk excluding infant formulae and breast milk) from a cow that had been fed a sub-clinical dose of 5000 mg/cow/day of ptaquiloside in bracken could receive a dose of up to 22.8 mg/person/day of ptaquiloside (or 2.62 mg/kg bw/day for an infant of the UK mean bodyweight of 8.7 kg). This gives an estimate of the maximum consumer intake of ptaquiloside from milk from bracken-exposed cows that show no clinical signs.

93. The estimated intake by infants of 22.8 mg/person/day of ptaquiloside is regarded as an extreme intake as it takes the highest measured amount of ptaquiloside in milk from cows given the highest tolerable dose of bracken and compares it with a high estimate of the cows' milk intake of infants. Most infants would be expected to have a lower intake of cows' milk than this as the UK Government advises that cows' milk should not be directly fed to infants of one year of age or less. Furthermore, most consumers would consume bulked milk, where the milk from any cows receiving such high intakes would be diluted with milk from cows with low or zero intakes of ptaquiloside. It is also conceivable that pasteurisation and other processing of milk would further reduce the levels of ptaquiloside present (although there are no data to confirm this).

94. The results of UK food surveys indicate that, after infants, the subpopulation with the highest per capita chronic intake of milk is the institutional elderly and those with the highest intake in relation to bodyweight are toddlers aged 1½-4½ years. Using the same information[142] on possible amounts of ptaquiloside in cows' milk as used to estimate the intake of infants, the intakes of ptaquiloside by high (97.5th percentile) consumers of milk have been estimated to be between 2.9 and 22.1 mg/person/day (0.047 to 0.36 mg/kg bw/day) for the institutional elderly and between 2.8 and 21.6 mg/person/day (0.19 to 1.49 mg/kg bw/day) for toddlers.

95. The above estimates of intake of ptaquiloside from milk represent the maximum intake that might be anticipated to occur as a result of drinking milk solely from cows receiving the maximum sub-clinical dose of ptaquiloside. It is possible that milk or edible tissues from bracken-poisoned cows could contain higher amounts of ptaquiloside. No quantitative data are available on the levels of exposure from sources other than milk.

---

[c] The information on UK intakes of milk was obtained using data on individual consumption that are compiled in the "Intake 2" programme. The data include intakes of cows', sheep's and goats' milk, milk in chocolate and milk used in recipes. Chocolate was assumed to be 25% milk.

The "Intake 2" programme used data from the following food intake surveys: Infants 86, Toddlers' Survey, Young Persons '98 Survey, Vegetarian 1994-95 Survey, Adults 2001 National Diet and Nutrition Survey and Free-Living and Institutional Elderly Surveys (Mills and Tyler, 1992; Gregory, *et al.*, 1995; Gregory, *et al.*, 2000; MAFF, 1996; Henderson, *et al.*, 2002 and Finch, *et al.*, 1998).

Summary of Exposure Data

96. Bracken is not eaten by humans in the UK, but it is uncertain whether this situation will remain unchanged. With the continuing interest in international cuisine, it is conceivable that in future there may be moves to introduce bracken as an exotic vegetable. If this were to be done, bracken would be regarded as a novel food and it is likely that it would need to be assessed for safety before authorisation could be given for its sale in the UK as a food.

97. There is a potential for exposure to component chemicals of bracken as residues in foods derived from animals that have eaten bracken. There is evidence that some animals readily eat bracken and there have been cases of bracken poisoning in farm animals. As component chemicals in bracken (eg. ptaquiloside) can cause systemic toxicity in farm animals, it is reasonable to assume that they have the potential to leave residues in edible tissues and other foods derived from animals that have eaten bracken. Little is known about the amounts of the component chemicals that can occur as residues in foods, but it is clear that ptaquiloside can pass into the milk of cows.

98. It has been estimated that, in the UK, the sub-population with the highest milk intake, infants, might be exposed to up to 2.62 mg/kg bw/day of ptaquiloside as a result of consuming milk from cows that had eaten bracken without showing clinical signs of poisoning. However, if the advice not to give cows' milk to infants below the age of one year is followed, the highest estimate of intake on a bodyweight basis is 1.49 mg/kg bw/day, for toddlers. Most consumers will be exposed to less than this as they drink less milk. Furthermore, most milk supplies will be bulked so any high concentrations in individual samples will be diluted.

99. No information was available on the amount of ptaquiloside or other components of bracken that can occur in milk derived from animals that have been poisoned by bracken.

100. No information was available on the amount of ptaquiloside or other components of bracken that can occur in meat and offal derived from animals that have eaten bracken.

101. No information was available on the rate at which residues of ptaquiloside or other components of bracken can be cleared from edible tissues. However, information on the rate of decrease of ptaquiloside residues in milk from bracken-exposed cows indicated that minimal amounts of residues would be present in the milk at 4 days or more after exposure ended.

Conclusions

102. The Committee agreed the following conclusions:

    i. Bracken is sometimes eaten by food-producing animals.

    II. Although no modern carcinogenicity bioassays have been performed on bracken, observations from farm animals, laboratory animals and mutagenicity studies suggested that it is carcinogenic and genotoxic. Few conclusions can be drawn from the small number of epidemiological studies of humans. It is prudent to regard bracken and at least one of its constituents (ptaquiloside) as being potentially carcinogenic to humans at all levels of ingestion.

III. Bracken contains some genotoxic or possibly genotoxic substances, including ptaquiloside, kaempferol and shikimic acid.

IV. Ptaquiloside from bracken ingested by food-producing animals (eg. dairy cows) can be passed into milk that might be consumed by humans.

V. Ptaquiloside from ingested bracken is likely to be present in meat and offal derived from animals that have recently eaten bracken.

VI. The level of consumer exposure to ptaquiloside and other bracken derived genotoxic substances, such as kaempferol, should be kept as low as reasonably practicable. Measures that could be considered to achieve this could include discarding milk or not slaughtering bracken-exposed animals for a length of time consistent with the clearance of residues of toxic substances.

VII. The available data suggest a withdrawal period of at least 4 days for ptaquiloside in milk. Current evidence does not provide a basis for specifying an adequate withdrawal period prior to slaughter for human consumption of meat and offal.

Recommendations

103. The Committee recommended the following actions to reduce uncertainty about the risk to consumers from bracken:

- Identify the amount of ptaquiloside and other harmful bracken constituents that can occur in meat and offal derived from animals that have been poisoned by bracken.
- Identify the amount of ptaquiloside and other harmful bracken constituents that can occur in meat and offal derived from animals that have eaten bracken without showing any signs of toxicity.
- Identify the rate at which residues of ptaquiloside and other harmful bracken constituents are cleared from edible tissues of food-producing animals (eg. those that are actively used to clear bracken).

104. It is recommended that priority should be given to identifying the rate of depletion of ptaquiloside from edible tissues of animals that had high exposure to bracken. Such information could be used by risk managers to help them decide with more confidence how long bracken-poisoned animals should be left before slaughter for human consumption.

COT Statement 2008/05
October 2008

References

1. Wolf PG, Sheffield E, Thompson JA and Sinclair RB, 1994, "Bracken taxa in Britain: A molecular analysis". In: Smith RT & Taylor JA (eds), "Bracken: An environmental issue", Chapter 3, pages 16-20 of special publication reporting the 2nd meeting of the International Bracken Group, held at the Institute of Earth Studies, Aberystwyth, UK. Published by International Bracken Group (co-ordinator Prof JA Taylor), Aberystwyth, Wales. ISBN 0 9525505.

2. Taylor JA, 1999, "Perceptions of bracken". In "Bracken perceptions and Bracken Control in the British Uplands", pages 1-7 of the Proceedings of the International Bracken Group Conference, Ceredigion, Wales, 10th September 1998.

3. Smith BL, Seawright AA, Ng JC, Hertle AT, Thompson JA and Bostock PD, 1994, "Concentration of ptaquiloside, a major carcinogen in bracken fern (Pteridium spp.) from eastern Australia and from a cultivated worldwide collection held in Sydney, Australia", Natural Toxins, 2: 347-353.

4. Rasmussen LH, Jensen LS and Hansen HCB, 2003, "Distribution of the carcinogenic terpene ptaquiloside in bracken fronds, rhizomes (*Pteridium aquilinum*) and litter in Denmark", J. Chem. Ecology, 29(3): 771-778.

5. Potter DM and Pitman RM, 1994, "The extraction and characteristics of carcinogens from bracken and the effect of composting". In: Smith RT & Taylor JA (eds), "Bracken: An environmental issue", Chapter 23, pages 110-115 of special publication reporting the 2nd meeting of the International Bracken Group, held at the Institute of Earth Studies, Aberystwyth, UK. Published by International Bracken Group (co-ordinator Prof JA Taylor), Aberystwyth, Wales. ISBN 0 9525505.

6. COM, 1993, "Committees on Toxicity, Mutagenicity, Carcinogenicity of Chemicals in Food, Consumer Products and the Environment: 1993 Annual Report", Department of Health, HMSO publications, ISBN 0-11-321808-7.

7. COT, 1996, "Committees on Toxicity, Mutagenicity, Carcinogenicity of Chemicals in Food, Consumer Products and the Environment: 1996 Annual Report", Department of Health, HMSO publications.

8. Livesey CT, 2007, personal communication to Derek Renshaw (FSA) from Chris Livesey of the Veterinary Laboratory Agency, New Haw, England. E-mail received 11th December 2007.

9. Rosenberger G, 1971, "Nature, manifestations, cause and control of chronic enzootic haematuria in cattle", Vet. Med. Rev., **2**: 189-206.

10. Cooper MR and Johnson AW, 1998, "Poisonous plants and fungi in Britain: Animal and human poisoning", published by The Stationary Office, Norwich, England. ISBN 011 242981 5.

11. Harwood DG, Palmer NMA, Wessels ME and Woodger NGA, 2007, "Suspected bracken poisoning in pigs", Vet. Record, **160**(26): 914-915.

12. van der Hoeven JCM, Lagerweij WJ, Posthumus MA, Veldhuizen A, Holterman HAJ, 1983, "Aquilide A, a new mutagenic compound isolated from bracken fern (*Pteridium aquilinum* (L.) Kuhn), Carcinogenesis, **4**: 1587-1590.

13. Hirono I, 1986, "Carcinogenic principles isolated from bracken fern", CRC Crit. Rev. Toxicol., **17**(1): 1-22.

14. Castillo UF, Ojika M, Alonso-Amelot ME and Sakagami Y, 1998, "Ptaquiloside Z, a new toxic unstable sesquiterpene glucoside from the neotropical bracken fern Pteridium aquilinum var. caudatum", Bioorganic and Medicinal Chemistry, **6**: 2229-2233.

15. Castillo UF, Wilkins AL, Lauren DR, Smith BL, Towers NL, Alonso-Amelot ME and Jaimes-Espinoza R, 1997, "Isoptaquiloside and caudatoside, illudane-type sesquiterpene glucosides from *Pteridium aquilinum* var. *caudatum*", Phytochemistry, **44**(5): 901-906.

16. Castillo UF, Wilkins AL, Lauren DR, Smith BL and Alonso-Amelot M, 2003, "Pteroside A2 – a new illudane-type sesquiterpene glycoside from *Pteridium caudatum* L. Maxon, and the spectrometric characterisation of caudatodienone", J. Agric. Food Chem., **51**: 2559-2564.

17. Alonso-Amelot ME and Oliveros A, 2000, "A method for the practical quantification and kinetic evaluation of cyanogenesis in plant material. Application to *Pterideum aquilinum* and *Passiflora capsularis*", Phytochem. Anal., **11**: 309-316.

18. Saito K, Nagao T, Matoba M, Koyama K, Natori S, Murakami T and Saiki Y, 1989, "Chemical assay of ptaquiloside, the carcinogen of *Pteridium aquilinum*, and the distribution of related compounds in the pteridaceae", Phytochemistry, **28**(6): 1605-1611.

19. Pamukcu AM, Yalçiner Ş, Hatcher JF and Bryan GT, 1980, "Quercetin, a rat intestinal and bladder carcinogen present in bracken fern (*Pteridium aquilinum*)", Cancer Res., **40**: 3468-3472.

20. Hatcher JF, Pamukcu AM, and Bryan GT, 1981, "AACR Abstracts #450: Quercetin (Q) and kaempferol (K) content of bracken fern (BF) and mutagenic activity in urine of rats ingesting Q, rutin (R) or BF", Carcinogenesis, ?:114.

21. Hirono I, Fushimi K and Matsubara N, 1977, "Carcinogenicity test of shikimic acid in rats", Toxicol. Lett., **1**: 9-10.

22. Alonso-Amelot ME, 2002, "The chemistry and toxicology of bioactive compounds in bracken fern (*Pteridium* ssp), with special reference to chemical ecology and carcinogenesis", Studies in Natural Products Chemistry, **26**: 685-739.

23. Wang CY, Pamukcu AM and Bryan GT, 1973, "Isolation of fumaric acid, succinic acid, astragalin, isoquercetin and tiliroside from *Pteridium aquilinum*", Phytochemistry, **12**: 1298-2299.

24. IARC (International Agency for Research on Cancer), 1986, "IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to humans – Some naturally occurring and synthetic food components, furocoumarins and ultraviolet radiation", Volume 40, representing the views and expert opinions of an IARC Working Group which met in Lyon, France, 15-22 October 1985. Published by World Health Organization, Geneva. ISBN 92 832 1240 1. ISSN 0250-9555.

25. Evans IA, Al-Samarrai AMH and Smith RMM, 1984, "Bracken toxicology: Identification of some water soluble compounds from crosier and rhizome", Res. Vet. Sci., **37**: 261-265.

26. Méndez J, 2005, "Dihydrocinnamic acids in *Pteridium aquilinum*", Food Chemistry, **93**: 251-252.

27. Evans IA and Galpin OP, 1990, "Bracken and leukaemia", Lancet, **335**: 231.

28. Hirono I, Sasoaka I, Shibuya C, Shimizu M, Fushimi K, Mori H, Kato, K and Haga M, 1975, "Natural carcinogenic products of plant origin", Gann Monogr., **17**: 205-217.

29. Pamukcu AM, Ertűrk E, Price JM and Bryan GT, 1972, "Lymphatic leukaemia and pulmonary tumours in female Swiss mice fed bracken fern (*Pteris aquilina*)", Cancer Res., **32**: 1442-1445.

30. Evans IA, Jones RS and Mainwaring-Burton R, 1972, "Passage of bracken fern toxicity into milk", Nature, **237**: 107-108.

31. Freitas RN, Brasileiro-Filho G, Silva ME and Pena SDJ, 2002, "Bracken fern-induced malignant tumors in rats: Absence of mutations in *p53*, H-*ras* and K-*ras* and no microsatellite instability", Mutat. Res., **499**: 189-196.

32. Santos RC, Brasileiro-Filho G and Hojo ES, 1987, "Induction of tumours in rats by bracken fern (*Pteridium aquilinum*) from Ouro Preto (Minas Gerais, Brazil)", Brazilian J Med. Biol. Res., **20**: 73-77.

33. Hirono I, Aiso S, Yamaji T, Niwa H, Ojika M, Wakamatsu K and Yamada K, 1984, "Hyperplastic nodules in the liver induced in rats fed bracken diet", Cancer Lett., **22**: 151-155.

34. Hirono I, Aiso S, Hosaka S, Yamaji T and Haga M, 1983, "Induction of mammary cancer in CD rats fed bracken fern diet", Carcinogenesis, **4**: 885-887.

35. Evans IA, 1968, "The radiomimetic nature of bracken toxin", Cancer Res., **28**: 2252-2261.

36. Ushijima J-I, Matsukawa K, Yuasa A and Okada M-A, 1983, "Toxicities of bracken fern in guinea-pigs", Jpn. J. Vet. Sci., **45**(5): 593-602.

37. Dawra RK, Kurade NP and Sharma OP, 2002, "Carcinogenicity of the fern *Pteridium aquilinum* collected from ezootic bovine haematuria-free hilly area in India", Current Sci., 83(8): 1005-1009.

38. El-Mofty MM, Sadek IA and Bayoumi S, 1980, "Improvement in detecting the carcinogenicity of bracken fern using an Egyptian toad", Oncology, **37**: 424-425.

39. Povey AC, Potter D and O'Connor PJ, 1996, "^{32}P-post-labelling analysis of DNA adducts formed in the upper gastrointestinal tissue of mice fed bracken spores", Brit. J. Cancer, **74**: 1342-1348.

40. Moura JW, Stocco dos Santos RC, Dagli MLZ, D'Angelino JL, *et al.*, 1988, "Chromosome aberrations in cattle raised on bracken fern pasture", Experientia, **44**: 785-788.

41. Lioi MB, Barbieri R, Borzacchiello G, Dezzi S, Roperto S, Santoro A, Russo V and Roperto F, 2004, "Chromosomal aberrations in cattle with chronic enzootic haematuria", J. Comp. Path., **131**: 233-236.

42. Peretti V, Ciotola F, Albarella S, Russo V, Di Meo GP, Iannuzzi L, Roperto F and Barbieri V, 2007, "Chromosome fragility in cattle with chronic enzootic haematuria", Mutagenesis, **22**(5): 317-320.

43. Santos RC, Silva ME, Brasileiro-Filho G, Recourso RC, Lindsay CJ, Frietas RN, Marliere CA and Galvao MAM, 1999. Abstract presented at the International Bracken Group Conference, Manchester, July 1999. Briefly reported by Alonso-Amelot, 2002.

44. Recouso RC, Stocco dos Santos RC, Freitas R, Santos RC, de Freitas AC, Brunner O, Beçak W and Lindsey CJ, 2003, "Clastogenic effect of bracken fern (*Pteridium aquilinum* v. *arachnoideum*) diet in peripheral lymphocytes of human consumers: preliminary data", Vet. Comparative Oncol., **1**(1): 22-29.

45. Hirono I, Fushimi K, Mori H, Miwa H and Haga M, 1973, "Comparative study of carcinogenic activity in each part of bracken", J. Natl.Cancer Inst., 50: 1367-1371.

46. Evans IA, Widdop B and Barber GD, 1967, "Carcinogenic activity of bracken", British Empire Cancer Campaign for Research, Annual Report, Part II: pages 411-412.

47. Roberts IM, Shaw DS and Evans WC, 1971, "A T4-bacteriophage reversion test for the naturally occurring mutagen present in bracken fern (Pteridium aquilinum)", Biochem. J., **124**: 13p.

48. Pamukcu AM, Ertűrk E, Yalçiner Ş, Milli U and Bryan GT, 1978, "Carcinogenic and mutagenic activities of milk from cows fed bracken fern (Pteridium aquilinum)", Cancer Res., **38**: 1556-1560.

49. İstanbulluoğlu E, Pamuçku T and Pamuçku M, 1981, "Mutagenic activity of milks from cows fed bracken fern", Vet. Fakult. Dergisi Ankara Universit., **28**(1-4): 137-143. (In Turkish with an English summary.)

50. Dean SW, 1991, "Study to determine the transmission of bracken into goats' milk", Microtest Report No. RMSREMAF.003, Hazleton Microtest, York. Reproduced as Annex 1 to MUT/93/8, Committee on the Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM).

51. Pamukcu AM & Price M, 1969, "Induction of intestinal and urinary bladder cancer in rats feeding bracken fern (*Pteris aquilina*)", J. Natl. Cancer Inst., **43**: 275-281.

52. Gounalan S, Somvanshi R, Kumar R, Dash S and Devi V, 1999, "Clinico-pathological effects of bracken fern (*Pteridium aquilinum*) feeding in laboratory rats", Indian J. Animal Sci., **69**(6): 385-388.

53. Gounalan S, Somvanshi R, Kataria M, Bisht GS, Smith BL and Lauren DR, 1999, "Effect of bracken (*Pteridium aquilinum*) and dryopteris (*Dryopteris juxtaposita*) fern toxicity in laboratory rabbits", Indian J. Exp. Biol., **37**: 980-985.

54. Villalobos-Salazar J, Meneses A, Rojas JL, Mora J, Porras RE and Herrero MV, 1989, "Bracken derived carcinogens as affecting animal health and human health in Costa Rica". In: Taylor JA (ed), "Bracken toxicity and carcinogenicity as related to animal and human health", Chapter 5, pages 40-50 of special publication reporting the 1st meeting of the International Bracken Group. Published by the Geography Department, University College of Wales, Aberystwyth, Wales.

55. Yasuda Y, Kihara T and Nishimura H, 1974, "Embryotoxic effects of feeding bracken fern (Pteridium aquilinum) to pregnant mice, Toxicol. Appl. Pharmacol., 28: 264-268.

56. Fenwick GR, 1988, "Bracken (*Pteridium aquilinum*) toxic effects and toxic constituents", J. Sci. Food Agric., **46**: 147-173.

57. Cranwell MP, 2004, "Bracken poisoning", Cattle Practice (BCVA), **12**(3): 205-207.

58. Cooper MR, Johnson AW and Dauncey EA, 2003, "Poisonous Plants and Funguses – An Illustrated Guide", second edition, published by the Stationery Office in association with Royal Botanical Gardens Kew, Guy's Medical Toxicology Unit and St. Thomas' hospital NHS Trust, London. ISBN 0 11 7028614.

59. Evans WC, Evans ETR and Hughes LE, 1954, "Studies on bracken poisoning in cattle", Brit. Vet. J., **110**(I, II & III), pages 295, 365 and 426.

60. Alonso-Amelot ME and Avendaño M, 2002, "Human carcinogenesis and bracken fern: a review of the evidence", Current Medical Chemistry, **9**: 675-686.

61. Rosenberger G and Heeschen W, 1960, "Bracken fern (Pteris aquilina) the cause of chronic bovine haematuria", Deutsche Tierärztliche Wochenschrift, **67**(8): 201-208. (In German with English summary.)

62. Lin Y, Kikuchi S, Tamakoshi A, Yagyu K, Obata Y, Inabata Y, Kurosawa M, Kawamura T, Motohashi Y and Ishibashi T, 2006, "Dietary habits and pancreatic cancer risk in a cohort of middle-aged and elderly Japanese", Nutr. Cancer, **56**(1): 40-49.

63. Hirono I, Shibuya C, Shimizu M and Fushimi K, 1972, "Carcinogenic activity of processed bracken used as human food", J. Natl. Cancer Inst., **48**: 1245-1250.

64. Haenszel W, Kurihara M, Locke FB, Shimuzu K and Segi M, 1976, "Stomach cancer in Japan", J. Natl. Cancer Inst., **56**: 265-274.

65. Kamon S and Hirayama T, 1975, "Epidemiology of cancer of the oesophagus in Miye, Nara and Wakayama Prefecture with special reference to the role of bracken fern", Proc. Jap. Cancer Assoc., **34**: 211 (abstract 770).

66. Hirayama T, 1979, "Diet and cancer", Nutr. Cancer, **1**(3): 67-81.

67. Marlière CA, Wathern P, Freitas SN, Castro MCFM and Galvão MAM, 1999, "Bracken fern (*Pteridium aquilinum*) consumption and oesophageal and stomach cancer in the Ouro Preto region, Minas Gerais, Brazil". Chapter 23 in "Bracken Fern: Toxicity, Biology and Control", edited by Taylor JA and Smith RT, pages 144-149 of the Proceedings of the International Bracken Group Conference, Manchester, 20-23 July 1999. Published by the International Bracken Group, Aberystwyth, Wales. August 2000. ISBN 0 9525505 2 0.

68. Alonso-Amelot ME and Avendaño M, 2001, "Possible association between gastric cancer and bracken fern in Venezuela: An epidemiologic study", Int. J. Cancer, **91**: 252-259.

69. Galpin OP and Smith RMM, 1986, "Bracken, stomach cancer and water supplies: Is there a link?", in Smith RT and Taylor JA (editors) "Bracken – Ecology, land use and control technology – The proceedings of the international conference – Bracken '85", Pantheon Publishing, Aberystwyth, Wales, pp 147-159.

70. Buckley, E, 1989, "Bracken, stomach cancer and the Gwynedd population". In: Taylor JA (ed), "Bracken toxicity and carcinogenicity as related to animal and human health", Chapter 7, pages 58-79 of special publication reporting the 1st meeting of the International Bracken Group. Published by the Geography Department, University College of Wales, Aberystwyth.

71. Galpin OP, Whitaker CJ. Whitaker R and Kassab JY, (1990), "Gastric cancer in Gwynedd: Possible links with bracken", Brit. J. Cancer, **61**: 737-740.

72. Evans IA, 1979 , "Bracken carcinogenicity", Res. Vet. Sci., **26**: 339-348.

73. Hirono I, Ito M, Yagyu S, Haga M, Wakamatsu K, Kishikawa T, Nishikawa O, Yamada K, Ojika M and Kigoshi H, 1993, "Reproduction of progressive retinal degeneration (bright blindness) in sheep by administration of ptaquiloside contained in bracken", J. Vet. Med. Sci., **55**: 979-983.

74. Yoshida M and Saito T, 1994, "Acute toxicity of braxin C, a bracken toxin, in guinea-pigs", J. Toxicol. Sci. **19**: 17-23.

75. Hirono I, Yamada K, Niwa H, Shizuri Y, Ojika M, Hosaka S, Yamaji T, Wakamatsu K, Kigoshi H, Niiyama K and Uosaki Y, 1984a, "Separation of carcinogenic fraction of bracken fern", Cancer Letters, **21**: 239-246.

76. Hirono I, Ogino H, Fujimoto M, Yamada K, Yoshida Y, Ikagawa M and Okumura M, 1987, "Induction of tumours in ACI rats given a diet containing ptaquiloside, a bracken carcinogen", JNCI, **79**(5): 1143-1149.

77. Shahin, M., Moore MR, Smith BL, Seawright AA and Prakash AS, 1998. "Acute and chronic toxicity induced by activated ptaquiloside in rats: Comparison of pathogenesis due to oral and intravenous administrations". Chapter 52, pages 255-259. In T. Garland & A. C. Barr (eds.). Toxic plants and other natural toxicants. CAB International, Wallingford Oxon, UK.

78. Shahin M, Smith BL, Worral S, Moore MR, Seawright AA and Prakash AS, 1998, "H-ras activation is an early event in the ptaquiloside-induced carcinogenesis: Comparison of acute and chronic toxicity in rats" Biochem. Biophys. Res. Commun., **250**: 491-497.

79. Nagao T, Saito K, Hirayama E, Uchikoshi K, Koyama K, Natori S, Morisaki N, Iwasaki S and Matsushima T, 1989, "Mutagenicity of ptaquiloside, the carcinogen in bracken, and its related illudane-type sesquiterpenes. I. Mutagenicity in *Salmonella typhimurium*", Mutat. Res., **215**: 173-175.

80. Matoba M, Saito E, Saito K, Koyama K, Natori S, Matsushima T and Takimoto M, 1987, "Assay of ptaquiloside, the carcinogenic principle of bracken, Pteridium aquilinum, by mutagenicity testing in *Salmonella typhimurium*", Mutagenesis, **2**(6): 419-423.

81. Burkhalter PW, Groux PMJ, Candrian U, Hübner P and Lüthy J, 1996, "Isolation, determination and degradation of ptaquiloside – a bracken fern (*Pteridium aquilinum*) carcinogen", J. Natural Toxins, **5**(2): 141-159.

82. Matsuoka A, Hirosawa A, Natori S, Iwasaki S, Sofuni T and Ishidate M Jr, 1989, "Mutagenicity of ptaquiloside, the carcinogen in bracken, and its related illudane-type sesquiterpenes. II. Chromosomal aberration tests with cultured mammalian cells", Mutat. Res., **215**: 179-185.

83. Shahin M, Smith BL, Moore MR and Prakash AS, 1995, "Development of a rat cancer model for ptaquiloside, a bracken carcinogen", Proceedings of the Australian Society for Clinical and Experimental Pharmacology and Toxicology, **3**: 174.

84. Mori H, Sugie S, Hirono I, Yamada K, Niwa H and Ojika M, 1985, "Genotoxicity of ptaquiloside, a bracken carcinogen, in the hepatocytes primary culture/DNA-repair test", Mutat. Res., **143**: 75-78.

85. Bonadies F, Borzacchiello G, Dezzi S, Nicoletti R and Roperto S, 2004, "Mass spectrometric analysis of ptaquiloside, the toxic sesquiterpene from bracken fern", Rapid Commun. Mass Spectrom., **18**: 825-828.

86. Fukuoka M, Kuroyangi M, Yoshihira K, Natori S, Nagao M, Takahashi Y and Sugimura T, 1978, "Chemical and toxicological studies on bracken fern *Pteridium aquilinum* var. *latiusculum*. IV. Surveys on bracken constituents by mutagenicity test", J. Pharm. Dyn., **1**: 324-331.

87. Ishidate M, Harnois MC and Sofuni T, 1988, "A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures", Mutat. Res., **195**: 151-213.

88. Evans WC, Korn T, Natori S, Yoshihira K and Fukuoka M, 1983, "Chemical and toxicological studies on bracken fern *Pteridium aquilinum* var. *latisculum*. VIII. The inability of bracken extracts containing pterosins to cause cattle bracken poisoning", J. Pharm. Dyn., **6**: 938-940.

89. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, 2006, "Statement on cyanogenic glycosides in bitter apricot kernels", Food Standards Agency.

90. Saito T and Mochizuki D, 1986, "Isolation of two active glucosides, braxin A1 and A2, from rhizomes of bracken fern", J. Toxicol. Sci., **11**: 15-27.

91. Tjatur Rasa FS, Saito T and Satoh H, 1998, "The haemolytic activity of bracken extracts in guinea-pigs", J. Vet. Med. Sci., **61**(2): 129-133.

92. Stavric B, 1994, "Quercetin in our diet: from potent mutagen to probable anticarcinogen", Clin. Biochem., **27**: 245-248.

93. Ngomuo AJ and Jones RS, 1996, "Cytotoxicity studies of quercetin, shikimate, cyclohexanecarboxylate and ptaquiloside", Vet. Human Toxicol., **38**(1): 14-18.

94. Campo MS, Jarrett WFH, Barron R, O'Neil BW and Smith KT, 1992, "Association of bovine papillomavirus type 2 and bracken fern with bladder cancer in cattle", Cancer Res., **52**: 6898-6904.

95. NTP, 1992, "Toxicology and carcinogenesis studies of quercetin (CAS No. 117-39-5) in F344 rats (feed studies)", report number TR-409 in the National Toxicology Program (NTP) of the National Institute of Environmental Health Sciences, US Department of Health and Human Services, Research Triangle Park, NC, USA.

96. Dunnick JK and Hailey JR, 1992, "Toxicity and carcinogenicity studies of quercetin, a natural component of foods", Fund. Appl. Toxicol., **19**: 423-431.

97. Hirono I, Ueno I, Hosaka S, Takanashini H, Matsushima T, Sugimura T and Natori S, 1981, "Carcinogenicity examination of quercetin and rutin in ACI rats", Cancer Lett., **13**: 15-21.

98. Takanashi H, Aisi S, Hirono I, Matsushima T and Sugimura T, 1983, "Carcinogenicity test of quercetin and kaempferol in rats by oral administration", J. Food Safety, **5**: 55-60.

99. Ito N, Hagiwara A, Tamano S, Kagawa M, Shibata MA, Kurata Y and Fukushima S, 1989, "Lack of carcinogenicity of quercetin in F344/DuCrj rats", Jpn. J. Cancer Res., **80**: 317-325.

100. Saito D, Shirai A, Matsushima T, Sugimura T and Hirono I, 1980, "Test of carcinogenicity of quercetin, a widely distributed mutagen in food", Teratogenesis Carcinogenesis Mutagenesis, **1**: 213-221.

101. Morino K, Matsukura N, Kaeachi T, Ohgaki H, Sugimura T and Hirono I, 1982, "Carcinogenicity test of quercetin and rutin in golden hamsters by oral administration", Carcinogenesis, **3**: 93-97.

102. Musonda CM and Chipman JK, 1999, "Carcinogenic and anti-carcinogenic potential of quercetin". Chapter 16 in "Bracken Fern: Toxicity, Biology and Control", edited by Taylor JA and Smith RT, Proceedings of the International Bracken Group Conference, Manchester, 20-23 July 1999. Special Publication #4 of the International Bracken Group, Aberystwyth, Wales. August 2000. ISBN 0 9525505 2 0.

103. Fazal F, Rahman A, Greensill J, Ainley K, Hadi SM and Parish JH, 1990, "Strand scission in DNA by quercetin and Cu(II): Identification of free radical intermediates and biological consequences off scission", Carcinogenesis, **11**(11): 2005-2008.

104. Sugimura T, Nagao M, Matsushima T, Yahaghi T, Seino Y, Shirai A, Sawamura M, Natori S, Yoshihira K, Fukuoka M and Kuroyanagi M, 1977, "Mutagenicity of flavone derivatives", Proc. Jpn. Acad., **53**(Ser. B): 194-197.

105. MacGregor JT and Jurd L, 1978, "Mutagenicity of plant flavonoids: Structural requirements for mutagenic activity in *Salmonella typhimurium*", Mutat. Res., **54**: 297-309.

106. Brown JP and Dietrich PS, 1979, "Mutagenicity of plant flavonoids in *Salmonella*/mammalian microsome test. Activation of flavonol glycosides by mixed glycosidases from rat cecal bacteria and other sources", Mutat. Res., **66**: 223-240.

107. Carver JH, Carrano, AV and MacGregor JT, 1983, "Genetic effects of the flavonols quercetin, kaempferol and galangin on Chinese hamster ovary cells *in vitro*", Mutat. Res., **113**: 45-60.

108. Maruta A, Enaka K and Umeda M, 1979, "Mutagenicity of quercetin and kaempferol on cultured mammalian cells", Gann, **70**: 273-276.

109. Leal AM, Ferraz OP, carvilho C, Freitas AC, Beniston RG, Beçak W, Campo MS and Stocco dos Santos RC, 2003, "Quercetin induces structural chromosomal aberrations and uncommon rearrangements in bovine cells transformed by the E7 protein of bovine papillomavirus type 4", Vet. Comparative Oncol., **1**(1): 15-21.

110. Sahu RK, Basu R and Sharma A, 1981, "Genetic toxicological testing of some plant flavonoids by the micronucleus test", Mutat. Res., **89**: 69-74.

111. Ngomuo AJ and Jones RS, 1996, "Genotoxicity studies of quercetin and shikimate *in vivo* in the bone marrow of mice and gastric mucosal cells of rats", Vet. Human Toxicol., **38**(3): 176-180.

112. MacGregor JT, 1979, "Mutagenicity of plant flavonoids *in vivo* and *in vitro*", Toxicol. Appl. Pharmacol., **48**: A47 (Abstract No. 94).

113. Watson WAF, 1982, "The mutagenic activity of quercetin and kaempferol in *Drosophila melanogaster*", Mutat. Res., **103**: 145-147.

114. Hardigree AA and Epler JL, 1978, "Comparative mutagenesis of plant flavonoids in microbial systems", Mutat. Res., **58**: 231-239.

115. Leach H, Barker GD, Evans IA and Evans WC, 1971, "Isolation of an active principle from the bracken fern that is mutagenic, carcinogenic and lethal to mice on Intraperitoneal injection", Biochem. J., **124**: 13p-14p.

116 O'Donovan MR, Brewster D and Jones RS, 1977, "Embryotoxic properties of shikimic acid", Int. Res. Commun. Syst., **5**: 514.

117. Evans IA and Osman MA, 1974, "Carcinogenicity of bracken", Nature, **250**: 348-349.

118. Jones RS, Ali M, Ioannides C, Styles JA, Ashby J, Sulej J and Parke DV, 1983, "The mutagenic and cell transforming properties of shikimic acid and some of its bacterial and mammalian metabolites", Toxicol. Lett., **19**: 43-50.

119. Meyer P, 1989, "Thiaminase activities and thiamine content of *Pteridium aquilinum, Equisetum ramonissimum, Malva parviflora, Pennisetum clandestinum* and *Medicago sativa*", Onderstepoort J. Vet. Res., **56**: 145-146.

120. Pamukcu AM, Yalçiner Ş, Price JM and Bryan GT, 1970, "Effects of the coadministration of thiamine on the incidence of urinary carcinomas in rats fed bracken fern", Cancer Res., **30**: 2671-2674.

121. Campo MS, 1997, "Bovine papillomavirus and cancer", Veterinary J., **154**: 175-188.

122. Australian Wine Research Institute, 2008, "The Australian Wine Research Institute's analytical service - analyses". At web-site http://www.awri.com.au/analytical_service/alalyses/colour/

123. Myer RO and Froseth JA, 1983, "Heat-processed small red beans (Phaseolus vulgaris) in diets for young pigs", J. Anim. Sci., **56**: 1088-1096.

124. IARC (International Agency for Research on Cancer), 1976, "IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to humans – Some naturally occurring substances", Volume 10. Published by World Health Organization, Geneva.

125. IARC (International Agency for Research on Cancer), 1987, "IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to humans – Overall evaluations of carcinogenicity: An updating of IARC Monographs Volumes 1-42", Supplement 7. Published by World Health Organization, Geneva.

126. Pamukcu AM, Ching-Yung Wang, Hatcher J and Byran GT, 1980a, "Carcinogenicity of tannin and tannin-free extracts of bracken fern", J. Natl. Cancer Instit., **65** (1): 131-136.

127. Shahin M, Smith BL, Worral S, Moore MR, Seawright AA and Prakash AS, 1998, "Bracken fern carcinogenesis: Multiple intravenous doses of activated ptaquiloside induce DNA adducts, monocytosis, increased TNFα levels and mammary gland carcinoma in rats" Biochem. Biophys. Res. Commun., 244: 192-197.

128. Shahin, M., Smith BL, Oelrichs PB, Moore MR, Worral S, Seawright AA and Prakash AS, 1998, "Induction of mammary gland carcinoma, monocytosis and type II pneumonocyte proliferation by activated ptaquiloside". Chapter 65, pages 329-333. In T. Garland & A. C. Barr (eds.). "Toxic plants and other natural toxicants". CAB International, Wallingford Oxon, UK.

129. Niwa H, Ojika M, Wakamatsu K, Yamada K, Hirono I and Matsushita K, 1983, "Ptaquiloside, a novel norsesquiterpene glucoside from bracken, *Pteridium aquilinum* var. *latisculum*", Tetrahedron Letters, **24**(38): 4117-4120.

130. Ojika M, Sugimoto K, Okazaki T and Yamada K, 1989, "Modification and cleavage of DNA by ptaquiloside, a new potent carcinogen isolated from bracken fern", J. Chem. Soc. Chem. Commun., **22**: 1775-1777.

131 Kushida T, Uesugi M, Sugiura Y, Kigoshi H, Tanaka H, Hirokawa J, Ojika M and Yamada K, 1994, "DNA damage by ptaquiloside, a potent bracken carcinogen: Detection of selective strand breaks and identification of DNA cleavage products", J. Am. Chem. Soc., **116**: 479-486.

132. Prakash AS, Pereira TN, Smith BL, Shaw G and Seawright AA, 1996, "Mechanism of bracken fern carcinogenesis: Evidence for H-ras activation via initial adenine alkylation by ptaqiloside", Natural Toxins, **4**: 221-227.

133. Borzacchiello G, Ambrosio V, Roperto S, Poggiali F, Tsirimonakis E, Venuti A, Campo MS and Roperto F, 2003, "Bovine papillomavirus type 4 in oesophageal papillomas of cattle from the south of Italy", J. Comp. Path., **128**: 203-206.

134. Borzacchiello G, Iovane G, Marcante ML, Poggiali F, Roperto F, Roperto S and Venuti A, 2003, "Presence of bovine papillomavirus type 2 DNA and expression of the viral oncoprotein E5 in naturally occurring urinary bladder tumours in cows", J. General Virol., **84**: 2921-2926.

135. Borzacchiello G, Ambrosio V, Galati P, Perillo A and Roperto F, 2003, "Cyclooxygenase-1 and -2 expression in urothelial carcinomas of the urinary bladder in cows", Vet. Pathol., **40**: 455-459.

136. Jarrett WFH, 1978, "Transformation of warts to malignancy in alimentary carcinoma in cattle", Bull. Cancer, **65**(2): 191-194.

137. Jarrett WFH, McNeil PE, Grimshaw WTR Selman IE and McIntyre WIM, 1978, "High incidence area of cattle cancer with a possible interaction between an environmental carcinogen and a papilloma virus", Nature, **274**: 215-217.

138. Campo MS, O'Neil BW and Jarrett WFH, 1994, "Experimental reproduction of the papillomavirus-carcinoma complex of the alimentary canal in cattle", Carcinogenesis, **15**: 1597-1601.

139. Campo MS, Beniston RG, Connolly JA and Grindlay GJ, 1999, "Synergism between papillomavirus and bracken fern in carcinogenesis of the upper gastrointestinal tract in cattle and humans: Quercetin and cell transformation". Chapter 17 in "Bracken Fern: Toxicity, Biology and Control", edited by Taylor JA and Smith RT, pages 116-122 of the Proceedings of the International Bracken Group Conference, Manchester, 20-23 July 1999. Published by the International Bracken Group, Aberystwyth, Wales. August 2000. ISBN 0 9525505 2 0.

140. Villalobos-Salazar J, Hernandez H, Meneses A and Salazar G, 2000, "Factors which may affect ptaquiloside levels in milk: Effects of altitude, bracken fern growth stage and milk processing". In "Bracken Fern: Toxicity, Biology and Control", edited by Taylor JA and Smith RT, Chapter 10, pages 68-74 of the Proceedings of the International Bracken Group Conference, Manchester, 1999. Published by the International Bracken Group, Aberystwyth, Wales.

141. Alonso-Amelot ME, Castillo U, Sánchez MD and De Jongh F, 1992, "Detection of ptaquiloside, the potent carcinogen of bracken fern (*Pteridium aquilinum*) in bovine milk", Abstr. Pap. Am. Chem. Soc., **203**(1-3): AGFD 89.

142. Alonso-Amelot ME, Castillo U and De Jongh F, 1993, "Passage of bracken fern carcinogen ptquiloside into bovine milk", Lait, **73**: 323.

143. Alonso-Amelot ME, Castillo U, Smith BL and Lauren DR, 1998, "Excretion, through milk, of ptaquiloside in bracken-fed cows. A quantitative assessment", Lait, **78**: 413-423.

144. Kumar KA, Kataria M and Somvanshi R, 2000, "Haematological evaluation of bracken (*Pteridium aquilinum*) and cheilanthes (*Cheilanthes farinose*) fern feeding in guinea-pigs", Indian J. Environ. Toxicol., **10**(1): 30-33.

145. Recouso RC, Stocco dos Santos RC, Freitas R, santos RC, de Freitas AC, Brunner O, Beçak W and Lindsay CJ, 2003, "", Vet. Comparat. Oncology, **1**(1): 22-28.

146. Harwood M, Danielewska-Nikiel B, Borzelleca JF, Flamm GW, Williams GM and Lines TC, 2007. "A critical review of the data related to the safety of quercetin and lack of evidence of *in vivo* toxicity, including lack of genotoxic/carcinogenic properties", Food Chem. Toxicol., **45**: 2179-2105.

147. Stoewsand GS, Anderson JL, Boyd JN, Hrazdina G, Babish JG, Walsh KM and Losco P, 1984, "Quercetin: A mutagen, not a carcinogen, in Fischer rats", J. Toxicol. Environ. Health, **14**: 105-114.

148. Hosaka S and Hirono I, 1981, "Carcinogenicity test of quercetin by pulmonary adenoma bioassay in strain A mice", GANN, **72**: 327-328.

149. Ertűrk E, Nunoya T, Hatcher JF, Pamukcu AM and Bryan GT, 1983, "Comparison of bracken fern (BF) and quercetin (Q) carcinogenicity in rats (R)", Proc. Am Assoc. Cancer res. Annu. Meet., **24**: 53 (abstract No. 210).

150. Mendall MA, Goggin PM, Molineaux N, Levy J, Toosy T, Strachan D and Northfield TC, 1992, "Childhood living conditions and *Helicobacter pylori* seropositivity in adult life", Lancet, **339**(8798): 896-897.

151. Barker DJP, Coggon D, Osmond C, Wickham C, 1990, "Poor housing in childhood and high rates of stomach cancer in England and Wales", Br. J. Cancer, **61**: 575-578.

152. Van der Hoeven JCM, Lagerweij WJ, Posthumus MA, Veldhuizen A, Holterman HAJ, 1983, "Aquilide A, a new mutagenic compound isolated from bracken fern (*Pteridium aquilinum* (L.) Kuhn), Carcinogenesis, **4**: 1587-1590.

153. White RD, Krumnerman PH, Cheeke PR, *et al*., 1983, "An evaluation of acetone extracts from six plants in the Ames mutagenicity test", Toxicol. Lett., **15**: 135-136.

154. Fukuoka M, Kuroyangi M, Yoshihira K, Natori S, Nagao M, Takahashi Y and Sugimura T, 1978, "Chemical and toxicological studies on bracken fern *Pteridium aquilinum* var. *latiusculum*. IV. Surveys on bracken constituants by mutagenicity test", J. Pharm. Dyn., **1**: 324-331.

155. Yoon J-Y and Lee S-R, 1988, "Mutagenic activity by Ames test of bracken grown in Korea", Korean J. Food Sci. Technol., 20(4): 558-562. (In Korean with summary, tables and figures in English.)

156. Symonds HW, 1991, "Transmission of bracken toxins in goat's milk", Study Number MAF 3/S, Department of Animal Physiology and Nutrition, University of Leeds, 17th July 1991. Reproduced as Appendix 10 to MUT/93/8, Committee on the Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM).

157. Almeida Santos M de F, Dorea JG and Luna H, 2006, "Bracken-fern extracts can be clastogenic or aneugenic depending on the tissue cell assay", Fd. Chem. Toxicol., **44**: 1845-1848.

158. European Food Safety Authority, 2004, "Opinion of the Scientific Panel on Food additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on hydrocyanic acid in flavourings and other food ingredients with flavouring properties", The EFSA Journal **105**: 1-28.

159. European Food Safety Authority, 2007, "Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to cyanogenic compounds as undesirable substances in animal feed", The EFSA Journal, **434**: 1-67.

160. Freitas RN, Silva ME, O'Connor PJ and Povey AC, 1999, "^{32}P-postlabelling analysis of DNA adducts in tissues obtained from rats treated with bracken fern (*Pterideum aquilinum* subsp. *caudatum*) from Brazil". Chapter 20 in "Bracken Fern: Toxicity, Biology and Control", edited by Taylor JA and Smith RT, pages 132-135 of the Proceedings of the International Bracken Group Conference, Manchester, 20-23 July 1999. Published by the International Bracken Group, Aberystwyth, Wales. August 2000. ISBN 0 9525505 2 0.

Appendix to COT statement on the risk to consumers of eating foods derived from animals that had eaten bracken

Literature search

Articles were identified and selected for review as follows.

Computerised literature search

Several computerised searches of the scientific literature published over the last 10-38 years were conducted by the Information Centre of the Food Standard Agency. The computerised searches performed were:

- Search on DART Developmental Toxicology Literature, NewsQuest, Food Science and Technology Abstracts 1969-2007, Foodlineweb 1972-2007, and National Library for Health (incorporating Biomed Central, Dialog Datastar, MyLibrary, NHL Evidence, NHL Guidance, NHL Specialist Libraries, Proquest and PubMed) for
  "**bracken**".

- Searches on Campden and Chorleywood Food Research Association (CCFRA) Site, Barbour Index, British Library Inside, Current Contents 1998-2007, FoodlineWeb (Leatherhead Food International Ltd.), Nature – journal, and New Scientist - journal for:
  (**bracken** or **bracken fern** or **Pteridium aquilinum** or **Pteridium**)
  and
  (**ptaquiloside** or **bracken toxin** or **sesquiterpene glycoside**)
  and
  (**toxicology** or **toxicological** or **cancer** or **carcinogenic** or **mutagenic** or **genotoxic** or **epidemiology**)

- Searches on TOXLINE and FoodlineWeb (Leatherhead Food International Ltd.), Current Contents 1998-2007 and Food Science and Technology Abstracts (FSTA) for:
  pterosin or **pterosin-B** or **isoptaquiloside** or **iso-ptaquiloside** or **caudatoside** or **thiaminase** or **pharmacokinetics** or **toxicology** or **toxicological** or **cancer** or **carcinogenic** or **mutagenic** or **genotoxic** or **epidemiology**

- Searches on TOXLINE 1900-2007, British Library Inside, Ingenta.com, Food Science and Technology Abstracts (FSTA), TOXLINE, Foodlineweb 1972-2007, and Current Contents 1998-2007 for:
  quercetin or **prunacin**
  and
  bracken

The computerised literature searches identified a large number of articles, many appearing on several of the databases. Printouts of details of articles including abstracts were read and articles that looked like they would be relevant to the consumer safety of bracken were ordered. The main criterion for ordering was that the article should deal with some aspect of the exposure to or toxicology of bracken and/or its component chemicals.

Secondary search of the literature

Review articles on bracken were read and from these further key articles were identified. This was particularly helpful for finding older articles.

The relevant articles were read and summarised for this COT paper. During this process, several further important articles that had been missed earlier were identified and ordered.

Selection of articles for review in TOX/2008/12

A large number of articles on the safety of bracken and on related issues were obtained by the Secretariat. As a result of time constraints, not all of the articles were read in detail. Some selection had to be made. It was quickly apparent that certain articles were too general or only dealt peripherally with the issues of interest. Such articles were left aside.

Sometimes several articles referred to the same piece of work. Wherever possible the primary source of information was the source that was cited. In some instances, information was pieced together from several sources and it was necessary to cite several articles for a single piece of information.

Criteria for selection of articles cited in TOX/2008/12

Articles were selected for inclusion in the review on the basis that they dealt with some aspect of the safety of bracken and its constituent chemicals to human consumers. This could be a direct reference to human safety by dealing with exposure, toxicological or epidemiological aspects or less direct by looking at effects in exposed animals or of exposure by routes other than the diet. Related issues of interest included the taxonomy and worldwide distribution of different sub-species of bracken ferns.

Some articles were excluded as they covered areas of work already dealt with in other articles. This was particularly the case concerning the large number of studies from the 1980s that reported the testing various fractions of bracken in standard short-term assays for mutagenicity or carcinogenicity in order to isolate the carcinogenic component of bracken.

There were numerous review articles available that added no new information. Most of these were not cited.

Articles Cited in the Statement

Not all of the articles cited in the review paper TOX/2008/12 were cited in the Statement on the Risk to Consumers of Eating Foods Derived from Animals that had Eaten Bracken. Some aspects covered in the review paper were not considered to be directly relevant to the Statement and thus articles cited in these parts of the review paper were not cited in the Statement. Also, when several articles dealt with the same issue, some of the articles that contributed no unique information were omitted from the Statement.

Articles Added to the Statement

After the COT had discussed the first draft of the statement, Members identified a few additional articles that had not been identified in the Secretariat's search of the scientific literature. These articles and some of the articles giving primary data that they had cited were added to later drafts of the Statement.

Statement on Pyrrolizidine Alkaloids in food

Introduction

1 Pyrrolizidine alkaloids (PAs) are a large group of natural toxins produced by plants, several of which are known to be highly hepatotoxic and have been shown to be carcinogenic in rats. They have been associated with a number of livestock diseases and with cases of human poisoning following consumption of herbal remedies or after contamination of staple foods. There is also potential for PAs to be transferred to other food products such as honey, milk, eggs and offal.

2. In 2007, the report of a Food Standards Agency funded project on PAs in honey from borage and ragwort was published. This report was provided to the Committee along with a number of risk assessments of PAs from other countries.

3. The Committee was asked for its view on the risk assessment of PAs in food and whether it considered potential human exposure, particularly via honey and milk, to be of concern.

Background

4. PAs are found in a large number of plants around the world including the families *Boraginaceae* (particularly *Heliotropium* and *Trichodesma* species), Compositae (*Asteraceae*) in the tribe *Senecioneae,* and *Leguminosae* (*Fabaceae*) in *Crotalaria* species. It is estimated that approximately 3% of the world's flowering plants contain one or more toxic PAs[1].

5. Cases of human toxicity have been shown to occur following contamination of staple foods, generally grain crops, and after consumption of some herbal remedies. Other possible food sources of exposure include milk, honey, offal and eggs, which have all been found to contain PAs in some instances[2], although cases of human poisoning resulting from exposure through these sources have not been reported. It is unknown whether PA residues are present in meat but the potential for exposure is thought to be slight due to the fast metabolism and elimination of PAs from the bodies of animals as determined experimentally[1,3].

6. In humans, veno-occlusive disease is the most prominent hepatic lesion resulting from PA poisoning. Classical symptoms and signs are abdominal pain and rapidly developing ascites. The effects of PAs can take time to develop and might result from long term low level exposure, although known cases of poisoning have usually presented as acute disease similar to Budd-Chiari syndrome[2].

7. Livestock poisonings have been reported worldwide, especially in cattle and horses, but also in some instances in sheep. One of the plants often associated with this is common or tansy ragwort (*Senecio jacobaea*)[2].

8. In animals, PA toxicosis is usually characterised by clinical signs relating to hepatic insufficiency including weakness, loss of appetite and wasting, jaundice and behavioural abnormalities. Extensive haemorrhagic necrosis of the liver is usually recorded in acute toxicity. Chronic disease, resulting either from a single sublethal dose or from repeated low level intake, is characterised by various

abnormalities including parenchymal megalocytosis, extensive fibrosis, obliteration of central and sub-lobular veins characteristic of veno-occlusive disease, bile duct proliferation and nodular regeneration[2].

9. Instances of poisoning in humans and livestock, combined with the results of studies in experimental animals indicate that there is variation between species in susceptibility to PAs. In general, cattle, horses, pigs, poultry, humans, rats and mice are considered to be sensitive while sheep, goats, rabbits and guinea pigs are less so[4,5].

Previous COT recommendations

Comfrey

10. The Committee last reviewed PAs in 1992[6], focussing on comfrey, a herb which at the time was available in tablet and capsule form as well as for tea and infusions. The recommendations of that review of comfrey were as follows:

- "the public should be warned of the potential dangers associated with the consumption of comfrey and products containing comfrey. This advice applies equally to commercial and home-grown comfrey and preparations made from it.
- "concentrated forms of comfrey such as tablets and capsules should no longer be available.
- "the public should be advised against the ingestion of comfrey root and leaves, and of teas and infusions made from comfrey root.
- "comfrey teas and tinctures may continue to be available to the public. However, this recommendation should not be construed as an endorsement of these products."

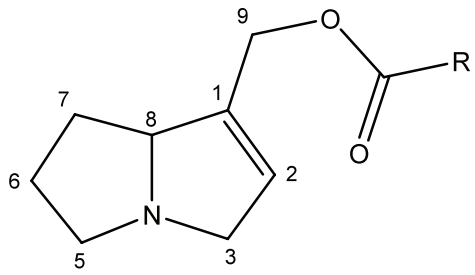
The COT advice was subsequently endorsed by the Food Advisory Committee. Department of Health (DH) and Ministry of Agriculture Fisheries and Food (MAFF) Ministers accepted the committees' advice and action was taken to implement it.

Chemistry

11. PAs are a group of more than 350 natural toxins sharing a basic structure derived from esters of 4 necine bases: platynecine, retronecine, heliotridine and otonecine. The acid moieties of the esters are termed necic acids. A number of structural features determine the potency of the PAs.

12. PAs associated with adverse effects are esters of 1-hydroxymethyl 1,2-dehydropyrrolizidine (Figure 1). There may be a second hydroxyl group at the C7 position. At least one of these hydroxyl groups must be esterified to exert toxicity and the acid moiety of the ester linkage must contain a branched chain. PAs can therefore exist as mono or open diesters or as a closed macrocyclic diester[2].

Figure 1: The generic structure required for PAs to cause toxicity



13. PAs are fairly stable chemically and require metabolic activation to exert toxicity[5].

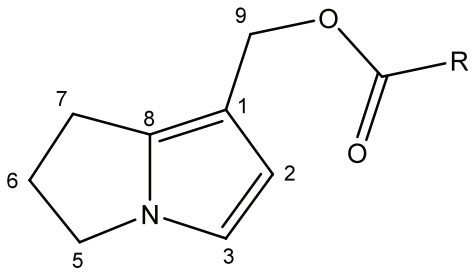
Metabolism

14. On ingestion of PAs, parent alkaloid or metabolites can be found in the serum and only later in the urine and faeces indicating that absorption across the gastrointestinal tract occurs[7,8,9,10,11,12,13,14]. Studies using a limited number of representative PAs have shown that three main pathways of metabolism occur[15].

Activation pathway

15. The activation pathway is oxidation of the PA to form the dehydropyrrolizidine derivative, which is biologically and chemically reactive (figure 2). Cytochromes P450 have been shown to be involved in this bioactivation of the PAs[16].

Figure 2: The generic structure of the dehydropyrrolizidine derivative of PAs



16. Dehydropyrrolizidine derivatives can undergo further biotransformation by enzymic or non-enzymic glutathione conjugation[1]. Alternatively, the dehydropyrrolizidine derivative can be hydrolysed further at the ester bond to form the dehydronecine, often referred to as dehydropyrrolizine (DHP)[16].

Detoxification pathways

17. Esterase cleavage of the PA releases the necine base and necic acid(s). No further metabolism occurs and this is seen as a detoxification pathway[15].

18. *N*-oxidation of retronecine- and heliotridine-type PAs is generally catalysed by a variety of enzymes including cytochromes P450 and flavin-containing monoxygenases. The *N*-oxides are highly water soluble and are rapidly excreted in the urine[5].

19. *N*-oxides are also often found in plant materials. While metabolism to PA *N*-oxides is usually seen as a detoxification pathway, upon ingestion these can be converted to the alkaloid forms in the gut[3].

20. The activity of the metabolic enzymes towards individual PAs plays an important role in determining toxicity and varies between species, sexes and at different developmental stages[15,17,18,19,20].

21. Following metabolism, rapid elimination occurs mainly via urine but some through the bile. It is considered unlikely that large amounts of the PAs and their metabolites remain in the liver in the long term[3]. PAs and their metabolites can be excreted in the milk and possibly, in the case of poultry, into eggs[1].

Toxicity

22. Much of the data on PA toxicity is derived from studies on plant constituents or extracts and is often derived from studies or reports of single dose administration. Information on the mechanism of toxicity has been taken from reviews, which have compiled data from a number of studies and do not quote the doses at which the individual effects occur. Where doses are given these are examples, generally of the lowest dose causing effect.

Human case reports

23. A 49 year-old woman, who had been consuming a herbal tea and comfrey-pepsin pills on a daily basis for 6 and 4 months respectively, was admitted to hospital with progressive swelling of the abdomen and extremities. This was diagnosed as veno-occlusive disease, a form of Budd-Chiari syndrome. A liver biopsy showed centrilobular necrosis and congestion. The hepatic venograms were consistent with moderate portal hypertension recording wedge pressure of 23 mmHg with corrected sinusoidal pressure of 17 mmHg. No demonstrable obstruction of outflow was observed but balloon distension of an intrahepatic venous tributary showed near obliteration of the smaller hepatic venules and extravasation of the dye into the hepatic parenchyma. There was no history of tumour or trauma, her last pregnancy was 22 years previously and no common causes of Budd-Chiari syndrome were evident. The herbal tea and comfrey-pepsin pills were analysed for PAs, based on monocrotaline as a standard and looking for alkaloids of the same molecular weight and assuming the same extinction coefficient for spectrophotometric detection. The subject's minimum daily PA intake was estimated as 15 µg/kg b.w. The researchers stated that it was possible she had other sources of exposure in the same period[21]. This report is one of the few where an attempt has been made to evaluate the exposure level of a person with PA poisoning.

24. A 5 day old female infant was referred to intensive care with jaundice, massive hepatomegaly and ascites. When the infant was 27 days old, a biopsy was taken. This showed centrilobular fibrosis, neovascularisation and iron deposition associated with widespread circumferential connective tissue occlusion of the small and medium size hepatic veins suggesting a diagnosis of hepatic veno-occlusive disease. The mother had had daily consumption of a herbal tea containing senecionine (including its *N*-oxide) at 0.60 mg/kg dry weight but no estimate of the dose to the mother or the fetus was given. A liver biopsy section was not obtained from the mother but her physical appearance and blood tests showed no abnormalities[22].

25. A pregnant woman was admitted to hospital in the 27th week of gestation with fetal ascites. A male infant was delivered by emergency caesarean section during week 32 but died 12 hours later. Autopsy found no internal or external malformations. Liver histology showed veno-occlusive disease. Tea used by the family was found to be free of PAs. However, a herbal mixture of which 2 g/day was used for cooking contained 6 mg/kg lycopsamine, 3.5 mg/kg interrimine and 3 mg/kg of their acetyl derivatives. Neither the maternal nor the fetal dose resulting from the use of this mixture was estimated. The dehydro-derivatives of these PAs were found in the fetal liver tissue[23].

26. Based on information provided by two paediatric liver centres to the Committee, it was noted that paediatric veno-occlusive disease was rare in the UK, and that cases were almost always attributable to other causes and therefore unlikely to be related to PA exposure.

Acute Studies

27. LD50 data obtained following intraperitoneal administration to male rats are available for some PAs and are given in Table 1[5,24]. These deaths, 3 to 7 days after administration, were associated with severe haemorrhagic liver necrosis[5].

28. Lesions in the lung following acute dosing include alveolar oedema and effects on the alveolar wall seen after a single dose of 60 mg/kg b.w. in rats given monocrotaline subcutaneously and at the same dose in dogs given monocrotaline intravenously. In the long term extensive pleural effusion occurs following intraperitoneal administration of a single dose of 50 mg fulvine/kg b.w. to female rats[5].

29. Necrotising pulmonary arteritis was observed following subcutaneous administration of monocrotaline at 120 mg/kg b.w. to male rats as a single dose or 30 mg/kg b.w. as 4 doses each 2 months apart in monkeys[5].

Table 1

Reported intraperitoneal LD50 values obtained for the male rat unless otherwise stated (Source: Cheeke and Shull, 1985 and World Health Organization (WHO), 1988)

Alkaloid	LD50 (mg/kg b.w.)
Retrorsine	34
Senecionine	50 – also quoted as 85
Heliosupine	60
Lasiocarpine	72
Seneciphylline	77
Jacobine	77 (mouse)
Riddelliine	105 (mouse)
Symphytine	130 – also quoted as 300
Heleurine	140
Jaconine	168 (female rat)
Monocrotaline	175
Echimidine	200
Spectabiline	220
Senkirkine	220
Heliotrine	300
Echinatine	350
Supinine	450
Europine	>1000
Heliotridine	1200
Intermedine	1500
Lycopsamine	1500

30. A study investigated the early pulmonary changes following PA exposure using monocrotaline pyrrole (the dehydropyrrolizidine derivative of monocrotaline) injected into the tail vein of Sprague Dawley rats. A single dose of 3.5 mg/kg b.w. in male rats caused changes in the lung from 4 hours after administration[25].

Repeated dose toxicity

31. In a study of riddelliine administered by gavage 5 days per week for 105 weeks to rats and mice, 0.033 mg/kg b.w./day in rats caused hepatocyte cytomegaly (NOAEL 0.01 mg/kg b.w./day) and at 0.33 mg/kg b.w./day regenerative hepatocyte hyperplasia was observed. In mice, focal necrosis of the liver was observed at 0.1 mg/kg b.w./day (lowest dose given) and hepatocyte cytomegaly and karyomegaly was observed at 0.3 mg/kg b.w./day[26].

32. Monkeys given monocrotaline at 60 mg/kg b.w. by subcutaneous injection at monthly intervals for 3 months showed varying degrees of occlusion of the centrilobular, sublobular and larger veins in the liver. Centrilobular haemorrhagic necrosis and megalocytosis were also observed[5].

33. Rats were given 8 mg/kg b.w./day of an alkaloidal extract of *Senecio nemorensis* ssp. fuchsii containing 50% fuchsisenecionine and 1% senecionine by gavage 5 days per week for 114 weeks. Fatty changes, single cell and focal necrosis, fibroses and granulomatous reactions were observed in the livers[27].

Mechanism of action

Hepatotoxicity

34. Animal studies have demonstrated that hepatic parenchymal cell and sinusoidal endothelial cell injury occur early in the process of PA-induced disease in the rat. Once cell injury has occurred, zonal necrosis ensues where the zone affected depends on the species and the pathway of metabolism for the PA in question[5].

35. Veno-occlusion is thought to occur because of damage to the sinusoidal and central vein endothelial cells leading to thickening and then collagenisation. Occlusion of the central vein occurs which is preceded by functional restriction of blood flow.

36. One of the typical features of PA toxicosis in animals is megalocytosis of hepatic parenchymal cells, though this has not generally been observed in humans. This is believed to occur where cells are stimulated to go through the cell cycle but do not divide[28].

37. The WHO stated that adverse long term effects are similar whether resulting from one relatively high dose, which is not acutely lethal, or multiple low level doses[5].

Pulmonary toxicity

38. Pulmonary toxicity is sometimes, but not always, seen with hepatotoxic PAs though in some instances higher doses are required to elicit pulmonary toxicity than cause hepatotoxicity. The structural requirements for toxicity in the lung are the same as those for toxicity in the liver and the same metabolites as are produced in the hepatocytes cause toxicity in the lung. In general, the more stable or persistent the dehydropyrrolizidine derivative is, the greater the possibility that it can be transported away from the liver to cause toxicity in other organs[5]. Some metabolism similar to that in the liver can occur in the pulmonary endothelial cells and type II pneumocytes of the lung[28].

39. Pulmonary toxicity manifests as pulmonary hypertension and can lead to cardiac right ventricular hypertrophy[5]. There may also be abnormal macrophages and a proliferation of mast cells. Initial damage is reported to be to the endothelial cells of the small blood vessels. This is followed by changes in the alveolar wall and then a reduction in the lumen of the small vessels[3].

Developmental studies

40. Studies in pregnant rats given heliotrine by intraperitoneal injection showed fetal malformations at doses above 100 mg/kg maternal b.w. along with subnormal maternal gestational weight gain. Fetal effects included retarded development, musculoskeletal defects, cleft palate and at high dose (300 mg/kg maternal b.w.) cessation of growth, immature fetuses and intrauterine deaths and resorptions. Litters exposed to 50 mg/kg maternal b.w. showed decreased weight and length following a temporary reduction in maternal weight after injection. However, little liver damage was observed in the fetuses suggesting that the fetal liver may be relatively more resistant to these toxic effects[29]. This is in contrast to the effects seen in the human case report[22] where a 5 day old infant showed liver pathology following daily consumption of a herbal tea by the mother in the absence of maternal toxicity (para 24).

41. Oral or intraperitoneal administration of two PAs to lactating rats did not result in maternal toxicity. The total dose, given singly or in multiple fractions, was 21 mg/kg or greater for retrorsine and 83 mg/kg or greater for lasiocarpine. However, liver biopsy samples from the pups showed marked changes. In pups that died aged 18 to 30 days, liver cells showed hydropic or fatty vacuolation. Pups dying after postnatal day 30 showed haemorrhagic necrosis and thickening of centrilobular veins in liver. Susceptibility of suckling rats was shown to be greater than that of their mothers in this study[30].

Mutagenicity, Genotoxicity and Carcinogenicity

42. Several PAs have been evaluated by the International Agency for Research on Cancer (IARC) and categorised either as Group 2B, possibly carcinogenic to humans, or Group 3, not classifiable as to its carcinogenicity to humans. Lasiocarpine, monocrotaline and riddelliine have been classified as Group 2B while hydroxysenkirkine, isatidine, jacobine, retrorsine, seneciphylline, senkirkine and symphytine have been classified as Group 3[31,32,33].

43. A review of the genotoxicity of PAs and the mechanisms involved was published in 2004[16]. Various PAs and PA-containing plant extracts have been shown to be mutagenic in *Salmonella typhimurium* TA100 strain with an S9 activated enzyme system. Seneciphylline, riddelliine, lasiocarpine, senecionine, retrorsine, heliotrine, senecivernine, senkirkine, petasitene, monocrotaline, clivorine, ligularidine, 7-acetyl intermedine, 7-acetyl lycopsamine, indicine, intermedine, jacoline and symlandine have been shown to be mutagenic in either Drosophila melanogaster or bacteria[34,35,36,37,38,39].

44. Male Drosophila flies were fed milk from lactating rats receiving 25 mg/kg b.w. seneciphylline at 0.5 ml per 10 male flies. The resulting number of sex-linked recessive lethals was compared to flies given seneciphylline directly and control flies receiving milk taken from the same rats before they were given seneciphylline. There was an increase in sex-linked recessive lethals compared to controls but not to as great an extent as flies receiving 10-5 M seneciphylline directly. The results indicated the presence of an indirect mutagen in the milk which the authors suggested to be unchanged seneciphylline[34].

45. PAs have been shown to have DNA binding and DNA to DNA or DNA to protein cross-linking abilities and cause sister chromatid exchange and chromosomal aberrations16. The cross linking potency of a sample of PAs (dehydrosenecionine, dehydromonocrotaline, dehydroseneciphylline and dehydroriddelliine) was shown to be correlated positively with differences in toxicity[40].

46. Mechanistic studies have shown that riddelliine induces liver tumours mediated at least in part by DHP-derived DNA adducts. It has been proposed that these could be used as biomarkers of tumourigenicity and that they could be responsible for the mutagenicity and teratogenicity of PAs20. Subsequently it has been suggested that monocrotaline formed DHP-derived DNA adducts either by hydrolysis of dehydromonocrotaline and then reaction with DNA or by dehydromonocrotaline interacting with DNA and then being hydrolysed to DHP[41]. Other PAs, namely riddelliine, lasiocarpine, clivorine, heliotrine and retrorsine have also been shown to form DHP-derived DNA adducts either *in vivo* or *in vitro*[42,43,44,45,46].

47. A number of animal studies on PAs and synthetically prepared pyrrolic metabolites have shown tumour development. In one study under the National Toxicology Program (NTP), using riddelliine administered by gavage 5 days per week for 105 weeks, hemangiosarcomas were observed in 0/50 female control rats and 0/50 females given 0.01, 0.033 and 0.1 mg/kg b.w./day, 3/50 at 0.33 mg/kg b.w./day ($p=0.118$) and 38/50 at 1.0 mg/kg b.w./day ($p<0.001$). In male rats, hemangiosarcomas were observed in 0/50 controls but in 43/50 given 1 mg/kg b.w./day ($p<0.001$). In male mice, hemangiosarcomas were observed in 2/50 controls, 1/50 at 0.1 mg/kg b.w./day, 0/50 at 0.33 mg/kg b.w./day and 2/50 at 1 mg/kg b.w./day but in 31/50 mice at 3 mg/kg b.w./day ($p<0.001$). No female mice in the control group showed hemangiosarcomas and only 1/50 given 3 mg/kg b.w./day (only dose given). Other tumours observed in rats at or above the doses causing hemangiosarcomas included hepatocellular adenomas and carcinomas, mononuclear cell leukaemia, alveolar and bronchiolar adenoma and carcinomas[26]. Similarly, a two year carcinogenicity study has been carried out under the NTP testing lasiocarpine in F344 rats with dietary administration for 104 weeks. High mortality was seen in both sexes at the high dose with all females dead by week 69 and males by week 88. Liver angiosarcoma was seen in 13 of 23 male ($p<0.001$) and 2 of 23 female (not significant) rats (though the authors suggested that only female rats surviving beyond 52 weeks should be used for the analysis so they quote this as 2 of 9 in the main report) following dietary administration at 30 ppm, in 11 of 23 males ($p<0.001$) and 7 of 24 females ($p=0.005$) at 15 ppm in the diet and in 5 of 24 males ($p=0.025$) and 8 of 22 females ($p=0.002$) at 7 ppm in diet. The authors concluded that this study had shown that lasiocarpine was carcinogenic in F344 rats[47].

48. Other PAs have also been shown to cause tumours in animals. Clivorine, petasitenine and symphytine all produce angiosarcomas in liver in non-standard carcinogenicity assays[48,49,50]. Senkirkine caused liver adenomas in a non-standard assay[50].

49. Plant extracts have also been tested for tumour formation, for example tumours have been observed in the pancreas when weanling male rats were given a single dose by stomach tube of 500 to 1500 mg/kg b.w. of a mixture of lycopsamine and intermedine as alkaloids extracted from tarweed (*Amsinckia intermedia* Fisch and Mey) seeds. Three rats of 15 in the treated group were found to have pancreatic tumours; 1 receiving 600 mg/kg b.w. had an islet cell tumour, and of those receiving 1500 mg/kg b.w. 1 had an islet cell adenocarcinoma and 1 had an adenoma of the exocrine pancreas[51].

Transfer to food

50. The PA content of plants has been reported as generally varying from 100 mg/kg dry weight to 40,000 mg/kg, although the highest reported is 180,000 mg/kg in *Senecio riddelli*. The amount of PAs present in a plant depends on the season and locality[3]. In addition, various parts of plants have different levels of PAs, some of which may be present in PA *N*-oxide form[5].

Feed

51. Although PA-containing plants are present throughout the world, the plants are usually unpalatable to livestock. Most cases of poisoning with fresh plant material occur when pastures are overgrazed or if there is a limited supply of forage[1].

52. Where feed is preserved, contamination with PA-containing plant material is not readily recognised by animals. Experiments carried out on hay indicate that the concentration of PAs does not decrease with storage. The evidence for silage is more equivocal with some experiments suggesting that levels do decrease while others find no change. Where decomposition occurs, this is mainly enzymic and levels remain stable once the crop is dry[5].

53. In 2004 the Department for Environment, Food and Rural Affairs (Defra) published a Code of Practice on How to Prevent the Spread of Ragwort. The aim is to control the spread of ragwort where there is an identifiable risk to vulnerable animals including through the production of forage[52].

Food

54. Humans are thought to be exposed to PAs through plant products (either herbal products or contamination of grain crops), or animal-derived products including honey, milk, eggs and offal[2].

Plant products

55. A number of reports of outbreaks of human PA poisoning exist from different parts of the world. These are generally as a result of contamination of cereal crops with PA-containing plants or deliberate intake of herbal remedies which contain PAs.

56. Where acute symptoms and deaths in human poisoning incidents have been reported, it is generally difficult to estimate the exposures responsible. As little or no follow-up has been carried out on those recovering from the illness or others involved in the outbreaks, it is also unclear whether there are long term effects of these poisoning events[5].

57. The Australian New Zealand Food Authority (ANZFA) has sampled various Australian grain commodities and found levels from <0.050 mg/kg to >6 mg/kg2. In the EU, legislation specifies a maximum level for weed seeds and unground and uncrushed fruits containing alkaloids in animal feed[1].

Milk

58. A number of studies have been carried out looking at transfer to milk as a possible route of excretion in lactating animals. The studies described above in paragraphs 41 and 44 show that lactational transfer of PAs occurs in rats.

59. Cows given ragwort containing 0.16% PAs by rumen cannula for 2 weeks at 10 g/kg b.w./day showed weight loss, reduced milk output and persistent diarrhoea. Liver biopsy sections showed megalocytosis and portal fibroplasia. Their calves showed no gross or microscopic lesions and appeared normal throughout the study. While the ragwort contained jacobine, seneciphylline, jacoline, jaconine and jacozine, the milk was found to contain only jacoline and following correction for recovery, the highest mean concentration was 0.840 mg/L[53].

60. Studies in goats have shown that PAs are also transferred into their milk. In one instance, a goat was fed ragwort containing 0.18% PAs as 25% of the feed, which was at the upper limit of acceptance of the plant by the goats. A pooled milk sample collected from the goat twice daily for 236 days contained 7.5x10-3 mg PAs/kg dried weight where the dry matter content of the milk was 12%[54].

61. A survey carried out by MAFF in 1988 analysed 21 retail bulked samples of milk from an area which had the highest reported incidence of ragwort poisoning in cattle for the 2 years beforehand. No senecionine, seneciphylline or jacobine were detected in any sample and it was concluded that detectable levels were unlikely to be present elsewhere in the UK[55].

62. The European Food Safety Authority (EFSA) noted that milk can be a relevant source of PAs when obtained from a single animal which has ingested considerable amounts of PAs1. However, common commercial practice in the UK is to bulk milk samples from all the cows at one farm and then also at the dairy, which results in dilution of the PAs if present.

63. EFSA also suggested that a possible source of human infant exposure is via their mother's milk[1].

Eggs

64. Free PAs were not detected in the eggs of laying hens fed up to 4% *Senecio vernalis*. The authors considered this may have been due to residues being below the level of detection, stated as 0.4 mg/ml dissolved residue, or the PAs being bound to egg protein, but they noted that reduced feed intake and egg production occurred at 2 and 4% of feed levels[56].

65. In contrast Edgar and Smith (2000) reported that in chickens fed contaminated wheat containing 26 mg/kg of PAs (heliotrine, europine and lasiocarpine), up to 0.168 mg/kg was detected in the eggs[57].

Meat

66. No published reports are available where PAs have been detected in meat from livestock which have ingested PA-containing plants. Results from experimental animals suggest that levels in tissues would fall rapidly after ingestion. Mattocks suggested that unless animals are killed soon after a large dose, PAs are not expected to be at a high level in tissues[3].

67. The ANZFA reported PA levels of <0.010 to 0.073 mg/kg in livers and kidneys of domestic animals[2].

Honey

68. In a 1994 UK survey, honey samples were collected from hives placed close to ragwort, or obtained from farmgate producers and a small independent retailer. Eight of 23 honey samples contained ragwort pollen and six of these had detectable levels of PAs. The two honey samples with the highest levels were dark, waxy samples, which were considered unpalatable and would not be used for blending with other honeys. Excluding these two samples, the highest detected level of PAs, was 0.06 mg/kg though the method used for this analysis was not reported. Using data on maximum honey consumption at any one time for adults (93g), children (60g) and infants (32g), the authors concluded that PA consumption from locally produced honey was not a cause for concern[58].

69. A 2002 review of PAs in honey noted that the highest identified level of 3.9 mg PAs/kg was in honey reported to be from ragwort. This value was not corrected for extraction efficiency. The authors recognised that where bees are used to pollinate plants such as borage, the resulting honey is likely to contain PAs, and data from the literature did not indicate that bees avoid PA-containing plants except ragwort as described by the Honey International Packers Association. Therefore it was thought likely that the PA content of a particular honey will depend on the number of PA-containing plants in the forage area[59].

70. The 2002 review considered exposure assessments which were carried out using a WHO database. The consumption data included non-consumers and therefore averages tended to underestimate consumption by consumers. In Europe average honey consumption is 1.3 g/day and high level (by the 95th percentile consumer) is 3.9 g/day. The estimated population average European dietary exposure resulting from honey containing 2 mg PAs/kg, which the review authors described as typical of a honey attributed to a single PA-containing plant, was 2.6 µg PAs/day[59].

71. In 2004, Food Standards Australia New Zealand (FSANZ) reported that Australian honey samples had levels up to 2 mg/kg PAs though it was noted that blending could substantially reduce this level. The highest levels were found in honey from Paterson's Curse/Salvation Jane (*Echium plantagineum*). The FSANZ considered that 2-4 year old children of approximately 17kg with high levels of consumption at 28.6 g honey/day would be the most vulnerable subgroup of the population. To keep this subpopulation within the ANZFA provisional tolerable daily intake (PTDI) of 1 µg/kg b.w./day, the honey consumed would need to contain no more than 0.594 mg PAs/kg. However as other food sources need to be considered, levels would need to be lower than this. As a result, the FSANZ advised that people consuming more than 2 tablespoons of honey every day (approximately 5% of the population) should not eat honey made exclusively from Paterson's Curse/Salvation Jane[60].

72. A recent Dutch study analysed honey samples for PA content of which 171 were retail samples of Dutch or imported origin and 8 were from hives deliberately placed in areas with high levels of groundsel, another PA-containing plant. Of the retail samples, 28% contained PAs at levels between 0.001 and 0.365 mg/kg. Four of the eight non-retail samples had detectable levels of PAs with the highest at 0.010 mg/kg. Pollen counts indicated that the bees had foraged on many other plants not just the groundsel[61].

73. The authors stratified Dutch honey consumers into groups depending on whether they consumed honey from different sources or from the same manufacturer which could coincidentally contain high levels of PAs. Each group was further subdivided into average (13 g honey/day) or high level consumers (30 g/day). The authors concluded that "only in cases of prolonged consumption of types of honey which contain high concentrations of PA is there any suggestion of a significantly increased risk of cancer and possibly acute liver damage." This was considered as rare so warning consumers of the risk was not felt to be useful[61].

Food Standards Agency funded project T01037 "Collection and Analysis of Honey Samples Potentially Contaminated with Pyrrolizidine Alkaloids from Ragwort and Borage"

74. This project aimed to investigate the potential for PA contamination of honey if bees forage on PA-containing flowers. Borage (Borago officinalis) and ragwort were the two flowers of interest and honey was produced in areas where either borage or ragwort was growing in abundance. While the PA concentrations in honey could not be quantified due to a lack of analytical standards, they could be compared from one honey sample to another and relative to the amount of PAs in a fixed weight of plant material[62].

75. Honey produced in areas with high levels of ragwort showed little difference in the PA profile compared to control sites except in honey from one site, which showed increased seneciphylline *N*-oxide levels. However conditions were very different at this site compared to sites for commercial honey production. Honey produced from ragwort is seen by beekeepers as a contaminant and is unlikely to be used for consumption. The authors concluded that the results indicated that even where there appears to be little else to forage on, the honey produced showed no conclusive evidence of ragwort contamination in terms of PA profile and pollen contained in the honey. The Honey International Packers Association has suggested that bees do not like foraging on ragwort or producing honey from it. They also state that the honey tastes unpleasant and therefore would not be consumed[62].

76. Honey produced in areas with high levels of borage showed the presence of one PA which could have been either intermedine or lycopsamine. Honey produced from borage has a distinctive taste and is seen as a speciality product so attracts a premium price[62].

77. This was a preliminary project to determine whether further quantitative analysis would be required for risk assessment. A standard for lycopsamine is now commercially available and the Food Standards Agency plans to fund further work to assess the levels of PAs in borage honey.

Previous risk assessments

World Health Organization (1988)

78. A WHO report provided a full account up to 1988 of experimental animal studies in addition to cases of livestock and human poisoning events. Using data from outbreaks of human disease, the authors

estimated total intake and length of exposure. Total doses in known outbreaks or cases of veno-occlusive disease were estimated to be 1 to 167 mg/kg b.w. Data from the Ridker *et al.* (1985) report on ingestion of comfrey indicated that ingestion of 15 µg PA/kg b.w./day, may lead to acute or subacute liver disease in humans. As comfrey contains echimidine and related alkaloids, the WHO used rat LD50 data to derive the equivalent heliotrine dose so exposures from different case reports of human disease could be compared. The heliotrine equivalent dose for this report was 9 µg/kg b.w./day. Therefore the WHO considered it prudent to conclude that a dose equivalent to 10 µg/kg b.w./day heliotrine may lead to disease in humans without providing further explanation[5].

79. The WHO considered that the dose estimates derived indicated that effects are cumulative at very low intakes and that chronic exposure even at low levels may present a health risk. It therefore recommended that exposure should be minimised if possible. Long term effects in humans might be liver cirrhosis or cancer but there had been a lack of long term follow up where exposure was known to have occurred[5].

Australia New Zealand Food Authority (2001)

80. This report provides a brief summary of the occurrence, chemistry, toxicity in livestock and humans, metabolism, mechanisms of toxicity, carcinogenicity and dose-response for chronic liver disease of PAs.

81. The ANZFA concluded that the major human dietary source of exposure is contaminated grains, with eggs, offal and honey being minor contributors. However the authors noted that the data available were very limited and it was not possible to estimate the potential dietary exposure to PAs from these food sources[2].

82. The conclusion of the ANZFA risk characterisation was: "On the basis of the limited human data on the incidence of veno-occlusive disease, a tentative NOEL for all PAs of 10 µg/kg b.w./day is suggested based on the human data reported by Ridker *et al.* (1985). Applying an uncertainty factor of 10 to this figure to take into account individual variation, the PTDI for PAs is 1 µg/kg b.w./day.

> "Further characterisation of the potential human health risk from exposure to PAs in food is not possible because there is currently inadequate dietary exposure information."[2]

83. Despite 10 µg/kg b.w./day being quoted as causing disease by the WHO, the ANZFA cited comments by Mattocks (1986) and Huxtable (1989) as suggesting that this dose "may well be close to the NOEL for humans"[2]. Both authors had highlighted the uncertainty stated in the original paper as to whether the woman had had exposure to PAs from other sources[3,63].

Dutch National Institute for Public Health and the Environment (2005)

84. The Dutch National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) established a virtually safe dose (VSD) for PAs of 0.00043 µg/kg/day, leading to an increased risk of at most one person in a million developing cancer. For non-cancer

effects, a tolerable daily intake (TDI) of 0.1 µg/kg b.w./day was derived from the rat NOAEL of 0.01 mg/kg b.w./day for non-neoplastic changes (hepatocyte cytomegaly) in the 105 week study cited in paragraph 31 above, and using an uncertainty factor of 100 (10 for interspecies and 10 for intraspecies variation)[64]. A 2007 report of the Dutch Food and Consumer Product Safety Authority (Voedsel en Waren Autoriteit, VWA) concluded that "only in cases of prolonged consumption of types of honey which contain high concentrations of PA is there any suggestion of a significantly increased risk of cancer and possibly acute liver damage"[61].

European Food Safety Authority (2007)

85. This is a report on the opinion of the Scientific Panel on Contaminants in the Food Chain on pyrrolizidine alkaloids as undesirable substances in animal feed. The report provides a summary of available data up to 2006. In relation to human exposure, EFSA recommended obtaining more data on carry over of PAs into milk as infants may have high exposure via this pathway. Also the need for quantitative assessment of the contribution of honey to human exposure was highlighted[1].

UK Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (2008)

86. The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) reviewed the mutagenicity and carcinogenicity of 7 PAs and 2 proposed metabolites. The COC concluded that riddelliine was genotoxic and carcinogenic and it would be prudent to assume at least part of the carcinogenic effect was through a genotoxic mechanism. Similarly, lasiocarpine is possibly a genotoxic carcinogen. There were sufficient data to conclude that monocrotaline was carcinogenic. There was limited evidence of carcinogenicity for clivorine, petasitenine and symphytine while for senkirkine there was insufficient evidence of carcinogenicity. A limited number of studies using non-oral routes of administration had been carried out on the two metabolites, dehydroretronecine and dehydroheliotridine. On balance, these did not indicate carcinogenic activity of the metabolites.

87. The COC agreed that the BMDL10 derived from the NTP studies on riddelliine and lasiocarpine would be an appropriate basis for a Margin of Exposure (MOE) approach to the risk assessment of PAs. The lowest $BMDL_{10}$ of 0.073 mg/kg b.w/day was derived from the study on lasiocarpine in male rats and included adjustment of the high dose to take into account cessation of dosing and termination of all animals by 88 weeks. Previously the COC have agreed that an MOE of less than 10,000 may be a concern, an MOE between 10,000 and 1,000,000 is unlikely to be a concern and above 1,000,000 is highly unlikely to be a concern[65]. This is similar to the 2005 EFSA Scientific Committee view that "an MOE of 10,000 or higher, if it is based on the $BMDL_{10}$ from an animal study, would be of low concern from a public health point of view and might be considered as a low priority for risk management"[66].

88. With evidence that the same rare tumour type, angiosarcoma, is produced by a number of PAs and that formation of the DHP metabolite has been demonstrated with various PAs, the COC felt that a cumulative assessment approach based on the most potent PA, lasiocarpine, as proposed by EFSA[67] would be appropriate for all PAs.

COT evaluation

89. The Committee noted that PAs are a large class of compounds with differing toxicities and that the variability in potency is an important consideration in the risk assessment of these toxins.

90. The Committee discussed the differing ANZFA and WHO assessments of the case report by Ridker *et al.*, together with the additional comments made by Mattocks and Huxtable. The ANZFA supported the WHO approach of extrapolation of the subject's dietary exposure to 10µg/kg b.w./day heliotrine equivalent. The authors of the case report recognised that the affected subject may have had further sources of PA exposure in addition to those identified. This source of uncertainty was further highlighted by Mattocks and Huxtable in their commentaries. While the ANZFA judged that this was sufficient to deem 10 µg/kg b.w./day a tentative NOAEL, the WHO concluded that this exposure level may cause disease.

91. The Committee concluded that a single human case report, with considerable uncertainties in the exposure assessment did not provide a reliable basis for deriving a TDI. Therefore use of the 2 year rat and mouse study with riddelliine administered by oral gavage was considered to be the most appropriate basis for assessing risks of the non-cancer effects of PAs. A NOAEL of 0.01 mg/kg b.w./day for hepatocyte cytomegaly in rats was observed in this study. Applying an uncertainty factor of 100, 10 for interspecies and 10 for intraspecies variation, indicated that non-cancer effects would not be expected at doses of riddelliine up to 0.1 µg/kg b.w./day. It was noted that this dose was 100 fold lower than the dose in heliotrine equivalents as defined by the WHO which the woman received in the report by Ridker *et al.*

92. In their review, the WHO used the ratio of LD50s to compare doses of different PAs. The LD50 data are based on intraperitoneal administration rather than oral dosing. However, as severe haemorrhagic necrosis of the liver caused death in the animals studied, the data are relevant to the impairment of hepatic function, which is the basis of the risk assessment. Therefore the Committee considered that using the ratio of LD50 values is acceptable to extrapolate between PAs. It was noted that where they occur, N-oxides should be considered as equivalent to their parent alkaloid, because of possible conversion to this form in the gut following oral administration. This does not occur with intraperitoneal administration, by which *N*-oxides are much less toxic than their parent alkaloid.

93. The Committee accepted the COC advice that PAs should be considered as a cumulative assessment group where it is prudent to assume that PAs are genotoxic carcinogens. As a result, the Committee used the $BMDL_{10}$ of 0.073 mg/kg b.w./day to assess margins of exposure (MOE) for any PA assuming an equivalent potency to lasiocarpine. In line with COC[65] and EFSA[66] opinions, the Committee considered that MOEs of 10,000 and above, corresponding to doses of up to 0.007 µg /kg b.w./day, would be unlikely to be of concern. Such doses are below the 0.1 µg/kg b.w./day identified as not expected to be associated with non-cancer effects of PAs. The Committee noted the COC's acknowledgement that a cumulative assessment group approach assuming equal potency for all PAs would be likely to be over-precautionary where little was known about the PAs in question.

94. Using the limit of detection for honey of 1 µg/kg honey reported by Betteridge *et al.* in 2005[68] and for milk of 10 µg/L milk reported by MAFF in 1994[55], hypothetical exposure assessments were carried out for all age groups based on the UK National Diet and Nutrition Surveys. The age group with highest PA exposure on a body weight basis in both instances would be infants.

95. High level (97.5th percentile) infant consumers have an intake of 6.97 g honey per day (equivalent to 1.14 g/kg b.w./day), which includes honey in other foodstuffs. This is despite FSA advice for infants not to consume honey due to the very small possibility of bacterial contamination that could cause infant botulism. Following consumption of honey with a PA concentration at the limit of detection (1µg/kg honey[68]), these high level infant consumers would receive 0.0011 µg PAs/kg b.w./day. This is 66,000 fold below the $BMDL_{10}$ and 90 fold below the dose of 0.1 µg/kg b.w./day, below which non-cancer effect would not be expected (this assumes that all the PAs present have equivalent potency to riddelliine, as no quantitative data on individual PAs present in honey are available). The maximum PA concentration in honey, which would still maintain an MOE of 10,000 compared to the $BMDL_{10}$, for high level infant consumers, would be 6.4 µg/kg honey.

96. There were limitations in the methods used in the Food Standards Agency funded study assessing honey samples potentially contaminated with PAs from ragwort and borage. The lack of analytical standards at the time of commissioning raised the possibility that where PAs were judged to be not present in the samples, this resulted from an inability to detect them. There was also concern that the PAs sought were the most prevalent in the plants but were not necessarily the most toxic PAs present. Overall, however, it was considered that the data from the project supported the hypothesis that honey produced in areas with a high concentration of ragwort is unlikely to be a concern for human health. The Committee noted that PAs had been found in honeys sampled around the world. However, in the absence of quantitative data on individual PAs present in UK commercial samples, it is difficult to assess the risk to the UK consumer.

97. For milk, high level (97.5th percentile) infant consumers receive 1054 g milk per day (equivalent to 187.6 g/kg b.w./day), where the specific gravity of milk is 1.03 kg/L. In milk with a PA concentration at the limit of detection (10µg/L milk[55]), infants would be exposed to 1.8 µg/kg b.w./day, which is only 40 fold below the $BMDL_{10}$. Hence the analytical method lacks sensitivity to detect levels in milk, which result in high level infant consumer exposure with a sufficient MOE to be of low concern.

98. Data on toxicity to young rats and calves following transfer of PAs to milk raised concern that human children and infants may be vulnerable following exposure to cows' milk and breast milk. Given the relatively low intake of PAs, the Committee doubted that levels in breast milk would be sufficiently high to cause significant effects in the neonate. In addition, the overall incidence of paediatric veno-occlusive disease in the UK appears to be extremely low with the majority of cases accounted for by other known causes such as cytotoxic drugs. Consumption of milk with PAs at the limit of detection would lead to exposure only 40 fold below the $BMDL_{10}$. Therefore the analytical method is insufficiently sensitive to identify concentrations of PAs in milk that would be of low concern following human exposure, particularly in high level infant consumers. However, the practice of bulking dairy milk supplies in the UK provides some reassurance that PA exposure through milk is unlikely to be a human health concern.

99. In addition to the precautionary nature of the assessment of carcinogenic potential, the Committee noted that the exposure assessments are precautionary as they assume that all foodstuffs consumed will be contaminated, which, while possible in the short term, is unlikely in the long term.

100. Data were not available on concentrations of PAs in grain, eggs or meat on the UK market and, therefore, an assessment of UK consumer exposure from these foodstuffs could not be carried out. The Committee noted that, if grain in the UK were contaminated to the same extent as the upper level identified in Australia, human exposure from this source could be significant. However, the absence of data prevented any further assessment of possible risk to the consumer.

101. It was noted that the Food Standards Agency research on PAs in honey is continuing while other organisations are looking at PAs in milk and other products. The Committee will monitor this research and other assessments made on PAs.

COT conclusions

102. PAs are known to cause veno-occlusive disease in humans. The available reports of human cases of poisoning do not provide sufficiently reliable exposure data to be used in establishing a health-based guidance value.

103. We conclude that the two-year study in rats administered riddelliine by oral gavage is the most robust basis for assessing the non-cancer effects of PAs. Applying uncertainty factors of 10 for interspecies and 10 for within species variability to the NOAEL of 0.01 mg/kg b.w./day for hepatocyte cytomegaly, indicates that 0.1 µg riddelliine/kg b.w./day would not be expected to result in non-cancer effects. The ratio of LD50 values can be used to convert other PAs to riddelliine equivalents for comparison with this dose.

104. We endorse the COC recommendation to assess all PAs as a cumulative assessment group using the $BMDL_{10}$ with an adequate MOE, while acknowledging the precautionary nature of this approach. A $BMDL_{10}$ of 0.073 mg/kg b.w./day was derived from a 2 year carcinogenicity study of lasiocarpine and should be used to assess exposure for any PA. Allowing an MOE of at least 10,000 indicates that PA doses of up to 0.007 µg /kg b.w./day are unlikely to be of concern for cancer risk. Such doses would also not be expected to result in non-cancer effects.

105. The maximum PA concentration in honey, to maintain an MOE of 10,000 for high level infant consumers, would be 6.4 µg/kg honey.

106. We note that consumption of milk with PAs at the limit of detection by high level (97.5th percentile) infant consumers leads to exposure only 40 fold below the $BMDL_{10}$. We conclude that there is a need for more sensitive analytical methods to detect PAs in milk to enable assessment of exposure with an adequate MOE compared to the $BMDL_{10}$. However, based on the available data on PA content in milk and the practice of bulking dairy milk supplies, which is likely to lead to dilution of any contamination present, we conclude that PAs in milk are unlikely to be a human health concern. There is a need for more information on the levels of PAs in grain in the UK to enable assessment of exposure and risk to consumers from this source.

COT Statement 2008/06
September 2008

References

1 EFSA. (25-1-2007). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the European Commission related to pyrrolizidine alkaloids as undesirable substances in animal feed. *The EFSA Journal* 447, 1-51.

2 ANZFA. (2001). Pyrrolizidine alkaloids in food. A Toxicological Review and Risk Assessment. *Technical Report Series No. 2*, 1-16.

3 Mattocks, A. R. (1986). *Chemistry and Toxicology of Pyrrolizidine Alkaloids.* London, Academic Press.

4 McLean, E. K. (1970). Pyrrolizidine (Senecio) Alkaloids. *Pharmacol Rev* 22, 429-483.

5 WHO. (1988). *Pyrrolizidine Alkaloids. Environmental Health Criteria* 80, 1-345.

6 COT, C. &. C. 1992 Annual Report.

7 Candrian, U., Lüthy, J., Schlatter, C. (1985). *In vivo* covalent binding of retronecine-labelled [^{3}H]seneciphylline and [^{3}H]senecionine to DNA of rat liver, lung and kidney. *Chem.-Biol, Interactions* 54, 57-69.

8 Panter, K. E. and James, L. F. (1990). Natural plant toxicants in milk: A review. *J.Anim.Sci.* 68, 892-904.

9 Williams, L., Chou, M. W., Yan, J., Young, J. F., Chan, P. C., Doerge, D. R. (2002). Toxicokinetics of Riddelliine, a Carcinogenic Pyrrolizidine Alkaloid, and Metabolites in Rats and Mice. *Toxicology and Applied Pharmacology* 182, 98-104.

10 Lüthy, J., Heim, T., Schlatter, C. (1983). Transfer of [^{3}H]pyrrolizidine alkaloids from *Senecio vulgaris* L. and metabolites into rat milk and tissues. *Toxicology Letters* 17, 283-288.

11 Chu, P.S., Lame, M.W., Segall, H.J. (1993). Invivo Metabolism of Retrorsine and Retrorsine-N-Oxide. *Archives of Toxicology* 67: 39-43.

12 White, I.N.(1977). Excretion of pyrrolic metabolites in the bile of rats given the pyrrolizidine alkaloid retrorsine or the bis-N-ethylcarbamate of synthanecine A. *Chem Biol Interact* 16: 169-180.

13 Pierson, M.L., Cheeke, P.R., Dickinson, E.O. (1977). Resistance of the rabbit to dietary pyrrolizidine (Senecio) alkaloid. *Res Commun Chem Pathol Pharmacol* 16: 561-564.

14 Brauchli, J., Lüthy, J., Zweifel, U., Schlatter, C. (1982). Pyrrolizidine alkaloids from *Symphytum officinale* L. and their percutaneous absorption in rats. *Experientia* 38, 1085-1087.

15 Prakash, A. S., Pereira, T. N., Reilly, P. E. B., Seawright, A. A. (1999). Pyrrolizidine alkaloids in human diet. *Mutation Research* 443, 53-67.

16 Fu, P. P., Xia, Q., Lin, G., Chou, M. W. (2004). Pyrrolizidine Alkaloids - Genotoxicity, Metabolism Enzymes, Metabolic Activation and Mechanims. *Drug Metabolism Review*s 36, 1-55.

17 Cheeke, P. R. and Pierson-Goeger, M. L. (1983). Toxicity of *Senecio jacobaea* and pyrrolizidine alkaloids in various laboratory animals and avian species. *Toxicology Letters* 18, 343-349.

18 Huan, J.-Y., Miranda, C. L., Buhler, D. R., Cheeke, P. R. (1998). Species differences in the hepatic microsomal enzyme metabolism of the pyrrolizidine alkaloids. *Toxicology Letters* 99, 127-137.

19 Huan, J.-Y., Miranda, C. L., Buhler, D. R., Cheeke, P. R. (1998). The Roles of CYP3A and CYP2B Isoforms in Hepatic Bioactivation and Detoxification of the Pyrrolizidine Alkaloid Senecionine in Sheep and Hamsters. *Toxicology and Applied Pharmacology* 151, 229-235.

20 Fu, P. P., Xia, Q., Lin, G., Chou, M. W. (2002). Genotoxic Pyrrolizidine Alkaloids - Mechanisms Leading to DNA Adduct Formation and Tumorigenicity. *Int.J.Mol.Sci* 3, 948-964.

21 Ridker, P. M., Ohkuma, S., McDermott, W. V., Trey, C., Huxtable, R. J. (1985). Hepatic Venocclusive Disease Associated With the Consumption of Pyrrolizidine-Containing Dietary Supplements. *Gastroenterology* 88, 1050-1054.

22 Roulet, M., Laurini, R., Rivier, L., Calame, A. (1988). Hepatic veno-occlusive diseaes in newborn infannt of a woman drinking herbal tea. *J.Pediatrics* 112, 433-436.

23 Rasenack, R., Müller, C., Kleinschmidt, M., Rasenack, J., Wiedenfeld, H. (2003). Veno-Occlusive Disease in a Fetus Caused by Pyrrolizidine Alkaloids of Food Origin. *Fetal Diagnosis and Therapy* 18, 223-225.

24 Cheeke, P. R. and Shull, L. R. (1985). Natural Toxicants in Feeds and Poisonous Plants. Westport, Connecticut, The AVI Publishing Compant, Inc.

25 Schultze, A. E., Wagner, J. G., White, S. M., Roth, R. A. (1991). Early Indications of Monocrotaline Pyrrole-Induced Lung Injury in Rats. *Toxicology and Applied Pharmacology* 109, 41-50.

26 NTP. (2003). Toxicology and carcinogenesis studies of riddelliine. *NTP Technical Report* 508.

27 Habs, H., Habs, M., Marquardt, H., Röder, E., Schmähl, D., Wiedenfeld, H. (1982). Carcinogenic and Mutagenic Activity of an Alkaloidal Extract of Senecio nemorensis ssp. fuchsii. *Arzneim.-Forsch./Drug Res.* 32, 144-148.

28 Stegelmeier, B. L., Edgar, J. A., Colegate, S. M., Gardner, D. R., Schoch, T. K., Coulombe, R. A., Molyneux, R. J. (1999). Pyrrolizidine alkaloid plants, metabolism and toxicity. *Journal of Natural Toxins* 8, 95-116.

29 Green, C. R. and Christie, G. S. (1961). Malformations in foetal rats induced by the pyrrolizidine alkaloid, Heliotrine. *British Journal of Experimental Pathology* 42, 369-378.

30 Schoental, R. (1959). Liver lesions in young rats suckled by mothers treated with the pyrrolizidine (senecio) alkaloids, lasiocarpine and retrorsine. *J.Path.Bact.* 77, 485-495.

31 IARC. (1976). Some Naturally Occuring Substances. *IARC Monographs on Evaluation of Carcinogenic Risks to Humans* 10.

32 IARC. (1983). Some Food Additives, Feed Additives and Naturally Occurring Substances. *IARC Monographs on Evaluation of Carcinogenic Risks to Humans* 31.

33 IARC. (2002). Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. *IARC Monographs on Evaluation of Carcinogenic Risks to Humans* 82.

34 Candrian, U., Lüthy, J., Graf, U., Schlatter, C. (1984). Mutagenic activity of the pyrrolizidine alkaloids seneciphylline and senkirkine in Drosophila and their transfer into rat milk. *Food Chem.Toxic.* 22, 223-225.

35 Mori, H., Sugie, S., Yoshimi, N., Asada, Y., Furuya, T., Williams, G. M. (1985). Genotoxicity of a Variety of Pyrrolizidine Alkaloids in the Hepatocyte Primary Culture-DNA Repair Test Using Rat, Mouse, and Hamster Hepatocytes. *Cancer Research* 45, 3125-3129.

36 Frei, H., Luthy, J., Brauchli, J., Zweifel, U., Wurgler, F.E., Schlatter, C. (1992). Structure/activity relationships of the genotoxic potencies of sixteen pyrrolizidine alkaloids assayed for the induction of somatic mutation and recombination in wing cells of Drosophila melanogaster. *Chem Biol Interact* 83: 1-22.

37 Rubiolo, P., Pieters, L., Calomme, M., Bicchi, C., Vlietinck, A., Vanden Berghe, D. (1992). Mutagenicity of pyrrolizidine alkaloids in the Salmonella typhimurium/mammalian microsome system. *Mutat Res* 281: 143-147.

38 Yamanaka, H., Nagao, M., Sugimura, T., Furuya, T., Shirai, A., Matsushima, T. (1979). Mutagenicity of pyrrolizidine alkaloids in the Salmonella/Mammalian microsome test. *Mutation Research* 68, 211-216.

39 Berry, D. L., Schoofs, G. M., Schwass, D. E., Molyneux, R. J. (1996). Genotoxic activity of a series of pyrrolizidine alkaloids in primary hepatocyte-mediated V79 cell mutagenesis and DNA repair assay. *Journal of Natural Toxins* 5, 7-24.

40 Kim, H.-Y., Stermitz, F. R., Coulombe, R. A. (1995). Pyrrolizidine alkaloid-induced DNA-protein cross-links. *Carcinogenesis* 16, 2691-2697.

41 Wang, Y.-P., Yan, J., Beger, R. D., Fu, P. P., Chou, M. W. (2005). Metabolic activation of the tumorigenic pyrrolizidine alkaloid, monocrotaline, leading to DNA adduct formation *in vivo*. *Cancer Letters* 226, 27-35.

42 Wang, Y.P., Fu, P.P., Chou, M.W. (2005). Metabolic activation of the tumorigenic pyrrolizidine alkaloid, retrorsine, leading to DNA adduct formation *in vivo*. *Int J Environ Res Public Health* 2: 74-79.

43 Xia, Q., Chou, M.W., Kadlubar, F.F., Chan, P.C., Fu, P.P. (2003). Human liver microsomal metabolism and DNA adduct formation of the tumorigenic pyrrolizidine alkaloid, riddelliine. *Chem Res Toxicol* 16: 66-73.

44 Xia, Q., Chou, M.W., Lin, G., Fu, P.P. (2004). Metabolic formation of DHP-derived DNA adducts from a representative otonecine type pyrrolizidine alkaloid clivorine and the extract of Ligularia hodgsonnii hook. *Chem Res Toxicol* 17: 702-708.

45 Xia, Q., Chou, M.W., Edgar, J.A., Doerge, D.R., Fu, P.P. (2006). Formation of DHP-derived DNA adducts from metabolic activation of the prototype heliotridine-type pyrrolizidine alkaloid, lasiocarpine. *Cancer Lett* 231: 138-145.

46 Xia, Q., Yan, J., Chou, M.W., Fu, P.P. (2008). Formation of DHP-derived DNA adducts from metabolic activation of the prototype heliotridine-type pyrrolizidine alkaloid, heliotrine. *Toxicol Lett* 178: 77-82.

47 NTP. (1978). Bioassay of Lasiocarpine for possible carcinogenicity. *NTP Technical Report* 39, 1-66.

48 Kuhara, K., Takanashi, H., Hirono, I., Furuya, T., Asada, Y. (1980). CArcinogenic activity of clivorine, a pyrrolizidine alkaloid isolated from Ligularia dentata. *Cancer Lett* 10: 117-122.

49 Hirono, I., Mori, H., Yamada, K., Hirata, Y., Haga, M. (1977). Carcinogenic activity of petasitenine, a new pyrrolizidine alkaloid isolated from Petasites japonicus Maxim. *J Natl Cancer Inst* 58: 1155-1157.

50 Hirono, I., Haga, M., Fujii, M., Matsuura, S., Matsubara, N., Nakayama, M., Furuya, T., Hikichi, M., Takanashi, H., Uchida, E., Hosaka, S., Ueno, I. (1979). Induction of Hepatic Tumors in Rats by Senkirkine and Symphytine. *JNCI* 63, 469-472.

51 Schoental, R., Fowler, M. E., Coady, A. (1970). Islet Cell Tumours of the Pancreas found in Rats given Pyrrolizidine Alkaloids from Amsinckia intermedia Fisch and Mey and from Heliotropium supinum L. *Cancer Research* 30, 2127-2131.

52 Defra. (2004). Code of Practice on How to Prevent the Spread of Ragwort. *Defra* .

53 Dickinson, J. O., Cooke, M. P., King, R. R., Mohamed, P. A. (1976). Milk Tranfer of Pyrrolizidine Alkaloids in Cattle. *JAVMA* 169, 1192-1196.

54 Goeger, D. E., Cheeke, P. R., Schmitz, J. A., Buhler, D. R. (1982). Effect of feeding milk from goats fed tansy ragwort (Senecio jacobaea) to rats and calves. *Am.J.Vet.Res.* 43, 1631-1633.

55 MAFF. (1994). Naturally occurring Toxicants in Food. *MAFF Food Surveillance Paper* 42, 18-29.

56 Eröksüz, H., Eröksüz, Y., Özer, H., Yaman, I., Tosun, F., Akyüz Kizilay, Ç., Tamer, U. (2003). Toxicity of Senecio vernalis to Laying Hens and Evaluation of Residues in Eggs. *Vet Hum Toxicol* 45, 76-80.

57 Edgar, J. A. and Smith, L. W. (2000). Transfer of Pyrrolizidine Alkaloids into Eggs: Food Safety Implications. Tu, A. T. and Gaffield, W. Natural and Selected Synthetic Toxins. Biological Implications. [8], 118-128. Washington D.C., Americal Chemical Society. ACS Symposium series 745.

58 MAFF. (1995). Surveillance for pyrrolizidine alkaloids in honey. *MAFF UK Food Surveillance Information Sheets* 52.

59 Edgar, J. A., Roeder, E., Molyneux, R. J. (2002). Honey from Plants Containing Pyrrolizidine Alkaloids: A Potential Threat to Health. *Journal of Agricultural and Food Chemistry* 50, 2719-2730.

60 FSANZ. (2004). Consumers advised to limit consumption of Paterson's Curse/Salvation Jane honey. *FSANZ*.

61 VWA. (5-11-2007). Advice on Pyrrolizidine Alkaloids in Honey. *VWA: Dutch Food and Consumer Product Safety Authority*.

62 LGC. (2007). Collection and Analysis of Honey Samples Potentially Contaminated with Pyrrolizidine Alkaloids from Ragwort and Borage. *Food Standards Agency Project* T01037.

63 Huxtable, R. J. (1989). Human health implications of pyrrolizidine alkaloids and herbs containing them. Toxicants of Plant Origin. Volume 1. [3], 41-86.

64 RIVM. (2005). Advisory report on pyrrolizidine alkaloids in herb preparations.

65 COT, C. &. C. (2008). Annual Report 2007.

66 EFSA. (2005). Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic. *The EFSA Journal* 282, 1-31.

67 EFSA. (2008). Opinion of the Scientific Panel on Plant Protection products and their Residues to evaluate the suitability of existing methodologies and, if appropriate, the identification of new approaches to assess cumulative and synergistic risks from pesticides to human health with a view to set MRLs for those pesticides in the frame of Regulation (EC) 396/2005. *The EFSA Journal* 704, 1-84.

68 Betteridge, K., Cao, Y., Colegate, S.M. (2005). Improved method for extraction and LC-MS analysis of pyrrolizidine alkaloids and their N-oxides in honey: application to Echium vulgare honeys. *J Agric Food Chem* 53: 1894-1902.

Statement on the review of the 1998 COT recommendations on peanut avoidance

1 The COT was asked by the Food Standards Agency (FSA) to evaluate the results of a literature review that the Agency had commissioned following a research call issued in June 2007. The aim of this review was to assess the scientific evidence available since 1998 concerning avoidance versus exposure to peanut during early life and possible influences on the development of sensitisation and clinical allergy to foods, with particular reference to peanut. In the light of this literature review, and taking account of any other relevant information, the COT was asked to consider whether the recommendations to pregnant mothers that were issued in the 1998 COT report on Peanut Allergy[1] should now be amended or remain in place.

2. The literature review had been conducted by the British Nutrition Foundation (BNF), with assistance, where necessary, on specific aspects of the evidence, from other scientific experts.

3. The COT was provided with the final technical report of the review of published literature[2], as well as summary information about unpublished and ongoing research. This information was supplemented, at the request of the COT, by several key review articles together with summary details and abstracts of relevant studies published since these reviews, on the evidence concerning early life dietary exposures and atopic diseases other than food allergy, such as allergic asthma and atopic eczema. This additional information was requested by the COT to enable the Committee to take into account any relevant information from the wider body of evidence relating to early life exposures and the development of allergic disease beyond food allergy specifically, that might inform the present review. This supplemental information was collated by the FSA.

4. The Committee was grateful for the advice of a number of external medical and scientific experts, which informed its discussion of the literature review conducted by the BNF and of the wider evidence base. These were: Prof. Stephen Holgate, MRC Clinical Professor of Immunopharmacology from the University of Southampton; Dr Andrew Clark, Consultant in Paediatric Allergy from Addenbrookes Hospital, University of Cambridge; Prof. Graham Devereux, Clinical Senior Lecturer at the University of Aberdeen and Respiratory Physician at Aberdeen Royal Infirmary; and Prof. Ian Kimber, Professor of Toxicology at the University of Manchester and Programme Advisor to the FSA Food Allergy & Intolerance Research Programme (T07).

Background

Peanut allergy

5. Allergy to peanuts is a serious health problem among UK children, with estimates of prevalence suggesting that between 0.2 and 1.8% of children may be affected (see Table 2 on page 143). Allergic reactions to peanut in both children and adults can be severe and can involve life-threatening symptoms (anaphylaxis). Fatal food allergic reactions are rare and are more common in teenagers and adults than in children, but peanuts are reported to be the commonest cause of such fatalities across all age groups[3]. Sensitivity varies significantly between individuals and the most sensitive can react to very small (mg or, in very rare cases, µg[4]) amounts of peanut protein.

6. Unlike certain other food allergies such as egg and milk allergy, which are common in young childhood but which tend to be outgrown, peanut allergy commonly persists throughout life[5]. In common with allergy to other foods, there are currently no established primary preventive strategies for peanut allergy. Moreover, the only means of managing the condition once it has developed is complete avoidance of peanut coupled with use of rescue medication (antihistamines and adrenaline) to treat the symptoms of a reaction once it has happened. Avoidance is both difficult to achieve in practice[6], and also has socioeconomic and quality-of-life consequences for affected individuals and their families[7].

7. Allergic reactions to foods can occur through several mechanisms but, in the great majority of instances, peanut sensitisation and peanut allergy are mediated via IgE antibody. In common with other forms of IgE mediated allergy, IgE mediated peanut allergy develops in two phases. In the first phase, sensitisation is acquired by a susceptible individual following exposure to peanut. Sensitisation is characterised by specific immunological priming involving the induction of a special type of immunoglobulin, IgE that is in this case specific for peanut allergen. This IgE is distributed systemically throughout the vascularised tissue and binds to mast cells. If the now-sensitised individual is exposed to sufficient quantities of the same allergen, the IgE on the surface of mast cells can be cross-linked and degranulation may be triggered, which will cause the release of various inflammatory mediators, leading to the clinical manifestations (symptoms) of allergy. Once this second stage has been reached then an individual is said to have clinical (symptomatic) allergy. In the case of peanut allergy, the inflammatory response may occur within a few seconds or minutes after contact with the food, leading to serious systemic responses that include anaphylactic shock, asthma, hives (urticaria and angioedema) and gastrointestinal reactions such as vomiting and diarrhoea. Usually reactions follow dietary consumption of peanut, but they can also result from inhalation or skin contact with the peanut or peanut products. As defined here, the key difference between sensitisation and allergy to foods is that sensitisation, which is a priming of the immune response, is not necessarily associated with symptoms when the food is consumed. Sensitisation is defined operationally by the presence of allergen-specific IgE antibody directed to particular parts of an allergen molecule (usually a protein or glycoprotein) and/or positive skin prick tests (SPT) when a very small dose of allergen is introduced through the skin. Allergy is diagnosed using a combination of patient history, the presence of allergen-specific IgE and/or skin prick test results, and, where there is any doubt, a controlled food challenge.

8. The factors that determine susceptibility to food allergy and control its development are of considerable scientific and clinical interest, and the subject of current research. A major predisposing factor in the acquisition of food allergy is inheritance of an atopic phenotype – characterised by the ability to mount vigorous IgE antibody responses to allergens commonly encountered in the environment, in food, the workplace, certain drugs and insect stings. There are also a number of other factors that have important influences on the development of food allergy and determine which foods lead to allergy in a particular individual. Of special importance are the route, timing, duration and extent of exposure.

9. Early life environmental and dietary experiences are of particular relevance in determining whether allergy will develop, or whether the subject will develop protective tolerance. In the latter case the individual will either fail to induce the class of immune response required for effective sensitisation or will develop other immunological mechanisms that serve to prevent or neutralise IgE-dependant allergic responses and, as a result, will be able to consume the particular foodstuff without triggering the allergic cascade. The possibility that sensitisation (priming) to peanuts might be acquired from exposure via the mother during pregnancy through the placenta or during lactation, is of particular relevance to peanut allergy since in many cases, peanut allergy becomes apparent in children when they display reactions following what is believed to be their first known dietary exposure to peanut products. This possible sequence of events was one of the important pieces of evidence considered by the COT Working Party on Peanut Allergy in 1998[8,9], and was influential in the development of the precautionary recommendations made by the COT at that time.

Previous COT recommendations

10. In 1996, as a result of the particular severity of allergic reactions to peanut and the possibility that the prevalence of this allergy was increasing especially in children, the COT convened a Working Group. The aim of this Working Group was to review the scientific evidence on peanut allergy and to advise on whether there was an association between early exposure to peanuts or peanut products and the occurrence of peanut allergy in later life. Two of the four scientific experts who assisted the COT with the present review, were also members of that previous COT Working Group: Prof Stephen Holgate and Prof Ian Kimber.

11. At the time the COT Working Group was established few published studies were available on this subject. However, what literature there was suggested that some food allergens contained in egg and milk could be transmitted intact to infants via breastmilk[10,11], possibly leading to sensitisation (priming) of the infant[12]. There was also evidence from immunological studies of human umbilical cord blood mononuclear cells (CBMC) taken at birth, showing proliferative and cytokine responses following culture *in vitro* with certain food allergens. This evidence was considered suggestive of *in utero* sensitisation and there were indications from the literature that such responses might be associated with the subsequent development of allergic disease[13]. Published reports available at that time describing children reacting on their first known dietary exposure to peanut[8,9] were thus interpreted as suggesting peanut allergen transfer *in utero* or during lactation as a route of sensitisation.

12. In considering the available published evidence on intrauterine immunological sensitisation and on other possible routes of exposure to allergens, the COT concluded in 1998 that there was some support for the suggestion that peanut allergy in infants could result from exposure *in utero* or during lactation, but that the available data were inconclusive. However, with regard to the mechanism of sensitisation and allergy, a link between peanut consumption by pregnant and lactating women and the occurrence of peanut allergy in the child was considered possible. On this basis, the COT considered that it would be unwise to discount the possibility of sensitisation of offspring resulting from exposure of the mother, and issued a number of precautionary dietary recommendations.

13. The dietary recommendations from the 1998 COT report on peanut allergy[1] were that:

" *(i) pregnant women who are atopic, or for whom the father or any sibling of the unborn child has an atopic disease, may wish to avoid eating peanuts and peanut products during pregnancy;*

(ii) breast-feeding mothers who are atopic, or those for whom the father or any sibling of the baby has an atopic disease, may wish to avoid eating peanuts and peanut products during lactation;

(iii) a) in common with the advice given for all children, infants with a parent or sibling with an atopic disease should, if possible, be breast-fed exclusively for four to six months;

b) during weaning of these infants, and until they are at least three years of age, peanuts and peanut products should be avoided;

(iv) infants or children who are allergic to peanuts should not consume peanuts or peanut products. "

14. The report also recommended that parents or those charged with the care of peanut allergic children or infants should:

" *(i) be vigilant in reading labels on all multi-ingredient foods and should avoid any for which doubt exists about the ingredients;*

(ii) be aware that even minute amounts of peanut allergens may result in severe reactions. They should therefore be alert to the possibility of accidental exposure and should ensure that cross-contamination of foodstuffs with peanut allergens does not occur;

(iii) be aware of the treatment for anaphylaxis should inadvertent exposure occur at, for example, school, or the homes of other children. "

Developments since 1998

15. The previous COT recommendations formed the basis of Government advice that has now been in place for 10 years. Since 1998, several studies (including some funded by the FSA), have been published on the subject of sensitisation and allergy to foods in relation to early life dietary (and to a lesser extent non-dietary) exposures which add to the evidence base that underpinned the 1998 COT recommendations. One significant development during the intervening period has been recognition that, in principle at least, sensitisation to peanut proteins could be acquired via routes of exposure other than dietary intake, notably through skin contact. Although the importance of skin exposure in driving sensitisation to peanuts (and possibly other food stuffs) is suspected, it has not been established with certainty. If it were confirmed, however, the implication would be that sensitisation in early life might not require passage of peanut allergen via the placenta or breastmilk. Since the COT recommendations were made, there have also been several studies published on the frequency of sensitisation and allergy to peanuts, which could inform understanding of whether the prevalence of this allergy is increasing. It was therefore considered timely to re-assess the current state of scientific knowledge in this area and, based on the evidence now available, to re-consider whether the 1998 COT dietary recommendations remain appropriate.

16. The COT was also informed that a recent House Of Lords Science and Technology Select Committee inquiry on allergy, published in September 2007 [14], included a recommendation to the UK Government, based on submitted evidence, to revoke the current advice regarding peanut consumption by pregnant women and infants. The Government response to this inquiry was that it was important to review carefully all the relevant evidence that related to the acquisition of peanut allergy in order to reassess whether or not the advice should be rescinded or revised. The response also noted that a literature review commissioned by the Food Standards Agency was already being undertaken.

Literature review of studies published since 1998

17. A literature review, carried out by the British Nutrition Foundation (BNF), sought to identify and review studies published since 1998 on the early life patterns of exposure to, and avoidance of, food allergens and the later development of sensitisation and clinical food allergy, with particular reference to peanut. To address this overarching aim, the researchers structured the literature review from three different types of evidence: 1) studies conducted in humans; 2) studies conducted in animals; and 3) *in vitro* immunological studies conducted on human umbilical cord blood. The reviewers specified several research questions against which to identify studies of relevance, as detailed in Table 1.

Methodology

18. The following is a summary of the methodology used for the three different elements of the literature review (human, animal and human umbilical cord blood studies). Full details of the methodologies, including lists of search terms and data extraction forms, can be found in the final technical report of the project [2].

19. The review of human studies was conducted according to standard systematic review procedures and searched MEDLINE, EMBASE, the Cochrane Library and CAB Abstract databases to identify relevant papers published between 1st January 1999 and 7th March 2008 inclusive. Inclusion and exclusion criteria were drawn up for each research question and used to develop appropriate search term lists. In general terms, inclusion criteria were based on including all study designs except case reports and therapeutic or treatment studies; exposures of interest included exposure to or avoidance of any of the 14 allergenic foods listed in current EU legislation[15] and also kiwi fruit; and outcome measures included allergic sensitisation to foods and clinical food allergy including that which is self-reported. Studies which reported only other allergy endpoints such as asthma, atopic eczema, rhinitis, atopic wheeze and other respiratory outcomes were excluded. Identified publications were assessed against the relevant inclusion/exclusion criteria and those of potential relevance were assessed independently by two reviewers to determine whether they should be included. Data extraction was performed in duplicate using modified versions of the extraction forms produced by the Scottish Intercollegiate Guidelines Network (SIGN) [16].

20. Twenty four separate studies were identified by the review of the human studies, including 9 clinical trials, 9 cohort studies, 4 case-control studies, 2 cross-sectional studies of associations between dietary exposure measures and allergy outcomes, and 2 prevalence studies (some investigations used more than one design).

Table 1

List of the research questions around which the BNF literature review was structured

Nature of evidence to be reviewed	Research questions to be addressed
Evidence from studies in humans	**Research question 1:** Does maternal dietary consumption of food allergens – or avoidance of dietary consumption of food allergens – during pregnancy/lactation have any impact on the subsequent development of sensitisation, or allergy to foods by the child?
	Research question 2: Does dietary consumption of food allergens – or avoidance of dietary consumption of food allergens in childhood – have any impact on the subsequent development of sensitisation or allergy to foods?
	Research question 3: Does non-dietary exposure to peanut in childhood, for instance via skin or the respiratory tract, have any impact on the subsequent development of sensitisation or allergy to peanuts?
	Research question 4: Has the current UK Government guidance on dietary consumption of peanuts and peanut products had any impact on sensitisation and allergy rates to peanuts in the UK?
Evidence from studies in animals	**Research question 5:** Does maternal dietary/oral exposure to allergen (peanut or ovalbumin) – or avoidance of dietary consumption of allergen – during pregnancy/lactation have any impact on the subsequent acquisition by offspring of sensitisation (IgE antibody), or allergy (other signs) to the same protein?
	Research question 6: Does dietary/oral exposure to allergen (peanut or ovalbumin) – or absence of dietary/oral exposure to allergen – have any impact on the subsequent development of sensitisation (IgE antibody) or allergy (other signs) to the same protein?
	Research question 7: Does non-oral/dietary exposure to allergen (peanut or ovalbumin), for instance via skin or the respiratory tract, have any impact on the subsequent development of sensitisation or allergy to the same protein?
Evidence from studies utilising human cord blood	**Research question 8:** Does intrauterine immunological sensitisation occur and is it associated with subsequent atopic disease?

21. The review of animal studies was conducted as two separate expert literature reviews. One review evaluated published experimental studies in animals which had examined the influence of maternal and/or early life dietary and dermal exposures to peanut on the development of sensitisation, symptoms of allergy or other immunologically relevant endpoints. The second identified similar studies that had employed ovalbumin (hens' egg allergen). In both cases the literature was searched systematically, followed by expert review of the relevant scientific evidence. Both reviews were carried out by Dr Rebecca Dearman, an expert in immunotoxicology and currently a Member of the COT. Search terms were drawn up and conducted in Pub Med to identify relevant articles published since 1980. This earlier starting date was chosen in order to encompass the entire body of relevant evidence from published animal studies, since such studies had not previously been reviewed by the COT. In fact, only 2 studies published before 1998 were identified as being of relevance to the review; the remainder being published from 1998 onwards. The searches resulted in a total of 30 papers being included in the review of studies on peanut, and 25 for ovalbumin*.

22. The review of studies on human umbilical cord blood was conducted as an expert review of research published since the 1998 COT report on human CBMC responses and sensitisation and allergy, which was set in the context of the state of scientific knowledge in this area in 1998 when the COT report was published. This review was written by Prof. Graham Devereux, an expert in the early life origins of allergic disease*.

Findings of the literature reviews

Evidence from studies conducted in humans

Maternal dietary intake

23. The BNF review identified seven studies meeting the inclusion criteria that had investigated an association between maternal dietary intake of allergenic foods during pregnancy or lactation and the development of sensitisation or clinical allergy to foods in the child. These comprised one non-randomised clustered trial[17], two cohort studies[18,19], and four case-control studies[20-23]. According to the SIGN criteria, these studies, which involved a heterogeneous range of dietary exposures, were not considered to be of high quality. In non-atopic women but not in atopic women, one of the case-control studies (Calvani *et al.*, 2006[20]) did report a statistically significant decreased risk of sensitisation to fish with increased maternal consumption of fish during pregnancy (consumption once a week versus once a month or less (OR 0.22 (95% CI 0.08-0.55), consumption 2-3 times a week or more than once a month versus once a month or less OR 0.23 (95% CI 0.08-0.69)). However, whilst the results had been adjusted for various possible confounding factors, this did not include those associated with breastfeeding; and the retrospective assessment of maternal diet, up to 18 years previously, could have led to significant recall bias. The non-randomised trial[17] found a higher rate of sensitisation to peanut (when assessed by positive SPT but not by peanut-specific IgE) and soy (when assessed by IgE but not by SPT), in mothers in the comparison group who were not avoiding allergenic foods when compared with the mothers in the intervention group who were avoiding eggs, cows' milk and fish for 3 months after delivery (soy OR 6.01 (95% CI 1.09-43.33), peanut OR 10.67 (95% CI 1.24-239.1)). However, the mothers in the intervention group were not instructed to avoid peanut or soy, which complicates the interpretation of these findings. Of the two case-control studies, one compared maternal consumption of peanut (during pregnancy and lactation) in a group of children who were sensitised to peanut, with that in a control group of children who were sensitised to egg or milk (but not peanut)[21]. The other study compared maternal consumption of peanut (during pregnancy and lactation) in a group of peanut allergic children, a control group of atopic (but non-peanut allergic) children, and a further control group of non-atopic children[22]. In the former study (Frank *et al.*, 1999) unadjusted odds ratios of between 2 and 4 were reported, but these were not found to be statistically significant (consumption of peanut by mothers during pregnancy more than once a week compared with less than once a week: unadjusted OR (peanut sensitisation as measured by positive peanut-specific IgE) 3.97 (95% CI 0.73-24.0); consumption of peanut by mothers during lactation more than once a week compared with less than once a week: unadjusted OR (peanut sensitisation as measured by positive peanut-specific IgE) 2.19 (95% CI 0.39 – 13.47))**. In the latter study (Lack *et al.*, 2003), no statistically significant associations between dietary consumption and peanut allergy in the child were found after adjustment for potential confounding factors.

* These expert reviews, including the list of references that they encompassed, can be found in the final technical report of FSA funded research project T07052 (http://www.foodbase.gov.uk)

** adjusted odds ratios were not reported

24. One other report included in the literature review, of a study aimed primarily at assessing prevalence of peanut allergy in young children, made reference to maternal peanut consumption and peanut sensitisation (defined as a positive SPT to peanut), in infants[6]. This study found no association between reduction in maternal consumption, or avoidance, of peanuts in response to the 1998 Government advice and whether or not their children were sensitised to peanuts, although it is unclear from the published report whether this statement related to intake during pregnancy, lactation or both.

Infant dietary intake

25. The BNF review identified nineteen studies meeting the inclusion criteria that had investigated dietary consumption or avoidance of allergenic foods in early childhood, and the association with subsequent development of sensitisation or allergy to foods. These included eight randomised controlled trials[24-31], ten cohort studies[19,22,32-39] and one case-control study[21]. Again, there were heterogeneous exposures and many of the studies did not adjust results for potential confounding factors. Most of the studies identified had not been designed specifically to look at consumption of peanut, or other allergenic foods, and food allergic outcomes, but instead focussed primarily on the impact of breastfeeding versus formula feeding, duration of breastfeeding, and/or timing of introduction of solids/specific foods, on a range of health outcomes including food allergy/sensitisation.

26. One general population study reported a statistically significant decreased risk of parental report of doctor-diagnosed food allergy when comparing cows' milk formula with breastfeeding, but the results were not adjusted for paternal history of atopy, breastfeeding was poorly assessed, and food allergy diagnosis was not confirmed by any objective testing[32]. Eight of the studies examined duration of breastfeeding and none found a statistically significant association between breastfeeding beyond 6 months and lower food allergy risk. In fact, results of the studies identified in this area, where statistically significant, tended to be in the opposite direction, i.e. increased duration of breastfeeding beyond six months was associated with increased rate of food allergy. Two studies[6,36] reported that breastfeeding compared with never breastfeeding was associated with an increased risk of food/peanut allergy in general populations, but these analyses did not adjust for possible confounding factors. One study[37] reported that children with a family history of allergy had an increased risk of food allergy, at both 5 and 11 years of age, with duration of exclusive breastfeeding of 9 months or longer compared with less than 9 months, (OR 5.3 (95% CI 1.2-24.1) for parental report of food allergy at 5 years of age, OR 7.9 (95% CI 1.4-50.0) for parental report of food allergy at 11 years of age). No such association was found for children without a family history of allergy. A study by Lack *et al.*, (2003)[22] reported an association between breastfeeding beyond 6 months and peanut allergy (based on positive peanut challenge), although this was not statistically significant after adjustment for potential confounding factors (maternal atopy, maternal diet during pregnancy, infant diet).

27. Of those studies that examined the timing of introduction of solids or specific foods into the weaning diet, one, conducted in a high risk population, reported a statistically significant increased risk of wheat allergy associated with a delayed introduction (beyond 7 months of age compared with before 6 months of age) of cereals into the diet[38] (OR 3.8 (95% CI 1.18-12.28) for parental report of wheat allergy at 4 years of age). Another study, conducted in an unselected general population, reported a

statistically significant increased risk of parental reports of doctor-diagnosed food allergy in children who had milk or egg introduced later then 6 months of age, but in adjusted analyses there was no statistically significant association between introduction of any foods later than 6 months of age compared with before 5 months and food sensitisation (OR 0.83 (95% CI 0.49-1.41) for parental reports of doctor-diagnosed food allergy at 2 years of age)[39]. A further general population-based study[35] reported a statistically significant association between consumption of fish during the first year of life and decreased risk of food sensitisation after adjustment for other factors (consumption more than once a week versus never OR 0.47 (95% CI 0.33-0.69)). Clinical food allergy was not assessed in this study. One case-control study (Frank *et al*., 1999)[21] reported earlier introduction of peanuts/peanut butter in children who went on to become peanut sensitised (mean age of introduction 12.5 (SD 6.4) versus 17.3 months (SD 5.5), p = 0.03), but the study did not account for inadvertent exposure to peanut in the child's diet provided by caregivers outside the immediate household, or exposure outside the home.

28. Three of the trials identified investigated multi-faceted interventions[29-31], combining dietary avoidance measures (such as avoidance of specified allergenic foods during pregnancy and/or in infant diet) with non-dietary avoidance measures (e.g. house dust mite avoidance, avoidance of environmental tobacco smoke, no pets) in high risk populations. These studies reported some statistically significant associations between interventions and food sensitisation and/or food allergy outcomes. However, the multi-faceted nature of the interventions means it is not possible to attribute the observed associations to the dietary part of the intervention, or to any specific food within the dietary intervention.

Non-dietary maternal/infant exposure

29. The BNF review identified only two studies meeting the inclusion criteria that had investigated the possible influence of non-dietary exposure to peanut in childhood, for instance via the skin or respiratory tract, on subsequent development of sensitisation or allergy to foods. One was a cohort study40 and the other was a case-control analysis[22].

30. The case-control study by Lack *et al.* (2003)[22], found a statistically significant increased prevalence of exposure to topical skin preparations containing peanut oil, in peanut allergic children (cases, n=23) compared with atopic (n=70) and non-allergic (n=140) control children. This result was still significant after adjustment for potential confounders (use versus no use of creams containing peanut oil and positive peanut challenge OR 8.34 (95% CI 1.05-66.1)), but the risk estimate may have been inflated by maternal recall bias due to the retrospective study design. No increased risk of peanut allergy in children was found for maternal use of such skin creams. The mean number of peanut oil preparations to which infants were exposed was also significantly higher for peanut allergic children (1.91) compared with atopic (0.93) and non-atopic controls (0.81) ($p<0.001$). The other study, in which peanut oil was used to deliver a vitamin supplement, conversely reported an increased risk of parental reported food hypersensitivity (cows' milk, egg, fish, soy, peanut and wheat allergy combined) for vitamins in water compared with vitamins in peanut oil in the first year of life (OR 1.87 (95% CI 1.32-1.65))[40].

Impact of the COT recommendations on dietary consumption of peanut and on sensitisation and allergy rates to peanut

31. The BNF review identified two general population studies that had specifically investigated the impact of the 1998 COT recommendations on maternal consumption/avoidance behaviour during pregnancy and that had measured rates of sensitisation and allergy to peanut in children[6,41]. These were prospective cohort studies (one carried out in Southampton and Manchester, and the other on the Isle of Wight) which assessed dietary consumption during pregnancy and breastfeeding, and subsequent rates of sensitisation and clinical allergy to peanut in children under 5 years old born after the COT recommendations were issued. In addition, the BNF review identified a number of other published studies reporting the prevalence of peanut sensitisation and/or peanut allergy in children of different ages, some born before the 1998 COT recommendations and some born after. Many of these additional studies were based on cohorts of children recruited from the Isle of Wight, but they also included cohorts from elsewhere in the UK as well as some cross-sectional surveys conducted in the US and in Canada.

32. The two cohort studies conducted on mothers and their children born after the COT recommendations were issued, reported levels of recall of the COT recommendations at 40 – 60% (42% of mothers recalled the recommendations in Dean *et al.*, 2007[41], 61% in Hourihane *et al.*, 2007[6]). However, the percentage of mothers changing their diet as a result of the recommendations was lower. In the study by Dean *et al.*, 21% of mothers changed their diet during pregnancy (reduced or avoided peanut consumption), as a consequence of the recommendations, and Hourihane *et al.* reported that 37% of mothers who recalled the recommendations changed their diet, although only 10% of the latter reported eliminating peanuts totally during pregnancy (i.e. only 3.8% of the whole group of mothers). Further, both studies reported no indication that the target group of women whose children had a family history of allergy were more likely to take up the COT recommendations than the general population of mothers. Hourihane *et al.* also examined the mean age of introduction of peanuts into the diet of children and reported this as being 32 months of age for peanut-sensitised children and 29 months for those not sensitised. There was no significant difference between these values ($p = 0.42$). The BNF review noted that an earlier study by Frank *et al.* [21], which had been conducted before the 1998 COT recommendations were issued, but published in 1999, reported an earlier age of introduction of peanuts in those who became sensitised (mean 12.5 months, SD 6.4) compared with the more recent study conducted by Hourihane *et al.* (2007).

33. The BNF literature review compared the available data from studies that were identified on the prevalence of sensitisation and/or allergy to peanuts in UK children born before and after the 1998 COT recommendations were issued. A summary of these data is presented in Table 2.

34. Data from studies conducted in the US and Canada are comparable with those presented in Table 2. Bock (1987)[49] reported the prevalence of peanut allergy confirmed by double blind placebo controlled food challenge (DBPCFC) at 3 years of age as 0.6% (3/480), and data collected from a random dial telephone survey, in which no objective testing was performed, indicated a peanut allergy prevalence of 0.4% in 1997 and 0.8% in 2002 for children under 18 years of age[50]. In Canada peanut allergy prevalence was estimated at 1.5% in 1999-2000 in children of average age 7 years[51].

Table 2. Summary details of data on prevalence of sensitisation and clinical allergy to peanuts from published UK studies identified by the BNF literature review

Study	Region	Year of birth of study population	Age of study population (yrs)	Diagnostic method(s) used (s= sensitisation, a = allergy)	Prevalence of peanut sensitisation (%)	Prevalence of peanut allergy (%)
Children born before 1997						
Pereira *et al.* 2005[42]	Isle of Wight	1987-1988	15	SPT	2.6% (17/649)	nd
Emmett *et al.* 1999[43]	Great Britain	1981-1985	10-14	Interviews	nd	0.9% males (9/989) 0.6% females (6/952)
Pereira *et al.* 2005[42]	Isle of Wight	1991-1992	11	SPT	3.7% (26/699)	nd
Emmett *et al.* 1999[43]	Great Britain	1986-1990	5-9	Interviews	nd	0.8% males (7/909) 0.6% females (5/909)
Tariq *et al.* 1996[44]	Isle of Wight	1989-1990	4-5	(s) SPT (a) SPT + clinical history	1.1% (13/1218)	0.5% (6/1218)
Grundy *et al.* 2002[45]	Isle of Wight	1994-1996	3-4	(s) SPT for sensitisation (a) SPT and OFC or known peanut allergy	3.3% (41/1246)	1.5% (18/1246)
Lack *et al.* 2003[22]	Avon	1991-1992	2-3	DBPCFC	nd	0.2%
Emmett *et al.* 1999[43]	Great Britain	1991-1995	0-4	Interviews	nd	0.5% males(5/1063) 0.3% females (3/882)
Children born 1997-1998						
Venter *et al.* 2006[46]	Isle of Wight	1997-1998	6	(s) SPT (a) SPT, OFC and known peanut allergy.	2.6% (18/700)	0.6% (5/798)
Children born after 1998						
Hourihane *et al.* 2007[6]	Southampton & Manchester	1999-2000	4-5	(s) SPT and IgE (a) SPT, DBPCFC and symptoms	2.8% (30/1072)	1.8% (20/1072)
Dean *et al.* 2007[41]	Isle of Wight	2001-2002	3	SPT	1.3% (7/543)	nd
Venter *et al.* 2008[47]	Isle of Wight	2001-2002	3	(s) SPT (a) SPT, OFC	2.0 (13/642)	1.2%* (11/891)
Dean *et al.* 2007[41]	Isle of Wight	2001-2002	2	SPT	2.0% (13/658)	nd
Venter *et al.* 2006[48]	Isle of Wight	2001-2002	1	SPT	0.4% (3/763)	nd

SPT = Skin Prick Test. DBPCFC = Double Blind Placebo Controlled Food Challenge. OFC = Open food challenge. IgE= Immunoglobulin E
nd = not determined/not reported

* prevalence calculated based on using the total cohort size as the denominator. (As a result of new information provided by the researchers to the FSA, this figure has been adjusted from the figure of 1.7% given in the technical report of the literature review conducted by the BNF, which was calculated using the number of children who had a SPT as the denominator).

Evidence from studies conducted in animals

Maternal intake

35. The expert reviews of studies conducted in experimental animals did not identify any relevant studies on maternal dietary intake of peanut allergen during pregnancy or lactation and sensitisation outcomes in the offspring. In relation to ovalbumin, two studies were identified that demonstrated suppression of specific IgE responses following intraperitoneal/subcutaneous immunisation with ovalbumin in the offspring of rats fed high oral doses of ovalbumin (200mg/day via the drinking water[52] or 21.5% of the animal's diet[53] respectively), during pregnancy or lactation. A more recent study was identified as demonstrating that intranasal or intragastric exposure of lactating mice to ovalbumin also resulted in reduced IgE antibody and reduced respiratory hypersensitivity responses in offspring on subsequent inhalation challenge[54].

Direct oral exposure during early life

36. The literature review identified a larger number of studies in animals that had investigated the direct impact (i.e. not via the mother) of oral exposure to peanut/ovalbumin on subsequent development of sensitisation. In relation to oral exposure to peanut, the review identified studies conducted in mice demonstrating that relatively low doses (0.02 to 0.2 mg) of peanut extract administered orally (either by gavage or in the diet), subsequently enhanced peanut-specific IgE responses to subcutaneous immunisation with peanut extract and adjuvant[55,56], whilst higher doses (100 mg) of peanut extract resulted in oral tolerance and inhibition of specific IgE responses following subcutaneous challenge[55]. In relation to ovalbumin, the review identified studies, conducted in mice and in dogs, that also found that low doses of oral exposure resulted in sensitisation responses, whereas high doses resulted in inhibition of sensitisation responses and reduced symptoms in response to subsequent challenge with the same allergen. The response pattern for ovalbumin was different from that for peanut, in that considerably lower doses (20 mg and above) of ovalbumin were found to result in tolerance[55,57]. In the case of ovalbumin it was also observed that mice of different strains exhibited varying degrees of tolerance to high doses of the allergen given orally[58].

37. Further studies were identified that confirmed that under some circumstances, oral exposure to either peanut extract or ovalbumin can lead to sensitisation rather than tolerance. For example in Brown Norway rats, repeated low dose oral exposure to ovalbumin induced anti-ovalbumin IgE responses in 50% of animals[59]. In relation to peanut allergen, repeated low dose oral exposure to peanut protein in BALB/c strain mice in the absence of adjuvant led to production of specific IgE over a 42 day period[60]. Finally, there were many studies demonstrating that oral (intragastric) administration of peanut extracts with adjuvant to various strains of mice induced the production of specific IgE antibody[61,62]. However, it was noted that the main aim of these latter studies was to induce vigorous IgE responses for further study, and not to replicate conditions of sensitisation in a human population. The nature of their experimental design, which included use of adjuvants to boost the immune response, complicates their extrapolation to oral exposure of the human population in which such adjuvants would not be present.

Non-dietary exposure

38. The literature review identified a number of studies which indicated that topical (dermal) exposure to protein allergens, including peanut and ovalbumin, may induce IgE-mediated immune responses. For example, topical exposure to low doses of peanut extract (0.1mg) through depilated intact skin of BALB/c strain mice augmented subsequent specific IgE responses to oral challenge with peanut and adjuvant[61]. Similarly for ovalbumin, mice exposed to 0.01 to 0.1 mg of ovalbumin via an occlusive patch on shaved skin, developed more vigorous specific IgE responses than did mice immunised by intraperitoneal injection of the same antigen in the presence of adjuvant[63]. Other investigators have confirmed these findings using similar exposure regimens (i.e. administration of antigen via an occlusive patch on shaved skin)[64-67]. Nedle *et al.* (2001) also demonstrated anaphylactic reactions upon oral challenge with ovalbumin following topical exposure to the same allergen[67]. Furthermore, studies with peanut extract have revealed that prior exposure to peanut through abraded skin prevented the tolerogenic effects of high dose oral exposure in BALB/c strain mice[56]. The subcutaneous route of exposure has also been shown to be effective for sensitisation of dogs to peanut extract[68,69], although the immunisation protocol used in these studies was vigorous, involving repeat injections in the presence of adjuvant (to boost the immune response) over a period of some months. The direct relevance of these data (deriving from experiments in which adjuvant has been incorporated) to humans is unclear.

Evidence from studies of human cord blood

39. The expert review of studies on human cord blood identified 16 relevant publications between 1999 and 2007. The review discussed the results and significance of these studies in relation to three key areas of continued scientific uncertainty since 1998:

- whether the fetus is exposed to maternally derived allergen
- whether allergen responsive cord blood mononuclear cells (CBMC) have been exposed to allergen *in utero*
- whether allergen responsive cord blood mononuclear cells have been primed by allergen *in utero*

40. The literature review identified several studies that collectively demonstrate that the fetus is exposed to tiny amounts of ubiquitous nutrient allergens derived from the mother's diet. However, the placental transfer of aeroallergens appears to be less efficient and much less frequent[70-73]. Few data were available on the association between maternal consumption of food allergens (especially peanuts) and immune responses to the allergens in the offspring. However, the review identified studies based predominantly on aeroallergens which indicated that maternal allergen exposure during pregnancy does not have a major influence on subsequent CBMC responses. A single study reported a weak association between maternal exposure to aeroallergen and CBMC responses in the offspring[74], but this association was derived from multiple comparisons during the analysis of the study. The vast majority of studies on maternal allergen exposure during pregnancy and CBMC responses failed to find an association[75,76]. A further study demonstrated that maternal allergen exposure during pregnancy only partly explains corresponding CBMC responses to that allergen. It was reported that only half of

CBMC samples responding to the pollen allergen Bet v1 allergen came from pregnancies exposed to birch pollen, indicating that CBMC samples were responding in the absence of previous exposure[77]. Conversely, a high proportion of CBMC samples not responding to this allergen came from pregnancies exposed to birch pollen, suggesting that CBMC responses after stimulation with aeroallergens do not reflect *in utero* exposure to the allergen. Finally, the review identified two recent studies which comprised detailed investigations of CBMC responses and the timing of allergen sensitisation, and reported that neonatal T cell responses to allergens differ markedly from those occurring later in life[78,79]. Thornton *et al.* (2004)[78], demonstrated that neonatal T-cells responding to allergens express CD45RA and CD38 and the majority undergo apoptosis (cell death) after stimulation. These authors concluded that responding neonatal T-cells are naive immature thymic emigrants with modified antigen receptors that interact non-specifically with protein antigens, providing short-lived cellular immunity that does not generate conventional T-cell memory. More recent work by the same group has indicated that stable IgE-associated Th2-cell memory to house dust mite and peanut occurs entirely postnatally and does not appear until after 6 months of age[79]. However, this latter finding has yet to be confirmed by other studies.

Other evidence considered by the committee

Recently published research

41. A paper describing a cross-sectional study of peanut allergy prevalence and infant weaning practices among Jewish children resident in the UK compared with Jewish children resident in Israel, was published in October 2008, during the course of the Committee's current review of the evidence[80].

42. This paper described a comparison between over 5,000 Israeli children (aged 4 to 18 years) and a comparable number of Jewish schoolchildren of similar age range living in the UK. The study was prompted by the observation that in Israel, peanut-based products are widely used as weaning foods, resulting in very high levels of dietary exposure during infancy. This is very different from practice in the UK where dietary exposure to peanut products during infancy is substantially lower. Using a validated food allergy questionnaire, the researchers compared the prevalence among these two groups of children, of allergy to each of 5 foodstuffs (peanut, sesame, tree-nuts, hens' egg and cows' milk). Although the prevalence of allergies to egg and milk in the two groups was similar, a significant difference in peanut allergy was observed (UK 1.85% versus Israel 0.17%), and the authors reported an adjusted risk ratio for peanut allergy between these two populations of 9.8 (95% CI 3.1-30.5). The authors speculated that these findings may reflect a direct relationship between the early introduction of peanut products into the diet and a lower rate of peanut allergy among the children, concluding that they raised the question of whether early and frequent ingestion of high-dose peanut protein during infancy might prevent the development of peanut allergy through the induction of oral tolerance to peanuts. However, it is notable that differences between the UK and Israel cohorts were also observed with regard to the prevalence of tree-nut allergy, which were not correlated with differences in the dietary consumption of tree-nuts by infants. For this reason the findings reported by DuToit *et al*[80] cannot be regarded as conclusive. Nor should the reported prevalence of peanut allergy among this selective UK population (at 1.85%) be regarded as representative of UK schoolchildren more generally.

Unpublished and ongoing research

43. The Committee also considered technical summaries of two relevant unpublished studies, together with a technical summary of a relevant published study whose published outputs had reported only selective aspects of the study and, therefore, had fallen outside the scope of the main literature review. All three studies had been funded by the FSA, under project codes T07043, T07028 and T07005. Final reports of these studies are available from the Agency's library[81-83].

44. The first (project T07043)81 was a retrospective case-control study that compared environmental exposure to peanut during the first year of life in children with peanut allergy and two groups of referents – children with egg allergy who had been referred to the same food-allergy clinic and children attending general paediatric clinics with non-allergic complaints. Environmental exposure to peanut was defined in terms of total consumption of peanuts and peanut-containing foods by all household members, and was assessed using a previously validated food frequency questionnaire[84] that included questions about portion size as well as frequency of consumption. Information was also collected on maternal consumption of peanut during pregnancy and whilst breastfeeding, and on other possible risk factors such as use of peanut-containing creams during infancy. To counter possible recall bias, potential cases and egg-allergic controls were recruited on first attendance at a food-allergy clinic from children whose parents at that time did not identify peanut as a suspected cause of their symptoms. Their exposures were then assessed before the specific nature of their allergy was established. The study found that median weekly household peanut consumption during the first year of life was 78.9 g for the cases as compared with 29.1 g for the non-allergic controls, and only 7.8 g for the controls with egg-allergy. These figures are equivalent to 18.8 g, 6.9 g and 1.9 g respectively of peanut protein. These differences were statistically significant ($p < 0.0001$), and could not be explained by differences in maternal consumption of peanut during pregnancy or lactation. The researchers hypothesised that they occurred because environmental exposure to peanut, occurring through cutaneous or inhalation routes, led to sensitisation in the absence of significant oral exposure.

45. The second and third studies (projects T07028[82] and T07005[83]) were both based on a randomised intervention trial of the effect of maternal dietary avoidance of egg during pregnancy and lactation, on food and aero-allergen sensitisation and on allergy outcomes up to 18 months of age, in a high risk population (where the mother or father had a history of allergy). Project T07005 evaluated the effects of maternal egg avoidance on allergy outcomes and project T07028 used the maternal dietary data collected from the trial together with that obtained from a retrospectively administered dietary questionnaire, to investigate associations between peanut consumption/avoidance during pregnancy and the development of peanut allergy in the infant by 18 months of age.

46. Project T07028[82] found no consistent relationship between estimated peanut intake during pregnancy and the subsequent development of peanut allergy in children at 6, 12 or 18 months of age. Levels of peanut-specific IgG and IgE in maternal blood and breastmilk, measured during pregnancy and breastfeeding, also did not appear to be related to peanut allergy in children at any of these time points[82]. However, the statistical power of this study was limited (maternal diet diaries were available for 227 women, dietary questionnaires were available for 165 women, and the number of children who were sensitised to peanut ranged between 7 (at 6 months of age) and 10 (at 18 months of age)).

47. Project T07005[83] found that despite intense dietetic input and support, only 16% of the mothers in the egg avoidance group managed to remain completely egg- free from recruitment to delivery. Overall there was no association between the maternal egg avoidance diet and prevention of either egg sensitisation or any other associated allergy and allergic disease by 18 months of age. However, there was a significant positive relationship between total egg intake from recruitment to delivery and atopy at 18 months of age, (p=0.041) (where atopy was taken as being skin prick test positive to any allergen and/or the presence of eczema). A moderate weekly egg intake (range 65-149 g, roughly the equivalent of 2 to 4 eggs) was significantly linked with development of atopy compared to a low weekly intake (range 0-78 g, roughly equivalent to between 0 and 2 eggs). When these results were analysed together with the atopic status of the parents, it was reported that whilst a moderate egg intake by atopic women, when compared with a low intake, was associated with development of atopy in the infant (although not statistically significantly); when the mother was not atopic but the father was, a very low intake compared with a moderate intake was associated with atopy in the infant (p= 0.006). A very low total egg intake compared with a moderate intake by non-atopic mothers was also associated with sensitisation to egg in the child at 1 year (p=0.019). Overall egg intake in the study (even among the control arm mothers who were not avoiding egg) was relatively low when compared with national averages. Therefore, it was not possible to evaluate the effect of high egg intake on allergic outcomes in this trial. Egg proteins were detected in breast milk and umbilical cord blood in a number of cases, sometimes in association with other antibodies and sometimes free. Free levels occurred more frequently where the mothers were attempting to avoid egg and this was associated with more allergy in the babies. The researchers concluded that egg avoidance diets are very difficult to sustain and that there was no overall association between avoidance and atopic outcomes, although there were complex interactions between dietary intake, maternal atopic phenotype and allergy outcomes in the baby.

48. The Committee was also provided with brief summaries and study protocols of two major ongoing clinical intervention trials* that were testing the hypothesis that early introduction of allergenic foods into the diet of children, promotes the acquisition of oral tolerance as opposed to clinical allergy, and therefore results in a reduced rate of allergy to the food(s). One of these studies was focussing on peanut and was using a high risk infant population. The other was focussing on early introduction of six allergenic foods in the general infant population.

49. Finally, the Committee was also informed about a major EU Framework 6 funded research project that is currently investigating the prevalence of food allergies (including peanut allergy) in infants, children and adults across Europe. The project is called EuroPrevall**. The birth cohort part of the project, which includes a UK sample of ~1200 infants, has the potential to deliver robust data on the cumulative prevalence of individual food allergies in the first 2 years of life in a combined cohort size of more than 10,000 infants. It is expected that these data will become available in the next 2 years.

Evidence relating to asthma and atopic eczema

50. To supplement the human data relating to food allergy outcomes, the Committee was provided with three recent review articles plus one editorial that had evaluated the published evidence on dietary

* Summary details about these two randomised intervention trials can be found at http://www.leapstudy.co.uk/ and at http://www.food.gov.uk/science/research/researchinfo/foodcomponentsresearch/allergyresearch/t07programme/t07projectlist/t07051/

** Further details about this project can be found on the project website www.europrevall.org/

exposure to/avoidance of allergenic foods and the development of allergic disease more generally than just food allergy[85-88]. Collectively, these publications covered studies published up to March 2006. Relevant papers published since that time were identified via a literature search conducted by the British Medical Association (BMA) on behalf of the FSA, and through consultation with the four medical and scientific experts who were assisting the COT in this review and who had expert knowledge of the literature in this area.

51. The literature search conducted by the BMA was conducted in OVID MEDLINE, using search terms similar to those used by the BNF, but including allergy, asthma, atopy, eczema/atopic dermatitis and wheeze as additional outcome measures. This search identified a further 1001 papers, which were assessed at title and then abstract stage against eligibility criteria for inclusion/exclusion. This reduced the selection to 49 published papers, 25 of which were subsequently excluded on the basis that they focussed on breastfeeding (duration/exclusivity/comparison with formula(s)) and allergy outcomes, rather than on exposure/avoidance of allergenic foods specifically. A further 12 papers were excluded as not being of interest to the review on other grounds, for example studies that looked at associations between overall dietary pattern, or nutritional content, and allergy outcomes. A written summary* covering the remaining 12 papers identified as relevant[89-100], plus three additional papers published since March 2006 and identified via consultation with the four experts as of potential relevance[101-103], were provided to the Committee for its consideration.

Committee Discussion

The literature review of human, animal and cord blood studies

52. The Committee considered that the literature review of human, animal and cord blood studies had been a significant and complex undertaking given the breadth of the subject areas to be covered. The review was considered to be of high scientific quality and rigour. It was noted that none of the studies identified in the review of human studies had been assigned high scores against the SIGN criteria used by the BNF, although the Committee had reservations about SIGN grading as an index of quality for observational data. Also, the overall evidence base was limited. Much of the relevant evidence was from studies not designed to investigate the influence of peanut in the diet and few data came from prospective studies.

53. Aspects of the design and scope of the human studies part of the literature review, could have limited the breadth of potentially relevant evidence that was captured and evaluated. The application of strict inclusion and exclusion criteria had resulted in a large number of studies being excluded, and it was possible that some of these studies contained information of indirect relevance to the review. In particular, it was considered that the exclusion of studies that had investigated exposure to or avoidance of allergenic foods and their possible influence on the development of allergic diseases other than food allergy, such as allergic asthma and atopic eczema, was a potential limitation of the BNF review. The Committee noted that the supplementary ascertainment of evidence in these latter areas was not fully systematic, but was able to consider recent review articles together with relevant papers published subsequently, in order to inform its discussions on the principal outcome of peanut allergy.

* This written summary formed part of the papers for the 14th October 2008 COT meeting, and can be found on the COT website at: http://cot.food.gov.uk/pdfs/tox200833annex2.pdf

Evidence from human studies

54. The Committee noted that the BNF review did not identify any high quality studies published since 1998 that had directly examined the effect of consumption versus avoidance of peanut or other allergenic foods **during pregnancy or lactation** on the development of sensitisation or food allergy in the child. No published randomised controlled trials were identified by this part of the review. It was also noted that the two case-control studies identified that had looked for associations between peanut consumption and peanut sensitisation or allergy[21,22], did not report any statistically significant associations after adjustment for confounders, although the variables accounted for in the analysis by Lack *et al.* were not specified in the published paper[22]. Frank *et al.*, reported odds ratios of 2-4 for peanut consumption more than once a week during pregnancy/lactation compared with less than once a week, but the associated confidence intervals were wide[21]. The lack of high quality studies carried out in humans coupled with the heterogeneous range of exposures that these studies compared (e.g. low versus high peanut consumption/ none versus any peanut consumption), and the small number of studies that had focussed on peanut consumption, precludes definitive conclusions as to whether maternal consumption or avoidance of allergenic foods during pregnancy or lactation, has any impact on the subsequent development of sensitisation or allergy to peanut in the child. The two unpublished studies reported to the Committee by the FSA (projects T07005 and T07028), do not provide any evidence that alters this conclusion.

55. The available evidence from studies in humans on the effects of consumption versus avoidance of dietary allergens **during infancy/early childhood** and the development of sensitisation or food allergy, is also very limited. A greater number of studies were identified in this area of the BNF literature review, including some randomised controlled trials. However, none of these was designed to investigate the effects of dietary consumption or avoidance of peanuts specifically, on food allergic outcomes. The majority were designed to compare breastfeeding with formula feeding, or to investigate the effect of duration of breastfeeding and/or timing of introduction of solids on allergic outcomes, and few looked at exposure to peanut. Therefore, the relevance of these studies to the present review is limited.

56. In the published literature that is currently available, there is limited evidence from one randomised controlled trial (RCT)[37] that, in children with (but not without) a family history of food allergy, breastfeeding for 9 months or longer is associated with increased risk of food allergy. The Committee noted that out of eight studies identified by the BNF review as assessing the effect of duration of breastfeeding, none found that breastfeeding beyond 6 months was associated with lower risk of food allergy. The Committee considers that the single case-control study that reported an earlier age of introduction of peanut into the diet in subjects who became peanut sensitised compared with those who did not[21], does not by itself provide sufficient evidence on which to base firm conclusions. The Committee also noted that two out of 3 multifaceted intervention trials identified by the BNF review, reported a statistically significant association between combined allergen avoidance and a reduced risk of sensitisation to foods (which included peanut), in the child. However, the individual factors responsible for the observed effects in these cases could not be disentangled and there was

no suggestion that such protective effects, if real, translated into a lower risk of clinical food allergy. Further, the third of these trials did not find a statistically significant protective effect of avoidance measures on sensitisation or allergy outcomes. Overall, the Committee considers that the available human data on whether dietary consumption or avoidance of allergenic foods in childhood has an impact on the development of allergy or sensitisation to allergenic foods are inconsistent. The two ongoing clinical intervention trials that are seeking to test the effect of early dietary introduction of high doses of peanuts, either alone, or in combination with other allergenic foods, on later allergic outcomes, have the potential to provide more conclusive data from humans on whether early high dose oral exposure to peanut and/or other allergenic foods leads to a reduction in sensitisation and food allergy. Results from these studies, will not, however, be available until 2013 at the earliest.

57. Currently, the available evidence from human studies regarding the possible importance of **non-dietary routes of exposure** is extremely limited. However, the findings of the two studies conducted by the research group led by Prof. Lack[22, 81], only one of which has yet been published in a peer-reviewed journal[22], together provide some indications that non-oral routes of exposure to peanut, such as through the skin, may be important in the development of sensitisation and peanut allergy during early childhood. Whether such a route of exposure might also be a risk factor for sensitisation in humans to other food allergens is unknown. The suggestion from the study by Lack *et al.* (2003)[22] that the use of infant skin creams containing peanut oil to treat inflamed skin is a risk factor for developing peanut allergy is of concern. However, the Committee understands that following the publication of the findings of that study, which were reviewed in 2002 by the Committee on Safety of Medicines (CSM), actions were taken by the then Medicines Control Agency to ensure clear labelling of peanut (arachis) oil whenever present in creams used for treating inflamed skin. The CSM also issued advice at that time that patients known to be allergic to peanut should not take/use medicines containing arachis oil (peanut oil) and such advice was also included in updated product labels and information leaflets issued from that time onwards. A general decline in the number of infant skin creams containing peanut oils has subsequently been observed.

58. The Committee also noted that the study by Lack *et al.* published in 2003, found that peanut allergy in the cohort of children studied was independently associated with intake of soya milk or soya formula in the first two years of age (OR 2.6, 95% CI 1.3-5.2). The authors suggested that this association could have arisen from cross-sensitisation between peanut and soya through shared immunoreactive epitopes, and concluded that consumption of soya formula in early life may be an additional risk factor for subsequent development of peanut allergy. However, *in vivo* cross reactivity between peanut and soya is relatively uncommon[104] and a confounding factor in the observed association could be a family history of milk allergy. A recent randomised controlled trial found that, during the first 2 years of life, use of a soya formula compared with an extensively hydrolysed formula did not increase the risk of developing peanut-specific IgE antibodies or of clinical peanut allergy, in infants with cows' milk allergy[105]. In addition, a recent cohort study of babies with a family history of allergic disease found that whilst children whose parents introduced soya formula or soya milk into their child's diet were more likely to be sensitised to peanuts, this relationship was explained by family or child history of milk allergy (OR after adjustment for family/child history of milk allergy = 1.34, 95% CI 0.64-2.79, p = 0.4)[106].

Evidence from studies conducted in animals

59. The available data from animal studies suggests that maternal oral or mucosal exposure to hens' egg ovalbumin during gestation and/or lactation, possibly with high doses of allergen, may protect offspring from the development of IgE-mediated responses to the same antigen. However, at this time, there are no comparable data for peanut. Experimental studies in rodents can provide valuable insights into the mechanisms of immune responses to food proteins. However, because of significant interspecies differences, it is difficult to replicate all aspects of human allergic disease in a single animal model. Therefore, caution should be used when attempting to extrapolate from animal models to the human situation. However, the findings do suggest that well designed, adequately powered, studies are warranted in humans to investigate the influence of high versus low doses of food allergen consumption during pregnancy/lactation on food allergy outcomes.

60. The available data from animal studies that have investigated the effect of dietary exposure to food allergens on immunological responses in the same animal indicate that oral exposure to either ovalbumin or peanut extract in low doses may induce sensitisation (measured as IgE antibody and immediate type hypersensitivity reactions). Conversely, higher doses of the allergens may result in oral tolerance and/or inhibition of sensitisation to subsequent immunisation with the same allergen. However, there are significant inter-strain differences in susceptibility to allergen, and the dose response relationships for the two allergens are different in that higher doses are required to generate tolerance to peanut compared with ovalbumin. Although not investigated in a systematic way, it is also probable that the age of animals at which a protein is first encountered via dietary or gavage exposure will impact on the effectiveness with which oral tolerance is induced[107]. For these reasons and those stated previously (para. 59), caution is required regarding extrapolation of these data to the human situation. However, they do suggest that dose, and possibly the time at which food allergens are first encountered orally, might be critical in determining the immunological outcome.

61. Potentially important evidence from studies in animals has emerged since the previous COT review relating to the skin as a possible route of sensitisation to food allergens. The available data in this area (all of which have been reported since 1998), provide evidence that relatively small amounts of peanut extract or ovalbumin when applied to skin that has been damaged to replicate eczematous skin, can induce strong IgE-mediated immune responses. These findings have been replicated in more than one animal species and in more than one study. Based on these investigations, the skin may be an important route of sensitisation to allergenic foods during early life. There is also limited evidence from these studies that where sensitisation is acquired via exposure to the skin, this may prevent the subsequent induction of tolerance that would normally be expected following oral exposure to the same antigen. In these studies, peanut protein was applied to damaged rather than intact skin, which may be an important factor. However, it is known that apparently healthy human skin often contains minor abrasions, and that barrier function in skin from atopic individuals is often compromised[108]. It remains to be confirmed whether abrasion, occlusion or impaired barrier function are necessary for the induction of allergic responses to proteins encountered at skin surfaces. Again, some caution is required in the extrapolation of these data from animal studies to the human situation, but there are already some parallels between the animal data and the associations between household (environmental) exposure to peanut and/or use of peanut oil-containing creams and peanut allergy in

children, that have been reported from the few human studies available (see paragraphs 30, 44, and 57). More evidence from studies in humans is needed to confirm whether or not the skin is an important route of sensitisation to peanut and other food allergens, and if so, to define the relevant immunological mechanisms involved.

Evidence from studies of human cord blood

62. The evidence base relating to whether intrauterine immunological sensitisation to allergens can occur and whether this is associated with subsequent allergic disease has also changed since the 1998 COT review. At that time, the view among the scientific community was that umbilical CBMC proliferative and cytokine responses reflected *in utero* exposure to, and sensitisation by, allergens. However, the data that have become available since then (discussed in para. 39-40), indicate that the associations between antenatal allergen exposure and CBMC responses are more complex than previously thought, and cast doubt on the assertion that observed CBMC responses to allergen are necessarily a consequence of maternal exposure to that allergen either during pregnancy or lactation. On the basis of the evidence base that is now available, the Committee consider that it is highly probable that the fetus is exposed to small (but variable) amounts of food proteins derived from the mother's diet and transported across the placenta, but it is unclear whether such fetal exposure results in *in utero* sensitisation of the fetal immune system. Furthermore, it is not possible to conclude that the *in vitro* CBMC responses observed after stimulation by food proteins necessarily reflect *in utero* exposure and/or sensitisation. Finally, the Committee consider that even if it could be established that *in utero* priming of fetal T-cells by maternal dietary proteins takes place, it would not necessarily follow that such priming results in clinical allergy in the infant.

Evidence relating to atopic asthma and atopic eczema

63. The Committee considers that the currently available evidence on dietary exposure to allergenic foods in early life and the risk of development of forms of atopic disease other than food allergy, such as allergic asthma and atopic eczema, is inconsistent and does not provide robust evidence of either harmful or beneficial effects associated with dietary exposure or avoidance. Most of the available literature in this area has not focused on the effects of exposure to peanut, but rather on exposure to other foods or on other possible dietary influences on these health outcomes, such as duration of breastfeeding, timing of introduction of solids, and use of infant formula. A single published study, based on a Dutch cohort of pregnant women and their babies, has reported a positive association between consumption of nut products during pregnancy and the asthma-related health outcomes of childhood wheeze, dyspnoea and 'asthma symptoms' (daily versus rare consumption of nut products OR (childhood wheeze) 1.42 (95% CI 1.06-1.89), OR (dyspnoea) 1.58 (95% CI 1.16 – 2.15), OR ('asthma symptoms') 1.47 (95% CI 1.08-1.99))[103]. However, whilst the sample size was large (n = 2832), there was no association of these endpoints with consumption of nuts as opposed to nut products, and there was also no association between nut or nut product consumption and any of the food allergy related endpoints that the researchers evaluated (skin prick test reactivity to milk, egg or inhalant allergens at 8 years of age and reported food allergy)[103]. In addition, the comparison was

carried out in a context of multiple statistical analyses, and the finding has not been replicated by other studies. A similar dietary assessment analysis of pregnant mothers in the UK, which was carried out prior to this study, found no associations with either nuts or nut products[90].

Evidence relating to prevalence of peanut sensitisation and allergy

64. Estimates from population-based surveys of the prevalence of peanut sensitisation and allergy are heterogeneous, both across different age ranges, and within similarly aged children from different studies. This variation precludes confident derivation of a single summary estimate of the prevalence of peanut allergy in UK children. However the available data suggest that the current prevalence of peanut allergy in UK children lies between 0.2% and 1.8%.

65. There are several limitations of the current evidence base that complicate the evaluation of temporal trends in UK prevalence of peanut sensitisation and peanut allergy in children. These include the use of different methodologies for the determination of clinical allergy and, to a lesser extent, sensitisation, with few studies having used the gold standard methodology for determining food allergy, the Double Blind Placebo Controlled Food Challenge (DBPCFC); low response rates in some studies with a potential for resultant bias; and (with the exception of the study by Lack *et al.* (2003)[22]), low statistical power to determine the prevalence of relatively rare health outcomes. Evaluation of trends is further complicated because studies have been conducted in different places and not always on children of the same ages.

66. Overall, the available data in the UK provide no clear evidence that age-specific prevalence rates of peanut sensitisation and peanut allergy among children have changed significantly during the past 20 years. If the data from the Isle of Wight studies of children aged 3 to 5 years are considered on their own[44,45,47], there is a suggestion that the rates of both peanut sensitisation and peanut allergy may have increased in that area between the late 1980's and the mid 1990's (before the 1998 COT recommendations were issued), but it is unclear whether this trend can be extrapolated to the wider UK population and there is no suggestion of a further increase since that time. Data on hospital admissions for food-related anaphylaxis, which are not specific for peanut[109] reveal a marked increase in England during the period 1990-2000, with a levelling off thereafter. However, it should be noted that this occurred in all age groups more or less in parallel, and even if driven by allergy to peanut (which is not known), appears to be a "period effect" and not a "cohort effect" of the type that would be expected if it reflected changes in exposure to peanut *in utero* or in infancy.

67. The Committee also noted the findings of the two published studies by Dean *et al.* (2007)[41], and Hourihane *et al.* (2007)[6], which examined the impact of the COT recommendations on children born after they were issued, and which were not able to discern any impact of the recommendations, either positive or negative, on the prevalence of peanut allergy in 3-5 year old children. The findings of those two studies on the low percentage take-up of the recommendations by mothers, coupled with evidence that the recommendations were taken up similarly by non-atopic as well as atopic women (the target group), are notable. They suggest that the recommendations have not been disseminated effectively, and/or that they have not been implemented by mothers as intended.

Conclusions and recommendations

68. From the evidence that was reviewed, the Committee has drawn the following conclusions:

    i. It is unclear whether prevalence rates of peanut sensitisation and allergy in the UK have changed since the previous COT recommendations were issued in 1998.

    ii. The new evidence that has become available since 1998 reduces the suspicion that maternal consumption of peanut or peanut products during pregnancy might predispose infants to the development of peanut sensitisation and allergy. In particular, it now appears that *in vitro* responses to allergens by umbilical cord blood mononuclear cells do not necessarily reflect maternal exposure to the allergens concerned. In addition, there is now limited human evidence, consistent with a larger body of animal data, suggesting that non-oral routes of exposure to peanut, such as via the skin, may be relevant to the development of peanut sensitisation and allergy during early childhood. This casts doubt on the previous assertion that reactions to peanut on first known dietary exposure are necessarily indicative of sensitisation *in utero* or and/or during lactation. Data from animal studies indicate that exposure of damaged skin to egg (ovalbumin) or peanut allergens can result in the induction of IgE-mediated systemic allergic responses.

    iii. Animal studies that have been reported since 1998 suggest that maternal oral exposure to the hens' egg allergen, ovalbumin, during gestation and/or lactation, particularly at high doses, may protect offspring from developing allergic responses to this allergen. There are no comparable data for peanut proteins in animals, or for humans, but the finding raises the possibility that maternal dietary consumption of peanut might in some circumstances reduce the risk of peanut allergy in offspring.

    iv. Overall, the evidence now available does not indicate whether maternal dietary consumption of peanut during pregnancy or lactation is more likely to increase or decrease the risk of sensitisation and allergy to peanut in the child. An effect in either direction is possible, and it is possible that the direction of effect could differ according to the level of intake. Alternatively, there could be no effect at all.

    v. Human data relating dietary consumption or avoidance of peanut or other allergenic foods in childhood to the development of sensitisation or allergy or tolerance to peanut, are limited and inconsistent. Data from animal studies suggest that, for peanut proteins and ovalbumin, the nature of the immune response may depend on dose, with high exposures tending to induce tolerance and low exposures sensitisation. However, there are no comparable published data for humans at this time.

69. The shift in the balance of evidence since 1998 is such that the Committee believes that the previous precautionary advice to avoid peanut consumption during pregnancy, breast feeding and infancy, where there is atopy or atopic disease in family members, is no longer appropriate.

70. However, the Committee considers that the basis of the more general recommendations made in 1998 is still justified and, therefore, recommends that:

(i) In common with the advice given for all children, infants with a parent or sibling with an atopic disease should be breast-fed exclusively for around 6 months;

and,

(ii) Infants and children who are allergic to peanuts or peanut products, should not consume them or foods that contain them;

and also recommends that:

(iii) those who are allergic to peanut should seek advice from medical professionals about avoidance strategies.

71. However, it should be recognised that there remains scientific uncertainty about the determinants of peanut sensitisation and allergy. Thus, further changes to this advice may be warranted in the future, as and when new data become available. In particular, studies are currently underway to investigate the impact on allergic outcomes of early dietary introduction of peanut and/or other allergenic foods into the infant diet, and these studies have the potential to provide more definitive data in the next 5 to 7 years.

72. In addition, the Committee recommends that further studies are needed in humans:

a. to determine whether and to what extent the skin and respiratory tract are important routes of sensitisation to peanut and other food allergens, and if they are, to determine the importance of timing and dose, and the underlying mechanisms; and

b. to investigate whether and how oral dose levels influence the development of sensitisation, allergy or tolerance to peanut and other allergenic foods.

73. The Committee also noted the need for clearer information on temporal trends in peanut consumption and the prevalence of peanut allergy in UK infants and children, as well as on infant weaning practices in the UK

COT Statement 2008/07
December 2008

References

1. COT report on Peanut Allergy. Department of Health. (1998) (http://www.food.gov.uk/science/ouradvisors/toxicity/cotreports/cotwgreports/cotpeanutallergy)

2. Thompson, R, Miles, L, Lunn, J, Buttriss, J. Systematic review of literature on early life patterns of exposure to, and avoidance of, food allergens and later development of sensitisation and clinical food allergy, with particular reference to peanut allergy. Final Technical Report of FSA research project T07052 (http://www.foodbase.org.uk)

3. Pumphrey, R, Gowland, H. (2007) Further fatal allergic reactions to food in the United Kingdom 1999-2006. Letter to the Editor. *Journal of Allergy and Clinical Immunology*, 119:1018-1019

4. Hourihane, J, Kilburn, S, Nordlee, J, Hefle, S, Taylor, S, Warner, J. (1997) An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: a randomized, double-blind, placebo-controlled food challenge study. *Journal of Allergy and Clinical Immunology*, 100: 596-600

5. Sicherer, S, Sampson, H. (2007) Peanut allergy: Emerging concepts and approaches for an apparent epidemic. *Journal of Allergy and Clinical Immunology*, 120(3): 491-505

6. Hourihane, J, Aiken, R, Briggs, R, Gudgeon, L, Grimshaw, K, DunnGalvin, A, Roberts, S. (2007) The impact of government advice to pregnant mothers regarding peanut avoidance on the prevalence of peanut allergy in United Kingdom children at school entry. *Journal of Allergy & Clinical Immunology*, 119: 1197-1202

7. Avery, N, King, R, Knight, S, Hourihane, J. (2003) Assessment of quality of life in children with peanut allergy. *Pediatric Allergy and Immunology*, 14(5): 378-382

8. Hourihane, J, Kilburn, S, Dean, P, Warner, J. (1997) Clinical characteristics of peanut allergy. *Clinical and Experimental Allergy*, 27(6): 634-639

9. Van Asperen, P, Kemp, A, Mellis, C, (1983) Immediate food hypersensitivity reactions on the first known exposure to the food. *Archives of Diseases in Childhood,* 56: 351-354

10. Kilshaw, P, Cant, A. (1984) The passage of maternal dietary proteins into human breastmilk. *International Archives of Allergy and Immunology,* 75:8-15

11. Fukushima, Y, Kawata, Y, Onda, T, Kitagawa, M. (1997) Consumption of cow milk and egg by lactating women and the presence of beta-lactoglobulin in breastmilk. *American Journal of Clinical Nutrition*, 651(1):30-35

12. Husby, S. (1988) Dietary antigens: uptake and humoral immunity in man. *Acta Pathol Microbiol Immunol Scand*: 96 (Suppl 1): 1-40

13. Warner, J, Miles, E, Jones, A *et al.*, (1994) Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clinical and Experimental Allergy*, 24: 423-430

14. Report of the House of Lords Science and Technology Committee on Allergy. 6th report of Session 2006-2007 (http://www.publications.parliament.uk/pa/ld200607/ldselect/ldsctech/166/166i.pdf)

15. The Food Labelling (Declaration of Allergens) (England) Regulations 2008 (http://www.opsi.gov.uk/si/si2008/uksi_20081188_en_1)

16. Scottish Intercollegiate Network (SIGN) guidelines on systematic literature review (http://www.sign.ac.uk/guidelines/fulltext/50/section6.html)

17. Hattevig, G, Sigurs, N, Kjellman, B. (1999) Effects of maternal dietary avoidance during lactation on allergy in children at 10 years of age. *Acta Paediatrica*, 88: 7-12

18. Sausenthaler, S, Koletzko, S, Schaaf, B, Lehmann, I, Borte, M, Herbarth, O, von Berg, A, Wichmann, H, Heinrich, J. (2007) Maternal diet during pregnancy in relation to eczema and allergic sensitization in the offspring at 2 years of age. *American Journal of Clinical Nutrition*, 85: 530-537

19. Wetzig, H, Schulz, R, Diez, U, Herbarth, O, Viehweg, B, Borte, M, Wetzig, H.(2000) Associations between duration of breast-feeding, sensitization to hens' egg and eczema infantum in one and two year old children at high risk of atopy. *International Journal of Hygiene and Environmental Health*, 203: 17-21

20. Calvani, M, Alessandri, C, Sopo, S, Panetta, V, Pingitore, G, Tripodi, S, Zappalà, D, Zicari, A. (2006) Consumption of fish, butter and margarine during pregnancy and development of allergic sensitizations in the offspring: role of maternal atopy. *Pediatric Allergy & Immunology*, 17:94-102

21. Frank, L, Marian, A, Visser, M, Weinberg, E, Potter, P. (1999) Exposure to peanuts *in utero* and in infancy and the development of sensitization to peanut allergens in young children. *Pediatric Allergy & Immunology,* 10: 27-32

22. Lack, G, Fox, A, Northstone, K, Golding, J. (2003) Avon longitudinal study of parents and children study T. Factors associated with the development of peanut allergy in childhood. *New England Journal of Medicine*, 348: 977-985

23. Ushiyama, Y, Matsumoto, K, Shinohara, M, Wakiguchi, H, Sakai, K, Komatsu, T, Yamamoto, S., (2002) Nutrition during pregnancy may be associated with allergic diseases in infants. *Journal of Nutritional Science and Vitaminology*, 48: 345-351

24. de Jong, M, Scharp-Van Der Linden, V, Aalberse, R, Heymans, H, Brunekreef, B. (2002) The effect of brief neonatal exposure to cows' milk on atopic symptoms up to age 5. *Archives of Disease in Childhood,* 86: 365-369

25. Halken, S, Hansen, K, Jacobsen, H, Estmann, A, Faelling, A, Engberg, A, Hansen, L, Kier, S, Lassen, K, Lintrup, M, Mortensen, S, Ibsen, K, Osterballe, O, Host, A. (2000) Comparison of a partially hydrolyzed infant formula with two extensively hydrolyzed formulas for allergy prevention: a prospective, randomized study. *Pediatric Allergy & Immunology*, 11: 149-161

26. Saarinen, K, Juntunen-Backman, K, Jarvenpaa, A, Kuitunen, P, Lope, L, Renlund, M, Siivola, M, Savilahti. (1999) Supplementary feeding in maternity hospitals and the risk of cows' milk allergy: A prospective study of 6209 infants. *Journal of Allergy & Clinical Immunology*, 104: 457-461

27. Schoetzau, A, Filipiak-Pittroff, B, Franke, K, Koletzko, S, von Berg, A, Gruebl, A, Bauer, C, Berdel, D, Reinhardt, D, Wichmann, H. (2002) Effect of exclusive breast-feeding and early solid food avoidance on the incidence of atopic dermatitis in high risk infants at 1 year of age. *Pediatric Allergy & Immunology*, 13:234-242

28. Szajewska, H, Mrukowicz, J, Stoiska, B, Prochowska, A (2004) Extensively and partially hydrolysed preterm formulas in the prevention of allergic diseases in preterm infants: a randomised, double-blind trial. *Acta Paediatrica,* 93:1159-1165

29. Arshad, S, Bateman, B, Sadeghnejad, A, Gant, C, Matthews, S. (2007) Prevention of allergic disease during childhood by allergen avoidance: the Isle of Wight prevention study. *Journal of Allergy & Clinical Immunology,* 119: 307-313

30. Chan-Yeung, M, Ferguson, A, Watson, W, Dimich-Ward, H, Rousseau, R, Lilley, M, DyBuncio,A, Becker, A. (2005) The Canadian Childhood Asthma Primary Prevention Study: outcomes at 7 years of age. *Journal of Allergy & Clinical Immunology*, 116: 49-55

31. Halmerbauer, G, Gartner, C, Schierl, M, Arshad, S, Dean, T, Koller, D, Karmaus, W, Kuehr, J, Forster, J, Urbanek, R, Frischer, T. (2003) Study on the prevention of the allergy in children in Europe (SPACE): allergic sensitization at 1 year of age in a controlled trial of allergen avoidance from birth. *Pediatric Allergy & Immunology,* 14: 10-17

32. Hilkino, S, Nakayama, H, Yamamoto, J, Kinukawa, N, Sakamoto, M, Hara, T. (2001) Food allergy and atopic dermatitis in low birthweight infants during early childhood. *Acta Paediatrica,* 90: 850-855

33. Gustafsson, D, Andersson, K. (2004) Effect of indoor environmental factors on development of atopic symptoms in children followed up to 4 years of age. *Paediatric and Perinatal Epidemiology*, 18: 17-25

34. Kull, I, Wickman, M, Lilja, G, Nordvall, S, Pershagen, G. (2002) Breast feeding and allergic diseases in infants-a prospective birth cohort study. *Archives of Disease in Childhood,* 87: 478-481

35. Kull, I, Bergstrom, A, Melen, E, Lilja , G, van Hage , M, Pershagen , G, Wickman, M. (2006) Fish consumption during the first year of life and development of allergic diseases during childhood. *Journal of Allergy & Clinical Immunology,* 118: 1299-1304

36. Milner, J, Stein, D, McCarter, R, Moon R. (2004) Early infant multivitamin supplementation is associated with increased risk for food allergy and asthma. *Pediatrics*, 114: 27-32

37. Pesonen, M, Kallio, M, Ranki, A, Siimes, M. (2006) Prolonged exclusive breastfeeding is associated with increased atopic dermatitis: a prospective follow-up study of unselected healthy newborns from birth to age 20 years. *Clinical & Experimental Allergy*, 36: 1011-1018

38. Poole, J, Barriga, K, Leung, D, Hoffman, M, Eisenbarth, G, Rewers, M, Norris, J. (2006) Timing of initial exposure to cereal grains and the risk of wheat allergy. *Pediatrics*, 117: 2175-2182

39. Zutavern, A, Brockow, I, Schaaf, B, Bolte, M, von Berg, A, Diez, U, Borte, M, Herbarth, O, Wichmann, H, Heinrich, J. (2006) Timing of solid food introduction in relation to atopic dermatitis and atopic sensitization: results from a prospective birth cohort study. *Pediatrics*, 117: 401-411

40. Kull, I, Bergstrom, A, Melen, E, Lilja , G, van Hage, M, Pershagen, G, Wickman, M. (2006) Early-life supplementation of vitamins A and D, in water soluble form or in peanut oil, and allergic diseases during childhood. *Journal of Allergy & Clinical Immunology*, 118: 1299-1304

41. Dean, T, Venter, C, Pereira B, Grundy, J, Clayton, C, Higgins, B. (2007) Government advice on peanut avoidance during pregnancy – is it followed correctly and what is the impact on sensitization? *Journal of Human Nutrition and Dietetics*, 19: 129-138

42. Pereira, B, Venter, C, Grundy, J, Clayton, C, Arshad, H, Dean, T. (2005) Prevalence of sensitization to food allergens, reported adverse reaction to foods, food avoidance, and food hypersensitivity among teenagers. *Journal of Allergy & Clinical Immunology*, 116:884-892

43. Emmett, S, Angus, F, Fry, J, Lee, P. (1999) Perceived prevalence of peanut allergy in Great Britain and its association with other atopic conditions and with peanut allergy in other household members. *Allergy*, 54: 380-385

44. Tariq, S, Stevens, M, Matthews, S, Ridout, S, Twiselton, R, Hide, D. (1996) Cohort study of peanut and tree nut sensitization by age of 4 years. British Medical Journal, 313: 514-517

45. Grundy, J, Matthews, S, Bateman, B, Dean, T, Arshad, S. (2002) Rising prevalence of peanut-allergic children: recent changes. *Pediatrics,* 120: 1304-1310

46. Venter, C, Pereira, B, Grundy, J, Clayton, C, Arshad, S, Dean, T. (2006) Prevalence and sensitization reported and objectively assessed food hypersensitivity amongst six-year-old children: a population based study. *Pediatric Allergy & Immunology*, 17: 356-363

47. Venter, C, Pereira, B, Voigt, K, Grundy, J, Clayton, C, Higgins, B, Arshad, S, Dean, T. (2008) Prevalence and cumulative incidence of food hypersensitivity in the first 3 years of life. *Allergy*, 63: 354-359

48. Venter, C, Pereira, B, Grundy, J, Clayton, C, Arshad, S, Dean, T. (2006) Prevalence of sensitization reported and objectively assessed food hypersensitivity in the first year of life. *Journal of Allergy and Clinical Immunology,* 117: 1118-1124

49. Bock, S. (1987) Prospective appraisal of complaints of adverse reactions to food in children during the first 3 years of life. *Pediatrics*, 79: 683-688

50. Sicherer, S, Munoz-Furlong, A, Sampson, H. (2003) Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: a 5-year follow-up study. *Journal of Allergy & Clinical Immunology*, 112: 1203-1207

51. Kagan, R, Joseph, L, Dufresne, C, Gray-Donald, K, Turnbull, E, St. Pierre, I, Clark, A. (2003) Prevalence of peanut allergy in primary school children in Montreal, *Canada. Journal of Allergy & Clinical Immunology,* 112(6): 1223-1228

52. Korotkova, M, Telemo, E, Yamashiro, Y, Hanson, L, Strandvik, B. (2004) The ratio of n-6 to n-3 fatty acids in maternal diet influences the induction of neonatal immunological tolerance to ovalbumin. *Clinical and Experimental Immunology*, 137: 237-244

53. Nicklin,S, Miller, K. (1987) Naturally acquired tolerance to dietary antigen: effect of *in utero* and perinatal exposure on subsequent humoral immune competence in the rat. *Journal of Reproductive Immunology*, 10: 167-176

54. Verhasselt, V, Milcent, V, Cazareth, J, Kanda, A, Fleury, S, Dombrowicz, D, Glaichenhaus, N, Julia, V. (2008) Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma. *Nature Medicine*, 14: 170-175

55. Strid, J, Thomson, M, Hourihane, J, Kimber I, Strobel, S. (2004) A novel model of sensitisation and oral tolerance to peanut protein. *Immunology,* 113: 293-303

56. Strid, J, Hourihane, J, Kimber, I, Callard, R, Strobel, S. (2005) Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization. *Clinical and Experimental Allergy*, 35: 757-766

57. Zemann, B, Schwaerzler, C, Griot-Wenk, M, Nefzger, M, Mayer, P, Schneider, H, de Weck, A, Carballido, A, Liehl, E. (2003) Oral administration of specific antigens to allergy-prone infant dogs induces IL-10 and TGF-beta expression and prevents allergy in adult life. *Journal of Allergy & Clinical Immunology,* 111:1069-1075

58. Lamont, A, Mowat, A, Browning, M, Parrott, D. (1988) Genetic control of oral tolerance to ovalbumin in mice. *Immunology*, 63: 737-739

59. Pilegaard, K, Madsen,C (2004) An oral Brown Norway rat model for food allergy: comparison of age, sex, dosing volume and dosing preparation. *Toxicology,* 196: 247-257

60. Dearman, R, Caddick, H, Stone, S, Basketter, D, Kimber, I. (2001) Characterisation of antibody responses induced in rodents by exposure to food proteins: influence of route of exposure. *Toxicology*, 167: 217-231

61. Adel-Patient, K, Ah-Leung, S, Bernard, H, Durieux-Alexandrenne, C, Créminon, C, Wal, J. (2007) Oral sensitization to peanut is highly enhanced by application of peanut extracts to intact skin, but is prevented when CpG and cholera toxin are added. *International Archives of Allergy and Immunology*, 143: 10-20

62. Cardoso, C, Teixeira, G, Provinciatto, P, Godoi, D, Ferreira, B, Milanezi. C, Ferraz, D, Rossi, A, Cunha, F, Silva, J.. (2007) Modulation of mucosal immunity in a murine model of food-induced intestinal inflammation. *Clinical and Experimental Allergy*, 38: 338-349

63. Spergel, J, Mizoguchi, E, Brewer, J, Martin, T, Bhan, A, Geha, R. (1998) Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerolized antigen in mice. *Journal of Clinical Investigation*, 101: 1614-1622

64. Akei, H, Mishra, A, Blanchard, C, Rothenberg, M. (2005) Epicutaneous antigen exposure primes for experimental eosinophilic isophagitis in mice. *Gastroenterology*, 129, 985-94

65. Herrick, C, MacLeod, H, Glusac, E, Tigelaar, R, Bottomly, K. (2000) Th2 responses induced by epicutaneous or inhalational protein exposure are differentially dependent on IL-4. *Journal of Clinical Investigation*, 105: 765-775

66. Hseih, K, Tsai, C, Wu, C, Lin, R. (2003) Epicutaneous exposure to protein antigen and food allergy. *Clinical and Experimental Allergy*, 33: 1067-1075

67. Nelde, A, Teufel, M, Hahn, C, Duschl, A, Sebald, W, Bröcker, E, Grunewald, S. (2001) The impact of the route and frequency of antigen exposure on the IgE response in allergy. *International Archives of Allergy and Immunology*, 124: 461-469

68. Schiessl, B, Zemann, B, Hodgin-Pickart, L., de Weck, A, Griot-Wenk. M, Mayer, P, Nefzger, M, Schneider, H, Liehl, E. (2003) Importance of early allergen contact for the development of a sustained immunoglobulin E response in a dog model. *International Archives of Allergy and Immunology*, 130: 125-134

69. Teuber, S, Del Val, G, Morigasaki, S, Rim Jung, H, Eisele, P, Frick, O, Buchanan, B. (2002) The atopic dog as a model of peanut and tree nut food allergy. *Journal of Allergy & Clinical Immunology*, 100: 921-927

70. Szepfalusi, Z, Loibichler, C, Pichler, J, Reisenberger, K, Ebner, C, Urbanek, R. (2000) Direct evidence for transplacental allergen transfer. *Pediatric Research*, 48: 404-407

71. Edelbauer, M, Loibichler, C, Nentwich, I, Gerstmayr, M, Urbanek, R, Szépfalusi, Z. (2004) Maternally delivered nutritive allergens in cord blood and placental tissue of term and preterm neonates. C*linical and Experimental Allergy*, 34: 189-193

72. Loibichler, C, Pichler, J, Gerstmayr, M, Bohle, B, Kiss, H, Urbanek, R, Szépfalusi, Z. (2002) Materno-fetal passage of nutritive and inhalant allergens across placentas of term and pre-term deliveries perfused *in vitro*. *Clinical and Experimental Allergy*, 32: 1546-1551

73. Vance, G, Lewis, S, Grimshaw, K, Wood, P, Briggs, R, Thornton, C, Warner, J. (2005) Exposure of the fetus and infant to hens' egg ovalbumin via the placenta and breast milk in relation to maternal intake of dietary egg. *Clinical and Experimental Allergy*, 35: 1318-1326

74. Hagendorens, M, Ebo, D, Bridts, C, L, Van de Water, De Clerck, L, Stevens, W. (2004) Prenatal exposure to house dust mite allergen (der p 1), cord blood T cell phenotype and cytokine production and atopic dermatitis during the first year of life. *Pediatric Allergy & Immunology*, 15: 308-315

75. Smillie, F, Elderfield, A, Patel, F, Cain, G, Tavenier, G, Brutsche, M, Craven, M, Custovic, A, Woodcock, A. (2001) Lymphoproliferative responses in cord blood and at one year: no evidence for the effect of *in utero* exposure to dust mite allergens. *Clinical and Experimental Allergy*, 31: 1194-1204

76. Chan-Yeung, M, Ferguson, A, Chan H, Dimich-Ward, H, Watson, W, Manfreda, J, Becker, A. (1999) Umbilical cord blood mononuclear cell proliferative response to house dust mite does not predict the development of allergic rhinitis and asthma. *Journal of Allergy and Clinical Immunology*, 104(2): 317-321

77. Szepfalusi, Z, Pichler, J, Elsässer, S, van Duren, K, Ebner, C, Bernaschek, G, Urbanek, R. (2000) Transplacental priming of the human immune system with environmental allergens can occur early in gestation. *Journal of Allergy and Clinical Immunology*,106: 530-536

78. Thornton, C, Upham, J, Wikstrom, M, Holt, B, White, G, Sharp, M, Sly, P, Holt, P. (2004) Functional maturation of CD4+CD25+CTLA4+CD45RA+ T regulatory cells in human neonatal T cell responses to environmental antigens/allergens. *Journal of Immunology*, 173: 3084-3092

79. Rowe, J, Kusel, M, Holt, B, Suriyaarachchi, D, Serralha, M, Hollams, E, Yerkovich, S, Subrata, L, Ladyman, C, Sadowska, A, Gillett, J, Fisher, E, Loh, R, Soderstrom, L, Ahlstedt, S, Sly, P, Holt, P. (2007) Prenatal versus postnatal sensitization to environmental allergens in a high-risk birth cohort. *Journal of Allergy & Clinical Immunology,* 119: 1164-1173

80. Du Toit, G, Yitzhak, K, Sasieni, P, Mesher, D, Soheila, M, Fisher, H, Fox, A, Turcanu, V, Amir, T, Zadik-Mnuhin, G, Cohen, A, Livine, I, Lack, G. (2008) Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *Journal of Allergy & Clinical Immunology*, 122(6): 978-985

81. Fox, A, Lack, G. Peanut allergy: routes of pre-natal and post-natal exposure. Final Technical Report of FSA research project T07043 (http://www.foodbase.org.uk)

82. Hourihane, J, Warner, J. The influence of dose and route of exposure on the early life origins of peanut allergy. Final Technical Report of FSA research project T07028 (http://www.foodbase.org.uk)

83. Warner, J. The effect of exposure to food proteins via maternal sources in the development of food allergy in infants. Final Technical Report of FSA research project T07005 (http://www.foodbase.org.uk)

84. Fox, A, Meyer, R, DuToit, G, Syed, H, Sasieni, P, Lack, G. (2006) Retrospective recall of maternal peanut consumption using a multiple food-frequency questionnaire. *South African Journal of Clinical Nutrition* 19(4): 154-160

85. Kramer, M, Kakuma, R. (2008) Maternal dietary antigen avoidance during pregnancy or lactation, or both, for preventing or treating atopic disease in the child (Review). The Cochrane Library Issue 3 Art. No.: CD000133. DOI:10.1002/14651858.CD000133.pub2

86. Greer, F, Sicherer, S, Burks, W and the Committee on Nutrition and Section on Allergy and Immunology (2008) Effects of early nutritional interventions on the development of atopic disease in infants and children: The role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formula. *Pediatrics* 121(1): 183-191

87. Tarini, B, Carroll, A, Sox, Christakis, D. (2006) Systematic review of the relationship between early introduction of solids to infants and development of allergic disease. *Archives of Pediatric and Adolescent Medicine,* 160: 502-507

88. Sicherer, S, Burks, W (2008) Maternal and infants diets for the prevention of allergic diseases: understanding menu changes in 2008. *Journal of Allergy and Clinical Immunology,* 122:29-33

89. Sausenthaler, S, Koletzko, S, Schaaf, B, Lehmann, I, Borte, M, Herbarth, O, von Berg, A, Wichmann, H, Heinrich, J. (2007) Maternal diet during pregnancy in relation to eczema and allergic sensitisation. *American Journal of Clinical Nutrition,* 85: 530-537

90. Willers, S, Devereux, G, Craig, L, McNeill, G, Wijga, A, Abou El-Magd, W, Turner, S, Helms, P, Seaton, A. (2007) Maternal consumption during pregnancy and asthma, respiratory and atopic symptoms in 5 year olds. *Thorax,* 62: 773-779

91. Sahakyan, A, Armenian, H, Breitscheidel, L, Thompson, M, Enokyan, G. (2006) Feeding patterns of babies and the development of atopic dermatitis in children after 12 months of age in Armenia: is there a signal? *European Jounal of Epidemiology*, 21: 723-725

92. Filipiak, B, Zutavern, A, Koletzko, S, von Berg, A, Brockow, I, Grübl, A, Berdel, D, Reinhardt, D, Bauer, C, Wichmann, H, Heinrich, J. (2007) Solid food introduction in relation to eczema: results from a four-year prospective birth cohort study. *Journal of Pediatrics,* 151: 352-358

93. Mihrshahi, S, Ampon, R, Webb, K, Almqvist, C, Kemp, A, Hector, D, Marks, G. (2008) The association between infant feeding practices and subsequent atopy among children with a family history of asthma. *Clinical and Experimental Allergy*, 37: 671-679

94. Zutavern, A, Brockow, I, Schaaf, B, von Berg, A, Diez, U, Borte, M, Kraemer, U, Herbarth, O, Behrendt, H, Wichmann, H, Heinrich, J. (2008) Timing of solid food introduction in relation to eczema, asthma, allergic rhinitis, and food and inhalant sensitisation at the age of 6 years: results from the prospective birth cohort study LISA. *Pediatrics,* 121:44-52

95. Fussman, C, Todem, D, Forster, J, Arshad, H, Urbanek, R, Karmaus, W. (2007) Cows'milk exposure and asthma in a newborn cohort: repeated ascertainment indicates reverse causality. *Journal of Asthma*, 44: 99-105

96. Kull, I, Bergström, A, Lilja, G, Pershagen, G, Wickman, M.. (2006) Fish consumption during the first year of life and the development of allergic diseases during childhood. *Allergy*, 61: 1009-1015

97. Dunlop, A,, Reichrtova, E, Palcovicova, L, Ciznar, P, Adamcakova-Dodd, A, Smith, S, McNabb, S. (2006) Environmental and dietary risk factors for infantile atopic eczema and Slovak birth cohort. *Pediatric Allergy and Immunology,* 17: 103-111

98. Snijders, B, Thijs, C, van Ree, R, van den Brandt, P. (2008) Age at first introduction of cow milk products and other food products in relation to infant atopic manifestation in the first 2 years of life: the KOALA birth cohort study. *Pediatrics*, 122: 115-122

99. Estrada-Reyes, E, Pardo-Castaneda, M, Toledo-Bahena, M, Lerma-Ortiz, M, del Rio-Navarro, B, Nava-Ocampo, A. (2007) A case control study of food hypersensitivity, timing of weaning and family history of allergies in young children with atopic dermatitis. *Allergology and Immunopathology,* 35: 101-104

100. Arshad, S, Bateman, B, Sadeghnejad, A, Gant, C, Matthews, S. (2007) Prevention of allergic disease during childhood by allergen avoidance: The Isle of Wight prevention study. *Journal of Allergy and Clinical Immunology*, 119: 307-313

101. Romieu, I, Torrent, M, Garcia-Esteban, R, Ferrer, C, Ribas-Fitó, N, Antó, J, Sunyer, J. (2008) Maternal fish intake during pregnancy and atopy and asthma in infancy. *Clinical and Experimental Allergy,* 37:518-525

102. Alm, B, Aberg, N, Erdes, L, Mollberg, P, Petterson, R, Norvenius, G, Goksor, E, Wennerberg, G. (2008) Early introduction of fish decreases the risk of eczema in infants. *Archives of Disease in Childhood*, DOI: 10.1136/adc.2008.140418

103. Willers, S, Wijga, A, Brunekreef, B, Kerkhof, M, Gerritsen, J, Hoekstra, M, de Jongste, J, Smit, H. (2008) Maternal food consumption during pregnancy and the longitudinal development of childhood asthma. *American Journal of Respiratory and Critical Care Medicine*, 178: 124-131

104. Sicherer, S, Sampson, H, Burks, A. (2000) Peanut and soy allergy: a clinical and therapeutic dilemma. *Allergy*, 55: 515-521

105. Klemola, T, Kalimo, K, Poussa, T, Juntunen-Backman, K, Korpela, R, Valovirta, E, Vamto, T. (2005) Feeding a soy formula to children with cow's milk allergy: The development of immunoglobulin E-mediated allergy to soy and peanuts. *Pediatric Allergy & Immunology*, 16: 641-646

106. Koplin, J, Dharmage, S, Gurrin, L, Osborne, N, Tang, M, Lowe, A, Hosking, C, Hill, D, Allen, K. (2008) *Journal of Allergy & Clinical Immunology*, 121: 1455-1459

107. Akei, H, Mishra, A, Blanchard, C, Rothenberg, M. (2005) Epicutaneous antigen exposure primes for experimental eosinophilic esophagitis in mice. *Gastroenterology*, 129: 985-994

108. Elias, P. (2008) Skin barrier function. *Current Allergy and Asthma Reports* 8(4): 299-305

109. Gupta, R, Sheikh, A, Strachan, D, Anderson, H. (2007) Time trends in allergic disorders in the UK. *Thorax*, 62(1):91-96

Appendix

Glossary of Terms and Abbreviations

This list defines the terms and abbreviations that appear in the Statement, as they have been used by the COT.

Adjuvant	A substance that non-specifically enhances immune responses
Allergen	Substance capable of inducing an allergic immune response
Allergy	Adverse health effects resulting from stimulation of a specific immune response
Anaphylaxis	Acute and severe allergic reaction characterised by urticaria, shortness of breath, rapid fall in blood pressure and swelling of the throat and lips. Without immediate treatment, anaphylaxis can be fatal
Antibody	Immunoglobulin which is specific for an antigen or allergen
Asthma	Chronic inflammatory disease of the airways which renders them prone to narrow too much. The symptoms include paroxysmal coughing, wheezing, chest tightness and breathlessness. The inflammation is commonly, but not always, associated with allergy and, therefore, occurs in individuals who are genetically predisposed to produce IgE antibodies
Atopic dermatitis	An allergic skin disorder, characterised by severe itching, a distinctive distribution of eczematous skin lesions, and, often, a personal or family history of atopic diseases.
Atopic disease	Asthma, eczema/atopic dermatitis or hayfever
Atopy	A predisposition to mount IgE antibody responses. Atopy is associated with allergic disease and, in practice, atopic individuals are commonly defined as those who exhibit sensitisation to two or more allergens.
CBMC	Cord Blood Mononuclear Cell. CBMC is the mononuclear fraction of umbilical cord blood and comprises monocytes and lymphocytes and is usually considered to be indicative of T-cell responses to antigens.
CI	Confidence Interval
Cytokine	Soluble mediators that influence immune, inflammatory and other biological responses. Produced and secreted by T and B lymphocytes, macrophages and by a wide variety of other cells

DBPCFC	Double Blind Placebo Controlled Food Challenge. An *in vivo* test in which the patient and doctor do not know which food is being tested until after the tests and the recording of responses have been completed. Often regarded as the "gold standard" of food allergy testing but infrequently used in peanut allergy due to the severity of reactions that can be associated with this allergy.
Eczema	A group of skin conditions characterised by dry, red, flaky, itchy skin. The most common form of eczema is allergic or atopic eczema (also atopic dermatitis)
EMBASE	An abstract and indexing biomedical database, which contains records from 1974 to present
Epitope	A discrete antigenic determinant within a protein that is recognized by antibody or lymphocytes
Food allergen	Substance found in food capable of inducing an allergic sensitisation and allergic disease
Food allergy	Adverse reaction to a food or food component that is mediated via immunological mechanisms
Food hypersensitivity	Heightened responsiveness induced by allergic sensitisation to food.
Gavage	Feeding via oral tube directly into the stomach.
IgE antibody	One of five classes of human immunoglobulin. IgE is involved in allergy and anaphylaxis as well as protecting against parasitic infection
Immunisation	The deliberate induction of an immune response by administration of foreign protein, often in the presence of adjuvant
Immunoglobulins	A family of proteins from which antibodies are derived. There are five main classes of immunoglobulin in humans known as IgM, IgA, IgD, IgE and IgG
Intragastric	Within the stomach
Intranasal	Within the nose
Intraperitoneal	Within the membrane that lines the abdominal cavity
In utero	Within the uterus
in vitro	In an artificial environment, rather than inside a living organism (*in vivo*) – usually implies in laboratory culture

MEDLINE	The US National Library of Medicine's bibliographic database that contains references to journal articles in the life sciences. It hold citations from 1950 to present
Occlusive patch	A patch that covers an application site on the skin and which enhances dermal absorption
OFC	Open Food Challenge. Challenging the patient with the food suspected to cause the adverse reaction, without any attempt to hide the nature of the challenge from the observer or patient
OR	Odds Ratio
Peanut ***(Arachis hypogea)***	Also known as the groundnut or monkey nut. Comes from the legume family. Related botanically to peas and beans. Not related to tree nuts such as brazil, hazel or almond. Used in a number of foodstuffs and also used to produce peanut oil
Peanut oil	Also known as arachis oil.
Phenotype	The physical constitution of an organism as determined by the interaction of its genetic constitution and the environment
Prevalence	The proportion of a specified population with an attribute (e.g. having a disease) at a stated point in time, or during a stated period
PubMed	PubMed is a service of the US National Library of Medicine that includes over 18 million citations from MEDLINE and other life science journals for biomedical articles back to the 1950s
Rhinitis	Literally, inflammation of the nasal passages. Symptoms of nasal irritation, sneezing, rhinorrhea (running nose) and nasal blockage
Sensitisation	Immunological priming to an allergen such that the sensitised subject may exhibit an adverse reaction following subsequent encounter with the same allergen
SD	Standard deviation of the mean
SIGN	Scottish Intercollegiate Guidelines Network
SPT	Skin Prick Test. A test of allergenicity commonly used in allergy clinics
Th2 cells	T helper (CD4+) lymphocytes of the type 2 subgroup which produce cytokines that promote IgE antibody production and allergic responses

T helper cells	In general, T cells (CD4+) which help B lymphocytes to produce antibodies. Two principal subtypes exist. Th1 cells produce IFN-γ amongst other cytokines and antagonise IgE responses. Th2-type cells produce interleukins that promote IgE production and allergic sensitisation
T lymphocytes	Thymus-dependent lymphocytes which, amongst other functions, help B lymphocytes during immunological responses and provide protection from intracellular microbial infection. Distinct sub-populations have been characterised - see T helper cells above
Tolerance	Specific immunological unresponsiveness or hyporesponsiveness resulting from exposure to antigen
Tolerogenic	Producing immunologic tolerance - see tolerance above
Wheeze	A high-pitched whistling sound during breathing. It occurs when air flows through narrowed breathing tubes

COT Statement on the 2006 UK Total Diet Study of Metals and Other Elements

Issue

1. The Food Standards Agency (FSA) has completed a survey of aluminium, antimony, arsenic, barium, bismuth, cadmium, chromium, copper, germanium, indium, lead, manganese, mercury, molybdenum, nickel, palladium, platinum, rhodium, ruthenium, selenium, strontium, thallium, tin and zinc in the 2006 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 any of the elements in the diet posed a risk to human health. The COT last evaluated population and consumer exposures to twelve of these elements (aluminium, arsenic, cadmium, chromium, copper, lead, manganese, mercury, nickel, selenium, tin and zinc) in 2003, using data from the 2000 TDS[1]. Eleven other elements (antimony, barium, bismuth, germanium, molybdenum, palladium, platinum, rhodium, ruthenium, strontium and thallium) were last analysed in the 1994 TDS and evaluated by the COT in 1998[2]; and indium was included for the first time in the 2006 TDS.

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, together with food consumption data from the National Diet and Nutrition Survey (NDNS), to estimate dietary exposures of the general UK population to chemicals in food, such as nutrients and contaminants, to identify changes or trends in exposure, and to make assessments on the safety and quality of the food supply. Such data can then be used as background information when considering issues such as the possible health impact of incidents of high-level contamination, and regulatory levels for nutrients and contaminants in various foodstuffs. Results from the TDS also indicate where there is a need for more targeted surveys, such as of arsenic levels in food, metals in infant food, and mercury levels in fish, all of which have been the subject of previous statements[3,4,5]. 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 elsewhere6 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 food purchase data from the National Food Survey (now the Expenditure and Food Survey, EFS). Foods are grouped so that commodities known to be susceptible to contamination (e.g. offal, fish) are kept separate, as are foods that are consumed in large quantities (e.g. bread, potatoes, milk)[6,7].

4. The survey data provided to the Committee related only to food prepared as for consumption. Information on exposure from other sources, such as drinking water and dietary supplements, is not captured by the TDS methodology. The Committee was informed on exposures from drinking water and dietary supplements using data from the Drinking Water Inspectorate (DWI) and the Expert Group on Vitamins and Minerals (EVM), respectively[8,9].

5. At present there are no specific limits on the levels of trace elements, minerals or other micronutrients that may be contained in supplements sold under food law, although the EU is currently in the process of setting maximum permitted levels for vitamins and minerals in dietary supplements. Industry guidance on upper levels of vitamins and minerals is available for manufacturers of supplements to ensure levels are not excessive. However, the supplements industry is not obliged to follow this guidance and is only bound by the provisions of the Food Safety Act, which make it an offence to offer for sale a food product that is injurious to health. The use of dietary supplements has increased during the last decade[10-12]. Vitamin and mineral supplements surveys suggest that between 20 and 40% of the UK adult population take supplements, with use most common among women aged 50-65 years[13-16]. The EVM has advised on supplemental amounts of minerals that even in conjunction with high exposure from food and drinking water would not result in safe upper levels of intake being exceeded[9]. Where supplements on the UK market exceed these amounts, the FSA has made recommendations for reformulation or labelling with advisory statements in advance of the EU regulations on maximum permitted levels[5].

6. Consideration of speciation of an element is an important component of risk assessment as it focuses the toxicological evaluation on the most relevant species, and allows a better understanding of the mechanisms of toxicity. Despite advances in speciation analysis in the past 20 years and the availability of methods for determination for some elements[17], in general, the TDS only determines the total concentration of elements. Elemental toxicity may vary according to the oxidation state, the formation of complexes, and the biotransformation of the element[17]. The relevance of speciation to health effects in humans has been demonstrated for a number of endpoints - for example, acute toxicity (lead), sensitisation (nickel), neurotoxicity (manganese), nephrotoxicity (cadmium), reproductive toxicity (mercury), genotoxicity (chromium), and carcinogenicity (arsenic)[17]. Where the 2006 TDS provided information on the chemical forms in which an element occurred in foods, the COT took this into account in its evaluation of potential risks to health. However, there were uncertainties in the risk assessment where this information was unavailable or published toxicological data did not relate to the same chemical forms of the element as occurred in food.

Concentrations of the elements in the foods surveyed

7. The full results of the 2006 TDS are published in a Food Survey Information Sheet[18]. In general, the concentrations of each of the elements in the food groups were lower than or similar to those reported in the 1994 and 2000 total diet studies, with the exception of aluminium, barium and manganese.

8. Most of the food groups had aluminium concentrations lower than or similar to those reported in the 2000 TDS, the exceptions being bread, meat products, and other vegetables groups. The miscellaneous cereals group had the highest mean concentration of aluminium (17.5 mg/kg), although this was lower than the concentration in the 2000 TDS (19 mg/kg). The miscellaneous cereals group was the main contributor to the population dietary exposure (42%) to aluminium. Possible sources of aluminium in this food group include aluminium compounds present naturally, aluminium-containing additives, and contamination from processing and storage of food in aluminium-containing utensils.

[5] http://www.food.gov.uk/foodindustry/guidancenotes/labelregsguidance/supplementreformguidance

9. Barium concentrations were similar to or lower than those reported in the 1994 TDS except for the nuts group, in which the mean concentration was 131 mg/kg compared to 56 mg/kg in 1994.

10. Manganese concentrations were similar to or lower than those reported in the 2000 TDS except for the bread, miscellaneous cereals and meat products groups. The largest increase (nearly 2-fold) was seen in the meat products group.

Dietary exposure assessment

11. The exposure assessments reported for the 2006 TDS were made by combining concentration data for the food groups with corresponding consumption data. The main source of data used by the FSA to estimate food consumption is the NDNS[15,19-22]. The NDNS was carried out as a series of cross-sectional surveys of diet and nutritional status; data from each of four age groups were collected over the years 1992-1993 (pre-school children aged 1.5-4.5 years, commonly referred to as toddlers), 1994-1995 (elderly), 1997 (young people), and 2000-2001 (adults). The Committee noted that these food consumption data used to estimate exposures might not reflect current dietary habits and did not include children under 18 months or sufficient data to estimate the intake of sub-groups such as ethnic minorities. The respondents in the surveys were asked to complete diaries of foods and beverages consumed over a 4 or 7 day period (depending on the survey), inside and outside the home. Quantities of foods consumed at home were assessed by weighing them with digital scales. Quantities of foods eaten outside the home were estimated from descriptions referenced to household measures. The dietary information was recorded "as consumed" so recipes were required to identify the food components. These recipes were obtained from the respondent's diaries, food manufacturers or published sources (e.g. recipe books and websites). The fieldwork covered a 12-month period to account for possible seasonal variations in eating habits. Other surveys such as the Expenditure and Food Survey[23] and the Dietary Survey of Vegetarians24 provided supporting information. The EFS is carried out annually and provides data on food purchases at a household level. This information is used to inform the quantities and relative proportions of each food that contributes to the total diet. The fieldwork for the Dietary Survey of Vegetarians was carried out during 1994-1995[24].

12. The vast majority of FSA dietary exposure assessments for chemicals are carried out using a bespoke in-house software package known as the Intake Programme. This programme estimates exposure by combining data on the concentration of a chemical in each food group with information on the distribution of individuals' food consumption patterns. Participants in the NDNS keep a diary of their food consumption, from which calculations are made of the total amount of each food group that each individual consumed. With the assumption that each food group contained an element at the concentration at which it was measured in the TDS, an estimate was made of the total daily amount of the element that each participant consumed. From the distribution of estimated exposures across all participants, values for mean- and high-level (97.5th percentile) exposure were then derived, which represent estimated exposures for individuals who consume average amounts of the element from food (mean-level consumers) and those who are among the highest consumers (high-level consumers). Where a chemical could not be detected in one or more food groups, two alternative calculations were made. In the first, all undetectable concentrations were assumed to be zero (lower bound), and in the second, they were all assumed to be at the limit of detection for the method of assay (upper bound)[6].

[6] In the calculation of upper bound exposures for inorganic arsenic, the concentration in the food groups was assumed to be equal to the concentration of total arsenic (since this was lower than the LOD for inorganic arsenic) except in the case of the poultry food group where it was considered to be equal to the LOD.

Mean- and high-level exposures were then each expressed as a range, with the lower bound derived under the first assumption and the upper bound under the second.

13. Table 1 compares the estimated dietary intakes of each element that was measured in the 2006 TDS for the consumer groups for which consumption data were available, and also summarises relevant tolerable intakes or other health based guidance values where they exist.

14. Estimates of dietary exposure for the different consumer groups 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 toxicological data on the elements[25]. The PTWI is used by JECFA to define tolerable intakes of food contaminants with the potential to accumulate in the body. In this statement, the PTWI has been divided by 7 to provide a tolerable daily intake (TDI) for comparison with the estimated daily dietary exposures (Table 1).

15. Exposure estimates were also carried out at the population level in order to assess trends in average exposure for the UK population as a whole. Such trends may reflect changes in food consumption patterns, changes in the concentrations of elements in foods, or both. Population dietary exposures were estimated by multiplying the average amount of each food group consumed (based on consumption data from the EFS survey and expressed in mg/day) by the corresponding elemental concentration in the food group from the TDS study, and then summing across all food groups. The EFS covers the total number of people in a household regardless of whether they ate specific foods or not, and so the EFS consumption data are averaged for the whole population. Tables 2a and 2b compare mean population dietary exposures to the 24 elements in the UK total diet studies dating back to 1976.

16. Exposures from drinking water were estimated for those elements included in The Water Supply (Water Quality) Regulations 2000[26]. Results from monitoring undertaken by each water company in 2007 provided a 1st percentile and a 99th percentile at consumers' taps, except where fewer than 100 samples were taken, when the figures are the actual maximum and minimum results8. The 99th percentile or maximum concentration was used to calculate exposure estimates from drinking water, based on UK data for chronic tap water consumption (mL/kg body weight/day, 97.5th percentile) of 158 pre-school children, 60 young people, 39 adults, and 28 elderly[15,19,21,22]. For those elements not included in The Water Supply (Water Quality) Regulations 2000, WHO Guidelines for Drinking-water Quality were used to estimate potential exposure from drinking water[27]. Exposure estimates from drinking water represent a worse case scenario because they are based on chronic water consumption at the maximum concentration of the element. Concentrations of elements in drinking water vary over time, even in the same region, and so it is highly unlikely that exposure to the maximum concentration would occur persistently over a prolonged period. Estimates of exposure from drinking water were carried out for aluminium, antimony, arsenic, barium, cadmium, chromium, copper, lead, manganese, mercury, molybdenum, nickel, and selenium (see Table 3).

COT evaluation

17. Health based guidance values were available for aluminium, antimony, barium, cadmium, chromium, copper, lead, manganese, mercury, nickel, selenium, tin, and zinc. There were no relevant tolerable intakes or reference doses by which to assess the safety of exposure to total or inorganic arsenic, bismuth, germanium, indium, molybdenum, palladium, platinum, rhodium, ruthenium, strontium or thallium.

Aluminium

18. Aluminium occurs naturally in the environment where it is present in its 3+ oxidation state and not in the metallic elemental state. Natural processes such as soil erosion and weathering of rocks, as well as human activities, result in the release and redistribution of aluminium compounds to other environmental compartments. Aluminium may form organic and inorganic compounds and is naturally present in varying amounts in most foodstuffs. The use of aluminium and aluminium compounds in processing, packaging and storage of foods, and as flocculating agents in the treatment of drinking water, all contribute to its presence in foods and drinking water. Medicinal antacid preparations can provide much larger aluminium doses, of up to 5 g per day.

19. Speciation is an important factor when considering the absorption of aluminium and it is widely assumed that soluble aluminium compounds are more bioavailable than insoluble compounds[28]. Absorption is also influenced by the presence or absence of particular foods and beverages (dietary ligands) in the intestines, and by acid digestion in the stomach. Citrate, which could be present in foods consumed at the same time as aluminium-containing foods, increases the absorption of aluminium[29]. The net absorption of aluminium from food is approximately 1%, although this varies based on the chemical forms present in the intestinal tract[28,29]. This low bioavailability is due to the formation of aluminium complexes as the pH increases from the stomach to the intestines.

20. The JECFA recently revised the PTWI for aluminium because of emerging evidence that aluminium compounds have the potential to affect the reproductive system and developing nervous system at doses lower than the NOAEL used in establishing the previous PTWI28. The PTWI was reduced from 7 mg/kg body weight to 1 mg/kg body weight, and applies to all aluminium compounds, including additives[28]. The PTWI incorporated a total uncertainty factor of 300 applied to the lower end of the range of LOAELs (50 mg Al/kg body weight/day) to allow for inter- and intra-species differences and deficiencies in the the database, notably the absence of NOAELs in the majority of the studies evaluated and the absence of long-term studies on the relevant toxicological end-points. The deficiencies were counterbalanced by the probable lower bioavailability of the less soluble aluminium species present in food. Overall, an additional uncertainty factor of three was considered to be appropriate. The JECFA noted that dietary exposure through foods containing aluminium compounds used as food additives represented the major route of aluminium exposure for the general population, but that further data were required on the bioavailability of different aluminium-containing food additives[28]. The EFSA also recently evaluated the safety of aluminium from dietary intake, basing its evaluation on the combined evidence from several studies showing adverse effects on testes, embryos and the developing and mature nervous system following dietary administration[29]. The EFSA derived the same TWI of 1 mg/kg body weight, noting that it was not possible to draw conclusions on the specific sources contributing to the aluminium content of a particular food[29]. Limitations of the studies

evaluated by the JECFA and the EFSA included lack of information on dose-response relationships, and on specific individual aluminium compounds or species present in food, and a failure in some studies to consider the basal aluminium content of the animals' feed.

21. Estimates of dietary exposure to aluminium (high-level intake for adults, pre-school children, young people, institutionalised elderly and vegetarian groups; and mean-level intake for pre-school children) exceeded the PTWI set by the JECFA and the EFSA (equivalent to 143 µg/kg body weight/day) by up to 2.4-fold. The current average population exposure to aluminium (5.4 mg/day) was increased compared to that reported in the 2000 and 1997 total diet studies (4.7 mg/day and 3.4 mg/day, respectively) but lower than previous estimates (10 mg/day and 11 mg/day in 1991 and 1994, respectively). In previously discussing the 2000 TDS, the Committee noted that 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 was seen in the miscellaneous cereals group and this was considered possibly to 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[1].

22. In the 2006 TDS most of the food groups had aluminium concentrations lower than or similar to those reported in the 2000 TDS, the exceptions being the bread, meat products, poultry, other vegetables, canned vegetables and fresh fruits groups, all of which had higher concentrations of aluminium compared to those reported in the 2000 TDS. The miscellaneous cereals group had the highest mean concentration of aluminium (17.5 mg/kg). This was lower than the concentration in the 2000 TDS (19 mg/kg) but three times greater than the concentration from the 1997 TDS (5.2 mg/kg). The levels of aluminium in this food group have varied from 4.8 mg/kg (1988 TDS) to 78 mg/kg (1994 TDS).

23. The miscellaneous cereals group, which comprises cakes, scones, biscuits, breakfast cereals, flour and rice, was the principal dietary contributor to the population dietary aluminium exposure (42%). Possible contributors to the relatively high aluminium concentration found in this group include naturally present aluminium compounds, aluminium-containing additives which are permitted for use in some bakery products[30,31], and contamination from processing and storage of food in aluminium-containing utensils.

24. The results of the 2006 TDS show an apparent increase in dietary exposure to aluminium, although this is within the estimated mean dietary exposure of European adults (1.6-13 mg/day)29. Variations in dietary exposure may be accounted for by differences in soil composition in the regions where food is produced, in individual dietary patterns and in consumption of foods with aluminium-containing food additives. It is acknowledged throughout Europe, that for certain groups of the population, exposure to aluminium will exceed the PTWI. This includes infants and young children, who have a higher food intake than adults when expressed relative to body weight[29]. Consumption of tap water has the potential to increase high-level exposure to aluminium by 3-7%, such that the worst case high level intake of pre-school children could exceed the PTWI by 2.7-fold.

25. The Committee noted that whilst the estimates of dietary exposure to aluminium were not markedly higher than previous estimates, they present uncertainty with regard to the safety of aluminium in food in the light of the recent reduction in the PTWI, which is exceeded by some population subgroups. There is a need for further information on possible sources and forms of aluminium in the diet and their bioavailability.

Antimony

26. Antimony was detected in most of the food groups except the oils and fats, eggs, and milk groups. The meat products group had the highest concentration of antimony (0.0099 mg/kg). The estimates of dietary exposure to antimony for all population subgroups were well below the TDI of 6 µg/kg body weight/day set by the WHO in 2003. The TDI was based on a NOAEL of 6 mg/kg body weight/day for decreased body weight gain and reduced food and water intake in a 90-day drinking water study in rats; and an uncertainty factor of 1000 (100 for inter- and intra-species variation and 10 for the short duration of the study)[27]. The toxicity of antimony is a function of the water solubility and the oxidation state of the species, with antimony (III) being more toxic than antimony (V) and inorganic compounds being more toxic than organic compounds[27]. No information was provided on how this TDI was set in relation to speciation, although, the WHO noted that antimony leached from antimony-containing materials would be in the form of the antimony(V) oxo-anion, which is the less toxic form. Intake of antimony from drinking water could be up to about twice that from high-level intake from food, but estimated worst case total intake is below the TDI.

27. The Committee concluded that the estimated dietary exposures to antimony were not of toxicological concern.

Arsenic

28. The toxicity of arsenic is dependent on the form (inorganic or organic) and the oxidation state of arsenical compounds. It is generally accepted that inorganic arsenic compounds are more toxic than organic arsenic compounds, with the toxicity being linked to the soluble inorganic trivalent forms[27].

29. In 2003 the Committee recommended that future surveys should measure both total and inorganic arsenic and include consideration of other sources of exposure such as water[1]. The 2006 TDS surveyed both total and inorganic arsenic but this information was not available for the arsenic content of water. Food is generally the principal contributor to the daily intake of total arsenic in non-occupationally exposed individuals32, but water can contribute more to the intake of inorganic arsenic. The levels of arsenic reported in drinking water in England & Wales for 2007 ranged between 0.3 and 7.9 µg/L[8]; and high-level intake from this source could be up to 1.2 µg/kg body weight/day for pre-school children, or 0.3 µg/kg body weight/day for adults. Since this is likely to be all in the inorganic form, it exceeds the intake from food. There is potential for significant exposure from work in some industries, although in the UK, arsenic and its compounds have been assigned a maximum airborne exposure limit of 0.1 mg/m^3 (averaged over an 8-hour period)[33]. For people who are not occupationally exposed, inhalation exposure can contribute up to approximately 10 µg/day in a smoker and about 1 µg/day in a non-smoker[32]. Other potential sources of exposure include contaminated soils and polluted atmospheres[32].

30. The Committee has concluded previously, when considering the 1999 TDS of total and inorganic arsenic, 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 COT considered that the approach used to establish the JECFA PTWI for inorganic arsenic (15 µg/kg body weight) in 1989 would now not be considered appropriate, in view of the evidence of genotoxicity and carcinogenicity[3]. When establishing the PTWI, the JECFA noted the epidemiological evidence of an association between overexposure of humans to inorganic arsenic from drinking water and an increased cancer risk, and also noted that skin cancer did

not occur in the absence of other toxic effects of arsenic[34]. The COT concluded that inorganic arsenic is genotoxic and a known human carcinogen and therefore exposure should be as low as reasonably practicable (ALARP)[3]. The European Commission has requested that the EFSA evaluate the risks to human health related to the presence of arsenic in foodstuffs (including drinking water), covering the ratios between inorganic and organic arsenic forms, the contribution of different foodstuffs to exposure, and the exposures of specific population groups. There is currently an open call for relevant data with the objective to collect all available data analysed during the time period from January 2003 to November 2008[35]. These data will then be used by the EFSA in its risk assessment of arsenic in food.

31. The estimates of average population dietary exposures to total arsenic in the 2006 TDS were comparable to those reported in the 1999 TDS of total and inorganic arsenic (0.061 - 0.064 mg/day and 0.055 mg/day, respectively[3]). The current population exposure to total arsenic was also similar to that reported in total diet studies since 1991 (see Table 2a). In discussing the 1999 and 2000 total diet studies, the Committee previously noted that fish was the major contributor to dietary exposure to arsenic and that the predominant form of arsenic in fish is organic[1,3]. Inorganic arsenic contributed less than 10% of the total dietary exposure to arsenic in 1999. Similarly, the results of the 2006 TDS indicate that fish was the major contributor to dietary arsenic exposure and that inorganic arsenic contributed less than 12% of the total dietary exposure.

32. With regard to population dietary exposures to total arsenic, since 1976 intakes have fluctuated but the general trend appears to be downwards. Therefore, the previous COT conclusion that the organic arsenic component is unlikely to constitute a hazard to health appears still valid. The average population dietary exposure to inorganic arsenic was 0.0014 - 0.007 mg/day and was comparable to the range reported in 1999 (0.0009 - 0.005 mg/day)[3] and therefore does not raise any new concern. Furthermore, although there is uncertainty about whether the JECFA PTWI for inorganic arsenic is sufficiently protective, estimated dietary exposures in all of the population groups examined were less than 20% of the PTWI (Table 1), and possibly less than 10% of the PTWI, taking into account the large number (18/20) of food groups with inorganic arsenic levels below the limit of detection.

33. The Committee concluded that the data on arsenic appear consistent with previous surveys of total and inorganic arsenic in food. Current dietary exposure to organic arsenic is unlikely to constitute a risk to health. The advice on inorganic arsenic continues to be that exposures should be ALARP.

Barium

34. Barium occurs in nature as a divalent cation in combination with other elements. The two most prevalent naturally occurring barium ores are barium sulphate and barium carbonate[36]. Barium sulphate is present in soils but only a limited amount accumulates in plants. The main route of exposure to barium compounds for the general population is oral intake via drinking water and food, with food being the primary source[36]. Where barium levels in water are high, associated with groundwater of low pH, drinking water may contribute significantly to barium intake[27]. No data are available on levels of barium in drinking water in the UK.

35. Case reports indicated that in humans, intentional or accidental ingestion of barium can cause gastroenteritis, hypokalaemia and hypertension. The WHO considered that the critical end-points for deriving a TDI for barium are hypertension and impaired renal function[36]. Hypertensive effects have

been observed in humans who ingested acute high doses of barium compounds and in workers who inhaled barium carbonate and dusts of barium ores. Hypertension has also been reported in rats exposed to barium chloride in drinking-water for 1 month at an estimated daily dose of 7.1 mg barium/kg body weight. Drinking water studies in rats and mice also indicated the kidney to be a sensitive target organ, with a lowest identified NOAEL of 45 mg/kg body weight in female rats given barium chloride in drinking water for 2 years. The WHO identified a NOAEL of 0.21 mg barium/kg body weight/day from a 10-week experimental study in humans (barium chloride in drinking water up to 10 mg/L) and an epidemiological study in populations living in communities with mean drinking water barium concentrations of 0.1 and 7.3 mg/L. Blood pressures were not significantly affected by barium exposure in either study. Applying an uncertainty factor of 10 to the NOAEL to allow for database deficiencies and differences between adults and children resulted in derivation of a TDI of 20 µg/kg body weight[36]. The WHO assigned medium confidence to this tolerable intake because neither study identified a LOAEL, and noted that there were uncertainties about the most sensitive toxic end-point in humans, and about whether there were differences in toxicity or toxicokinetics between adults and children.

36. In its Guidelines for Drinking Water, the WHO used the NOAEL of 7.3 mg/L from the epidemiological study described above in which a population with drinking water containing a mean barium concentration of 7.3 mg/L were compared with a population whose water contained a barium concentration of 0.1 mg/L. Subjects were selected randomly from a pool that included every person 18 years of age or older that had lived in the community for more than 10 years. There were no significant differences between the two populations in the mean systolic or diastolic blood pressures, or in history of hypertension, cardiovascular disease, or kidney disease, and thus no LOAEL was identified. An uncertainty factor of 10 was applied to the NOAEL to allow for intra-individual variation, resulting in a guideline value of 0.7 mg/L[37]. Assuming a 60 kg adult drinking 2 litres of water per day, this guideline value is equivalent to 23 µg/kg body weight/day which is comparable to the TDI established by the WHO in 2001, as described in paragraph 35. Both of these reference doses apply to barium as an element, and were derived from studies with barium chloride.

37. As with the results from 1994, the highest levels of barium in the 2006 survey were reported in nuts (131 mg/kg) and bread (0.81 mg/kg). All other foodstuffs contained lower levels than in bread. Levels of barium in nuts were double those reported in 1994 (131 mg/kg and 56 mg/kg, respectively). Estimated average population dietary exposures to barium have increased by approximately 46% since the last TDS in 1994. The estimates of dietary exposure to barium for pre-school children (mean- and high-level dietary intakes) and of high-level intakes by adults, young people, free living elderly, and vegetarians, exceeded the WHO TDI of 20 µg/kg body weight/day by up to 4.3-fold. Consumption of tap water has the potential to increase high-level exposure to barium by 60-130%, which could result in a total dietary exposure for high-level intake in pre-school children of 980% of the WHO TDI. However, because barium levels in water vary over time and given that the drinking water estimated exposure was based on the WHO guideline value and not a measured concentration, it is highly unlikely that this worst case scenario would occur over a prolonged period.

38. The population exposures that most exceeded the TDI were the high-level intakes for adults (~220% of the TDI), young people and vegetarians (~320% of the TDI), and pre-school children (~420% of the TDI). The mean population group exposures were below or in the region of the WHO TDI. Since the TDI is derived from studies in which no statistically significant effects were observed, the LOAEL could have been very much higher than the dose identified as a NOAEL, and hence the TDI may be over-precautionary. Therefore, the Committee concluded, that the estimated exposures, which exceeded the TDI by up to 4-fold, were not necessarily a toxicological concern. The Committee noted the uncertainty regarding the lack of information on effect levels and on the bioavailability of barium in the principal food group (nuts).

Bismuth

39. Bismuth was analysed previously in the 1994 TDS. Since 1994, estimated population dietary exposures have increased by 5-fold from 0.4 µg/day to 2 µg/day. There are no health based guidance values for bismuth. Bismuth is widely used in many medical applications, such as in compounds used in the treatment of diarrhoea, nausea and other gastrointestinal disturbances, and suppressants of lupus erythmatosus. Insoluble bismuth salts have also been used in cosmetics. No data are available on levels of bismuth in drinking water in the UK, and a WHO drinking water guideline value has not been set.

40. In 9 patients being treated with tripotassium dicitratobismuthate for 6 weeks, Gavey *et al.*[38] found that a daily oral dose of 432 mg/day was without adverse effect. This dose is equivalent to approximately 7000 µg/kg body weight/day for a 60kg adult. The margin of exposure between this human therapeutic dose and the highest estimated dietary exposure (0.217 µg/kg body weight/day; high-level intake by pre-school children) is 32300 (rounded to the nearest 100). This margin of exposure indicates a low concern for human health at the highest high-level dietary exposure. The Committee noted that doses used in medicines are very much larger than the estimated dietary exposure. The Committee concluded that dietary exposures to bismuth were unlikely to be of toxicological concern.

Cadmium

41. The major route of exposure to cadmium for the non-smoking population is via food, due to contamination of soil and water. Gastrointestinal absorption is influenced by the type of diet and nutritional status. For example, low iron status increases the uptake of cadmium. Cadmium was present at low concentrations in ten of the food groups, with the highest concentrations in the offal (0.084 mg/kg) and nuts (0.065 mg/kg) groups. The estimates of dietary exposure to cadmium for all population subgroups were below the PTWI of 7 µg/kg body weight (equivalent to 1 µg/kg body weight/day) set by the JECFA in 1989[4]. The PTWI was based on the risk of kidney damage at levels of cadmium in excess of 50 µg/g in the renal cortex. Assuming an absorption rate of 5% and a daily excretion of 0.005% of body burden, the JECFA concluded that total intake should not exceed 1 µg/kg body weight/day continuously for 50 years; the PTWI was maintained in 2003[74]. No information was provided on how the PTWI was set in relation to speciation. Cadmium toxicity arises only when the chelation capability of metallothionein in the critical organs or tissues is used up[17]. Consumption of tap water has the potential to increase high level dietary exposure to cadmium by about 10-25%, but estimated worst case total intake is below the TDI.

42. The Committee concluded that the estimated dietary exposures to cadmium were not of toxicological concern. This conclusion might need to be reviewed after completion of a risk assessment by the EFSA.

Chromium

43. The toxicity of chromium varies depending on the valency state, with hexavalent chromium being more toxic than trivalent chromium, which is an essential trace element. Ingested trivalent chromium has a low level of toxicity, due partly to its poor absorption. Hexavalent chromium and its compounds are oxidizing agents capable of directly inducing tissue damage, and epidemiological studies have found an association between exposure to hexavalent chromium and lung cancer[39]. The EVM considered that for guidance purposes, an intake of 150 µg/kg body weight/day trivalent chromium would be expected to be without adverse health effects[9].

44. Almost all of the sources of chromium in the earth's crust are in the trivalent state, and chromium compounds in the hexavalent state are almost always derived from human activities[39]. Hexavalent chromium in the soil tends to be reduced to trivalent chromium by organic matter; and studies with gastric juices have demonstrated that hexavalent chromium is reduced to the trivalent form in the gastrointestinal tract[40]. It was noted that chromium in food is likely to be largely, if not entirely, in the trivalent form. Analysis of hexavalent chromium in a range of infant foodstuffs did not detect any hexavalent chromium, although these observations were limited by the relatively high limit of detection (300 ng/g)[41].

45. The estimates of dietary exposure to chromium (mean- and high-level intakes) for all consumer groups were within the EVM guidance level for trivalent chromium of 150 µg/kg body weight/day. Results from total diet studies indicate that average dietary exposures to chromium have been steadily declining since 1991. The estimated population dietary exposure to chromium from the 2006 TDS was 0.022-0.029 mg/day, reduced from 0.046 mg/day in 2000. The Committee on Medical Aspects of Food and Nutritional Policy (COMA) did not set reference nutrient intakes (RNIs) for chromium but suggested that an adequate level of intake lies above 0.025 mg/day for adults (equivalent to 0.4 µg/kg body weight/day for a 60 kg adult) and between 0.1 and 1 µg/kg body weight/day for children and adolescents[42]. The current population dietary exposure was comparable to the COMA suggested adequate level of intake for adults. Consumption of tap water has the potential to increase high-level dietary exposure to chromium by 50-120%. In addition dietary supplements could provide up to 0.6 mg/day (10 µg/kg body weight/day for a 60kg adult)[9]. Worst case total intake from food, water and supplements is well below the EVM guidance level.

46. The Committee concluded that current dietary exposures to chromium were unlikely to be of toxicological concern.

Copper

47. Copper is an essential trace element that has two valency states, copper (I) and copper (II). Copper was present in all of the food groups analysed in the 2006 TDS. The offal (52.5 mg/kg) and nuts (9.15 mg/kg) groups contained the highest concentrations of copper. Although copper is an essential trace element, high levels can cause acute gastrointestinal effects. This may be a direct irritant effect of copper in

water and is not so apparent when copper is present in the food matrix[9]. The estimates of dietary exposure for all population subgroups were well within the PMTDI of 50-500 µg/kg body weight/day set by the JECFA in 1982[43] and the safe upper level of 160 µg/kg body weight/day set by the EVM[9]. The JECFA PMTDI was initially proposed in 1973 on the basis of human epidemiological and nutritional data related to background exposure to copper. The EVM safe upper level was based on a 13-week feeding study of copper sulphate in rats in which the NOAEL was 16 mg/kg body weight/day, with effects on the liver, kidney and forestomach seen at higher doses. Reported minimum and maximum tap water concentrations for 2007 were 0.015 and 4.25 mg copper/L, respectively[8]. Consumption of tap water has the potential to increase high-level dietary exposure to copper by up to three-fold in pre-school children, which could result in a total dietary exposure of 190% of the EVM safe upper level. However because levels of copper in water vary over time, even in the same region, it is highly unlikely that this worst case scenario would occur over a prolonged period. In addition dietary supplements could provide up to 2 mg/day (33 µg/kg body weight/day for a 60kg adult)[9].

48. The Committee concluded that the estimated mean- and high-level dietary intakes of copper were unlikely to be of any toxicological concern.

Germanium

49. Germanium can exist in valency states of 2 and 4, and was last analysed in a TDS in 1994. Since 1994, average population dietary exposures have decreased from 4 µg/day to 0.1-1.5 µg/day. Based on population dietary exposures estimated from the 1994 TDS, the COT previously concluded that the estimated dietary intakes of germanium in adults did not give cause for concern[2]. There are no health based guidance values for germanium but the EVM concluded that naturally occurring germanium present in food does not appear to be associated with any adverse effect, though there were insufficient data to define a NOAEL for chronic exposure[9]. No information was available on what forms of germanium are naturally present in foods or on potential intake from drinking water.

50. The Committee noted that population dietary exposures have decreased significantly since 1994 and given that germanium was not detected in most (18/20) of the food groups analysed in the 2006 TDS, the current dietary exposures to germanium were unlikely to be of toxicological concern.

Indium

51. Indium has not previously been included in a TDS. A food survey conducted in 1979 found concentrations in nine food groups to be low, with only fats and green vegetables showing concentrations of indium above the limit of detection. The mean daily dietary intake of indium was estimated as between 5 and 27 µg[44]. In the 2006 TDS, the average population dietary exposure to indium was comparable at 5 - 19 µg/day. With the exception of the canned vegetables and fruit products groups, indium concentrations were below the limit of detection of 0.003 – 0.02 mg/kg. For these two food groups, indium concentrations were 0.096 mg/kg (canned vegetables) and 0.031 mg/kg (fruit products). In 1998 the COT evaluated the results from a multi-element survey of cows' milk and vegetables produced near industrial sites, concluding that the intakes of indium in adults were very low[45]. There are no health based guidance values for indium. No information is available on indium in drinking water in the UK and a drinking water guideline has not been set by the WHO.

52. There are no data or reports of human toxicity from oral exposure to indium. Developmental toxicity was reported following oral gavage administration of indium chloride to rats at doses of 100 mg/kg body weight/day on days 6-15 of gestation[46] and 300 mg/kg body weight on day 9 of gestation[47]. Indium was found to cross the placenta resulting in a direct cytotoxic action on the embryo[46]. A lifetime drinking water study in mice conducted with indium chloride suggested a LOAEL of 250 µg/kg body weight/day for growth suppression[48]. The margin of exposure between this chronic mouse LOAEL and the highest estimated dietary exposure (0.93-1.48 µg/kg body weight/day; lower-bound to upper-bound estimate for high-level intake in pre-school children) is 170 - 270 (rounded to the nearest 10). There are no data on indium toxicity from food and the implications of the estimated dietary exposures to indium and margins of exposure are uncertain.

53. The Committee concluded that, although there is uncertainty, the sparse data available did not suggest that the estimated dietary exposures to indium give cause for toxicological concern.

Lead

54. Lead is dispersed throughout the environment as a result of human activities and food is one of the major sources of exposure[27,49,50]. Lead in foods may be derived from the environment in which the food is grown (air pollution from nearby industrial sources) or from preparation of foods with lead-contaminated water and/or utensils[27]. The most critical effect of lead at low concentrations is impaired cognitive development and intellectual performance in children, and studies have shown an association between blood lead concentrations and reduced intelligence quotient (IQ) in children exposed pre- and post-natally[51]. Young children are especially vulnerable to the effects of lead, because they absorb a higher percentage of ingested lead and are more susceptible to its neurotoxicity. The Committee noted that there could be subgroups of children with increased susceptibility to lead, such as those with calcium deficiencies or living in areas of high social deprivation.

55. The concentration of lead in blood is the most widely used biomarker of exposure and is typically reported in micrograms per decilitre (µg/dL). No threshold for intellectual deficits has been identified but there is evidence of an association at blood lead concentrations below 10 µg/dL[51]. Surveys of blood lead concentrations have indicated reductions in mean blood lead concentrations since the late 1970s[49,51-53]. Current mean levels in children in developed countries are in the region of 3 µg/dL[52]. This reduction has been attributed to the reduced use of lead in petrol and to programmes aimed at reducing exposure from other sources (such as phasing out the use of lead-based paints, eliminating the use of lead in food containers, and the replacement of lead water pipes with non-lead alternatives).

56. In 1999 the JECFA performed a quantitative risk assessment of the effects of dietary lead intakes on IQ in children. In order to correlate dietary intake with blood lead levels, the JECFA assumed that a dietary intake of 1 µg/kg body weight/day would result in an increase in blood lead concentration of 1 µg/dL (this being the upper estimate for infants), and that this relationship was valid over the long-term (*in utero* and for the first 10 years of life)[51]. There have been a number of epidemiological studies published since the 1999 JECFA assessment. Taken together, the available epidemiological data suggest that an IQ deficit of between 1 and 5 points occurs for each 10 µg/dL increase in blood lead level[49]. Recent studies have suggested that the dose-effect relationship is steeper than this at blood lead levels below 10 µg/dL, but the precise shape of the dose-effect relationship at lower blood lead levels remains uncertain[49,54,55]. There therefore remains no identified threshold for toxicity.

57. Using the JECFA correlation of dietary intake to increase in blood lead level, and assuming an IQ deficit of between 1 and 5 IQ points per 10 µg/dL increase in blood lead level, it is possible to quantify approximately the IQ deficit resulting from exposure to lead in infants and young children at the level of the PTWI. Dietary lead intake at the PTWI may be expected to increase the blood lead level in a young child by 3.6 µg/dL, with a resulting indicative mean IQ deficit of between 0.36 and 1.8 IQ points. Because of the uncertainties, this can only be regarded as an approximation of the degree of effect. Uncertainties include the true steepness of the dose-effect relationship at blood lead levels of <10 µg/dL; the nature of the dose-effect relationship below the lowest blood lead levels which have been studied in epidemiological studies (<1 µg/dL); variation between individual children; and those that arise because studies have assessed different aspects of cognitive and motor performance (such as distractibility, poor organisational skills, impulsivity, inability to follow sequences of directions, and short attention span). Limits to the precision of analytical and psychometric measurements further increase uncertainty in estimates of the effect of blood lead concentrations below 10 µg/dL.

58. The highest estimate of dietary exposure to lead was 0.42 µg/kg body weight/day (for high-level intake by pre-school children). This is comparable to the estimate from the 2000 TDS (0.47 µg/kg body weight/day) and is approximately 12% of the JECFA PTWI (equivalent to 3.6 µg/kg body weight/day). The JECFA PTWI of 25 µg/kg body weight for infants and children was originally set in 1986[50]. At the time of that evaluation, the PTWI was considered to be a level of exposure from all sources that was not expected to cause an increase in blood lead concentration in young children (the historical background being blood lead levels in UK infants at birth in the early 1980s). The JECFA again evaluated lead in 1993 when the Committee estimated what blood lead level the PTWI would lead to. As this was below levels known to be associated with intellectual deficits in children at the time, the PTWI of 25 µg/kg body weight for infants and children was re-confirmed and extended to all age groups[56]. The review of the health effects of lead in 1993 was based on an assessment of lead that had been performed by an International Programme on Chemical Safety Task Group, which was subsequently published[57]. In the most recent evaluation by the JECFA, the Committee assessed the risk of dietary exposure of infants and children, with special emphasis on the most critical effect, which was considered to be impaired neurobehavioural development. The PTWI was not re-considered[51].

59. A UK study of lead intake in children aged 2 years 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[58]. Thus, if dietary exposure to pre-school children is less than 30% of the JECFA PTWI (i.e. less than 1.08 µg/kg body weight/day), total intake is unlikely to exceed the PTWI. Reported minimum and maximum tap water concentrations for 2007 were 0.44 and 102 µg lead/L, respectively[8]. Consumption of tap water has the potential to increase high-level dietary exposure to lead by up to 10-fold in pre-school children. In 2003 the COT commented on a survey of metals in infant food[4]. The maximum estimated intake of lead was lower than for the previous survey and approximately 17% of the JECFA PTWI. The COT welcomed the apparent decline in lead exposure since the previous survey and concluded that efforts should continue to reduce lead exposure from all sources[4].

60. Table 2a illustrates that average population dietary exposures have declined considerably since 1976, with the current population exposure at its lowest level (7 µg/day compared to 26 µg/day in 1997). Although the JECFA PTWI for lead cannot be considered to be fully protective (i.e. there is an indicative minimal effect at the PTWI), all population groups' dietary exposures were well below the PTWI

(Table 1). However, drinking water has the potential to increase exposure further in some areas, with high-level total intake by pre-school children exceeding the PTWI by up to 23%. Lead levels in water vary over time, even in the same region, and therefore it is highly unlikely that this worst case scenario would occur over a prolonged period. Estimated worst case total intakes for all other population groups were below the PTWI.

61. The Committee concluded that adverse effects, if any, are likely to be small at the estimated dietary exposures to lead. 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.

Manganese

62. Manganese is an essential trace element that can exist in a variety of oxidation states. It is neurotoxic at high levels of occupational inhalation exposure, but there is limited evidence of neurological effects at lower doses. The extent of neurotoxicity is determined by the oxidation state, with Mn (III) being more toxic than Mn (II)[17]. 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[9]. Children might be particularly susceptible to the neurotoxicity of manganese. There is insufficient information to determine whether there are risks associated with dietary exposure to manganese and no available health based guidance value.

63. The EVM considered that, based on the results of epidemiological studies of neurological effects associated with concentrations of manganese in drinking water, total manganese intakes of 12.2 mg/day for the general population (equivalent to 200 µg/kg body weight/day for a 60kg adult) and 8.7 mg/day for older people (equivalent to 150 µg/kg body weight/day) would not result in adverse health effects[9]. This conclusion was based on a number of assumptions since neither of the two studies used to establish these guidance values recorded water consumption or dietary manganese intake. The WHO derived a TDI of 60 µg/kg body weight/day in the Guidelines for Drinking Water Quality[59]. This was based on the upper range value of manganese intake of 11 mg/day, identified using dietary surveys, at which there were considered to be no observed adverse effects. An uncertainty factor of 3 was applied to take into consideration the possible increased bioavailability of manganese from water. No information was provided on how these reference doses were set in relation to speciation.

64. The estimated high-level dietary exposure of pre-school children exceeded the EVM guidance value by approximately 50%. All other estimated dietary exposures were within the EVM guideline values. In the UK, intake from drinking water would have a minimal effect on total exposure to manganese. Dietary supplements provide up to 10 mg/day[9], which if added to the high level dietary exposure results in a total intake of 290 µg/kg body weight/day in a 60kg adult, representing 145% of the EVM guidance value.

65. The Committee concluded that there was insufficient information to determine whether there are risks associated with dietary exposures to manganese. However, the population dietary exposures to manganese (Table 2b) have remained fairly constant from the time manganese was first included in a TDS in 1983 (4.6 mg/day) to the 2006 TDS (5.24 mg/day), and there is no basis for assuming any concern for health.

Mercury

66. Mercury exists in multiple forms and in three oxidation states (elemental mercury, mercurous mercury, and mercuric mercury). The properties and chemical behaviour of mercury strongly depend on its oxidation state and its chemical form. Mercurous and mercuric mercury form numerous inorganic and organic chemical compounds. Organic forms of mercury are the most toxic following ingestion as they are absorbed more effectively in the gastrointestinal tract than elemental mercury or inorganic mercury compounds[17]. Food is the major source of exposure to mercury in the general population, particularly methylmercury in fish. There have been no reports of methylmercury being detected in drinking water[27].

67. Estimates of average population exposure to mercury have decreased since 1976 (0.005 mg/day), with the 2006 TDS population dietary exposure (0.001-0.003 mg/day) comparable to that in 2000, when levels were at their lowest (0.0012-0.0015 mg/day). Mercury concentrations were similar to those reported in the 2000 TDS except for the fish group, in which the concentration had decreased to 0.056 mg/kg from 0.071 mg/kg in 2000.

68. The estimates of dietary exposure to mercury (mean- and high-level intakes) for all consumer groups were within or in the region of the PTWI for methylmercury set by the JECFA in 2003 to protect against neurodevelopmental effects (equivalent to 0.23 µg/kg body weight/day), and endorsed by the COT[5]. The estimate for high-level consumption by pre-school children exceeded the JECFA PTWI for methylmercury by 13%. 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. The Committee concluded that current dietary exposures to mercury were unlikely to be of toxicological concern.

Molybdenum

69. Molybdenum is an essential trace element. It does not exist naturally in the metallic state, but occurs in association with other elements. The predominant form of molybdenum occurring in soil and natural waters is the molybdate anion, $MoO4^{-2}$ [9]. Estimated average population dietary exposures to molybdenum were comparable to previous estimates (0.123-0.125 mg/day vs. 0.11 mg/day in 1985, 1991 and 1994). There are no health based guidance values for molybdenum and there are few reliable data on its oral toxicity. The EVM noted that intakes of >1 mg/day could be associated with an increased incidence of gout-like symptoms but concluded that the maximum molybdenum intake from the UK diet and drinking water, estimated to be 0.23 mg/day (approximately 4 µg/kg body weight/day for a 60 kg adult), was not expected to present any risk to health[9]. There were insufficient data on the safety of molybdenum intakes in excess of those naturally occurring in the diet for the EVM to provide further guidance on supplementary intake. Dietary supplements can provide up to 333 µg/day (about 5 µg/kg body weight/day for a 60 kg adult)[9]. No data were available for molybdenum in drinking water in the UK, but the WHO noted that levels are usually less than 0.01 mg/L, which was the value used by EVM in reaching its conclusions.

70. The Committee concluded that the sparse data on the oral toxicity of molybdenum do not suggest that the estimated dietary exposures, excluding supplements, give cause for toxicological concern.

Nickel

71. Nickel is an abundant metallic element that can exist in valency states of 0, +1, +2, and +3. Nickel is usually analysed in food as total nickel. Therefore the chemical form is unknown, although nickel in food is normally considered to be in the form of complex bound organic nickel, which may be less bioavailable than other forms[27]. The estimates of dietary exposures to nickel for mean - and high-level intake by pre-school children and high-level intake by young people exceeded (by up to about 2-fold) the total nickel intake level of 4.3 µg/kg body weight/day, considered by the EVM as a dose that would not result in effects in non-sensitised individuals[9]. However, these estimated exposures were within the WHO TDI of 12 µg/kg body weight/day. Nickel in drinking water could increase high-level dietary exposure by 20-30%, with a potential total high-level intake of 11.1 µg/kg body weight/day for pre-school children. Dietary supplements can provide up to 5 µg/day (0.08 µg/kg body weight/day for a 60 kg adult)[9].

72. The value identified by EVM was based on the LOAEL of 1.3 mg/kg body weight/day from a multigeneration study in rats given nickel chloride in drinking water, and incorporated an uncertainty factor of 300. However, the EVM also noted that UK dietary intake of nickel in food was not expected to result in harmful effects. The WHO TDI was established on the basis of a study in which 20 nickel-sensitised patients ingested a single dose of 12 µg/kg body weight 61Ni in solution on a fasted stomach with abstinence from food maintained for a further 4 hours. Nine out of the 20 patients developed flare-up of symptoms after 12 hours. This dose was considered to be the acute LOAEL and a dose much higher than would normally be possible through drinking-water and/or with the presence of food in the stomach. Deriving the total acceptable intake for oral challenge from studies using drinking water on an empty stomach in fasted patients was, therefore, considered a worst-case scenario[27].

73. Previously the COT concluded that the estimated dietary exposure to nickel from the 2000 TDS was unlikely to be of any toxicological concern for consumers[1]. Population dietary exposures to nickel have decreased since 1976 (0.33 mg/day), with the current average dietary exposure at its lowest level (0.127-0.129 mg/day) and comparable to results from the 2000 TDS (0.13 mg/day). Nickel may exacerbate contact dermatitis/eczema in pre-sensitised individuals but the COT has concluded previously that pre-school children are less likely than adults to be sensitised and would therefore not be considered to be a sensitive sub-group[1]. The Committee therefore concluded that dietary exposures to nickel were unlikely to be of toxicological concern.

Palladium

74. The platinum group of metals, which includes palladium, rhodium, and ruthenium, are used in catalytic converters, which have been fitted to the engines of all new vehicles since 1993. Research has shown an increase in the concentration of these metals in roadside dust[60]. There is little information about the biological effects of platinum group metals in food and at present there is no evidence in relation to possible adverse health effects from these metals in the general environment[61].

75. Palladium was last analysed in a TDS in 1994. Since 1994, estimated average population dietary exposures have decreased slightly from 1 µg/day to 0.7 µg/day. Based on the estimated population dietary exposures from the 1994 TDS, the COT previously concluded that from the available data, there was no reason to believe that intakes of palladium from the diet posed a risk to health[2]. However, the COT did

note that the toxicological database on palladium metal and its compounds was extremely limited[2]. There are no health based guidance values for palladium. No data are available on levels of palladium in drinking water in the UK.

76. The WHO concluded that the main source of concern regarding palladium is the sensitisation risk; and that the available data did not allow identification of a NOAEL for sensitisation in humans[62]. The WHO noted that in an unpublished 28-day gavage study in which rats were dosed with tetraamine palladium hydrogen carbonate at 1.5, 5 or 150 mg/kg body weight/day[63], treatment-related abnormalities, confined to histopathological changes in the spleen and glandular region of the stomach, were observed at 5 and 150 mg/kg body weight/day. The study authors considered 1.5 mg/kg body weight/day to be the NOAEL, but significant increases in absolute brain and ovary weights were observed in females of this dose group. The margin of exposure between this sub-chronic rat NOAEL/LOAEL and the highest estimated dietary exposure (0.056 µg/kg body weight/day; high-level intake by pre-school children) is about 9700.

77. The Committee concluded, based on the limited database and the evidence that exposure had not increased since 1994, that there was no reason to believe that current intakes of palladium from the diet pose a risk to health. Analysis of dietary palladium was no longer considered to be a high priority for future study.

Platinum

78. Platinum was last analysed in a TDS in 1994, when the estimated population dietary exposure was 0.2 µg/day. Platinum was not detected in any of the food groups analysed in the 2006 TDS, resulting in an estimated average population exposure of 0-2.3 µg/day based on the lower-bound to upper-bound approach, which is not clearly different from 1994. There are no health based guidance values for platinum and it is not known what form of platinum, if any, is present in foods. No data are available on levels of platinum in drinking water in the UK.

79. In 1996, the COT reviewed organometallic platinum compounds in the context of their use as diesel fuel catalysts. The Committee considered the proposed usage and the projected emissions and noted that, if the majority of the emissions were in the form of the metal, there would be no risk to health; and that the platinum emissions from the catalyst were unlikely to be in an allergenic form[64]. The most significant health effect from exposure to soluble platinum salts is sensitisation, though there are no studies of sensitisation by the oral route in humans[65]. Hypersensitivity reactions to platinum-based chemotherapy are frequently encountered, including anaphylactic shock[66-68]. Reactions usually occur after several courses of treatment, although the pathogenic mechanisms are not fully understood. From the limited available data from experimental animals, a NOAEL of 13 mg platinum/kg body weight/day can be tentatively identified from a study in which rats were given $PtCl_4$ in drinking water for 30 days. The margin of exposure between this subchronic rat NOAEL and the highest estimated dietary exposure (0.130 µg/kg body weight/day; upper bound estimate for high-level intake in pre-school children) is 100000. This margin of exposure indicates a low concern for human health at the highest high-level dietary intake.

80. The Committee concluded that the very low dietary exposures to platinum did not suggest a reason for concern.

Rhodium

81. Rhodium was last analysed in a TDS in 1994, when the average estimated population dietary exposure was 0.3 µg/day. The chemical nature of rhodium in the diet is unknown. Rhodium was not detected in any of the food groups analysed in the 2006 TDS, resulting in an estimated population exposure of 0-2.3 µg/day (lower-bound to upper-bound range), which is not clearly different from 1994. There are no health based guidance values for rhodium. No data are available on levels of rhodium in drinking water in the UK.

82. There are no data in the literature relating to the acute or chronic health effects of rhodium or its compounds in man and few data from studies in experimental animals. When considering the results of the 1994 TDS, the COT concluded that there were insufficient experimental and human toxicological data to be able to make an appraisal of the toxicity of rhodium and its compounds, although, rhodium compounds would appear to be less potent than their platinum counterparts[2]. The Committee concluded that the very low dietary exposures to rhodium did not suggest a reason for concern.

Ruthenium

83. Ruthenium was last analysed in a TDS in 1994. Since 1994, the estimated average population dietary exposure has decreased from 4 µg/day to 0.03-0.81 µg/day (lower bound to upper bound range). Based on the estimated population dietary exposures from the 1994 TDS, the COT previously concluded that from the available data, there was no reason to believe that intakes of ruthenium from the diet posed a risk to health[2]. However, the COT did note that there were insufficient data for a full evaluation[2]. There are no health based guidance values for ruthenium. No data are available on levels of ruthenium in drinking water in the UK.

84. There are no data on the human toxicity of ruthenium compounds and limited experimental toxicological data, although there is some clinical usage as a candidate chemotherapeutic agent. Ruthenium compounds such as NAMI-A and KP1019 have displayed antitumour activity in Phase I clinical trials, with data indicating ruthenium compounds to be less potent in toxicity than their platinum counterparts[69-71]. The Committee concluded that the very low dietary exposures to ruthenium did not suggest a reason for concern.

Selenium

85. Selenium is an abundant element that can exist in 4 oxidation states (-2, +1, +2, and +6). In foods, selenium is generally present as the amino acid derivatives selenomethionine and selenocysteine[9]. Selenium was present in 14 of the 20 food groups analysed in the 2006 TDS. The offal (0.77 mg/kg) and fish (0.42 mg/kg) groups contained the highest concentrations of selenium. Selenium is an essential trace element. Selenium in drinking water has the potential to increase dietary exposure by approximately 20-30%. Adding potential intake from drinking water to the highest estimated dietary exposure (for pre-school children) indicates a possible highest total exposure of 5.3-5.6 µg/kg body weight/day (lower bound to upper bound range), which is below the safe upper level of 7.5 µg/kg body weight/day set by the EVM in 2003[9]. This safe upper level was based on a LOAEL of 0.91 mg/day, derived from an epidemiological dietary study in which signs of selenosis (prolonged prothombin time, morphological changes in the nails, and increased white blood cell count) were observed in individuals with selenium blood levels of 1.054 to 1.854 mg/L, which were calculated to represent a selenium intake of 0.91 mg/day.

An uncertainty factor of 2 was applied to extrapolate from the LOAEL to a NOAEL. A larger uncertainty factor was not considered necessary because the intake of 0.91 mg/day produced only slight effects and was close to a NOAEL. Dietary supplements can provide up to 0.3 mg/day (5 µg/kg body weight/day for a 60 kg adult), which together with intake from food and water would not result in the safe upper level being exceeded in adults.

86. The Committee concluded that the estimated dietary exposures to selenium were not of toxicological concern.

Strontium

87. Strontium occurs in nature chiefly as the minerals celestite (SrSO4) and strontianite (SrCO3), which are widespread in rocks and waters. Strontium is present in small quantities in most plants. Strontium was last analysed in a TDS in 1994, when the estimated average population dietary exposure was 1.3 mg/day, which the COT concluded to be of no health concern[2]. The population dietary exposure estimate for 2006 was comparable (1.2 mg/day). There are no health based guidance values for strontium. No data are available on levels of strontium in drinking water in the UK.

88. There are no epidemiological data concerning the health effects of strontium, although there is a long history of clinical use of strontium in the treatment and prevention of osteoporosis, and relatively high levels of strontium (1700 mg/day) have been given without any clear evidence of toxicity. This dose is equivalent to 28 mg/kg body weight/day for a 60kg adult. The Medicines and Healthcare products Regulatory Agency (MHRA) issued a warning in November 2007 related to hypersensitivity reactions to the molecule, strontium ranelate (also known as protelos), a drug used to treat postmenopausal osteoporosis[72]. The mechanism of this hypersensitivity is unknown and therefore it is uncertain whether it is related to the strontium ion, the molecule as a whole or a specific component. In rat studies, NOAELs of 190 mg/kg body weight/day (bone changes, 20-day study) and 15 mg/kg body weight/day (increased thyroid and pituitary weights, and increased thyroid activity, 90-day study) have been reported. The margin of exposure between the human therapeutic dose and the highest estimated dietary exposure (71.1 µg/kg body weight/day; high-level intake by pre-school children) is 400 (rounded to the nearest 10). The Committee concluded that current dietary exposures to strontium were unlikely to be of toxicological concern.

Thallium

89. Thallium is ubiquitous in nature and occurs in sulphide ores of various heavy metals (zinc, copper, iron and lead) at low concentration (<2 mg/kg)[73]. Thallium has two oxidation states, 1+ and 3+, both of which can have effects on the central and peripheral nervous systems, the skin, the gastrointestinal tract, the cardiovascular system, and the kidney. The more water-soluble salts are considered to have greater toxicity than the salts of lower water solubility[17]. In areas with a naturally high concentration of thallium in soil (such as Macedonia), the majority of vegetables, fruits and meat contain less than 1 mg/kg[73].

90. Thallium was last analysed in a TDS in 1994. Since 1994, estimated population dietary exposures have decreased from 2 µg/day to 0.7-0.8 µg/day. The COT previously concluded that there was no evidence that dietary intake of thallium by the UK population was harmful to health[2]. There are no health based guidance values for thallium. No data are available on levels of thallium in drinking water in the UK.

91. The WHO considered that exposures causing urinary thallium concentrations below 5 µg/L were unlikely to cause adverse health effects in humans[73]. In the range of 5-500 µg/L the magnitude of the risk and severity of adverse effects were uncertain, while exposures giving values over 500 µg/L had been associated with clinical poisoning[73]. The estimated daily oral intake corresponding to a urinary thallium concentration of 5 µg/L was determined to be approximately 10 µg/day as a soluble form of thallium, or 0.17 µg/kg body weight/day for a 60kg adult. The margin of exposure between this daily oral human intake and the highest estimated dietary exposure (0.046 µg/kg body weight/day; high-level intake by pre-school children) is approximately 4. The Committee concluded that current dietary exposures to thallium were unlikely to be of toxicological concern.

Tin

92. Tin is rarely found as the metallic element in nature but is more usually found combined with other substances, most commonly as the dioxide[9]. It has oxidation states of II and IV. Inorganic tin is of low toxicity, whereas some organotin compounds are potent neurotoxicants, though these are not normally present in food, beverages or food supplements[9,17]. No data are available on levels of tin in drinking water in the UK.

93. The estimates of dietary exposures to tin for high-level intake by pre-school children were lower than the JECFA PTWI of 2000 µg/kg body weight/day, but exceeded the EVM guidance level of 220 µg/kg body weight/day by approximately 55%. All other estimated subgroup dietary exposures (mean- and high-level intakes) were within the EVM guidance level. Dietary supplements can provide up to 10 µg/day (0.17 µg/kg body weight/day for a 60 kg adult), which would not lead to the guidance level being exceeded by adults. The PTWI, originally set as a provisional maximum tolerable daily intake in 1982, is not directly applicable to long term dietary exposures since it appears to be based on intakes associated with acute toxicity (the threshold concentration for manifestation of gastric irritation). The EVM guidance level was based on a NOAEL of 1000 mg/kg diet of stannous chloride (corresponding to an intake in the range of 22-33 mg tin/kg body weight/day) from a sub-chronic study in rats, in which anaemia and changes to liver cells were observed at higher doses. The EVM used the lower end of the NOAEL range (22 mg/kg body weight/day) and an uncertainty factor of 100 to derive the guidance level of 220 µg/kg body weight/day[9]. The Committee concluded that the small exceedance of this guidance level is within an area of uncertainty, but that current dietary exposures were unlikely to be of toxicological concern.

Zinc

94. Zinc is an essential trace element, occurring in nature as the sulphide, the silicate, and the oxide[9]. It is found in virtually all food and potable water. Zinc concentrations in tap water can be much higher than those of surface and ground waters as a result of the leaching of zinc from piping and fittings[27]. The WHO noted that drinking water makes a negligible contribution to zinc intake unless high concentrations of zinc occur as a result of corrosion of piping and fittings. The WHO did not derive a guideline value for drinking water quality but noted that drinking water containing zinc at levels above 3 mg/L may not be acceptable to consumers[27]. Excessive zinc intake interferes with copper absorption, potentially leading to copper deficiency, which can result in conditions such as anaemia and bone abnormalities. The current estimated dietary exposures to zinc for all subgroups were below or in the

region of the EVM safe upper level (700 µg/kg body weight/day) and within the JECFA PMTDI of 1000 µg/kg body weight/day. Dietary supplements can provide up to 50 mg/day (833 µg/kg body weight/day for a 60 kg adult), which exceeds the safe upper level before taking into account the diet. The Committee concluded that current dietary exposures to zinc, excluding supplements, were unlikely to be of toxicological concern.

Conclusions

95. We *conclude* that current dietary exposures to antimony, cadmium, copper and selenium are not of toxicological concern. We note that this conclusion with respect to cadmium might need to be reviewed after the current risk assessment by the European Food Safety Authority (EFSA) is published.

96. We *conclude* that current dietary exposures to bismuth, chromium, germanium, mercury, nickel, strontium, thallium, tin and zinc are unlikely to be of toxicological concern.

97. We *note* that whilst the estimates of dietary exposure to aluminium are not markedly higher than previous estimates, they present uncertainty with regard to the safety of aluminium in food in light of new data that led to the recent reduction in the Provisional Tolerable Weekly Intake (PTWI), which is exceeded by some population subgroups. There is a need for further information on possible sources and forms of aluminium in the diet and their bioavailability.

98. The data on arsenic appear consistent with previous surveys of total and inorganic arsenic in food, which we reviewed in 2003. We *reaffirm* our previous conclusions that current dietary exposure to organic arsenic is unlikely to constitute a risk to health. Our advice remains that exposure to inorganic arsenic should be as low as reasonably practicable (ALARP).

99. We *note* that the tolerable daily intake (TDI) for barium is based on studies in which no effects were observed and thus may be over-precautionary. Therefore, the estimated exposures, which exceeded the TDI by up to 4-fold, are not necessarily a toxicological concern. We *recommend* that further research be carried out to allow a TDI to be set with more confidence and to investigate the bioavailability of barium; especially from foods with relatively high levels such as nuts.

100. Population dietary exposures to indium and molybdenum are similar to previous studies and although there is uncertainty, the sparse data on the oral toxicity of indium and molybdenum do not suggest that the estimated intakes give cause for toxicological concern.

101. We *note* that estimates of dietary exposure to lead have not increased since the previous survey. At these dietary intakes, adverse effects, if any, are likely to be very small. 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.

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

103. The toxicological database on palladium metal and its compounds is extremely limited. However, we *conclude* that from the available data, there is no reason to believe that current intakes of palladium from the diet pose a risk to health.

104. Despite a dearth of information on the effects of low doses of platinum, rhodium and ruthenium, we *conclude* that current dietary exposures do not suggest a reason for concern as the levels present in the food samples tested were very low or undetectable.

105. We *recommend* that in future research and surveys of elements in food, priorities should include:

- Information on the forms of aluminium in food and their bioavailability.
- Clarification of the large variability in aluminium concentrations in food and whether these represent an increasing trend.
- Assessment of the bioavailability of barium in nuts compared to barium chloride in water.
- A long-term human study with a large number of subjects to examine the effect of barium on blood pressure and to investigate renal end-points following oral exposure to barium in drinking water, to allow a TDI to be set with more confidence.
- Information on the bioavailability of manganese, particularly from beverages that are the principal contributing food group.

COT statement 2008/08

December 2008

References

1. COT (2003). COT Statement 2003/07 Statement on twelve metals and other elements in the 2000 Total Diet Study. Available at: http://cot.food.gov.uk/cotstatements/cotstatementsyrs/cotstatements2004/cotstatements2004metals

2. COT (1998). Statement on the results of multielement surveys in various items of the diet. COT Statement 1998. Annual Report 1998 Committees on: Toxicity Mutagenicity Carcinogenicity of Chemicals in Food, Consumer Products and the Environment.

3. COT (2003). Statement on Arsenic in food: Results of the 1999 Total Diet Study. Available at: http://cot.food.gov.uk/cotstatements/cotstatementsyrs/cotstatements2003/arsenicstatement

4. COT (2003). Statement on a Survey of Metals in Infant Foods. Available at: http://cot.food.gov.uk/cotstatements/cotstatementsyrs/cotstatements2003/statementmetals

5. COT (2003). Statement on a Survey of Mercury in Fish and Shellfish. Available at: http://cot.food.gov.uk/cotstatements/cotstatementsyrs/cotstatements2003/cotmercurystatement

6. Peattie, M.E., Buss, D.H., Lindsay, D.G. and Smart, G.Q. (1983). Reorganisation of the British Total Diet Study for Monitoring Food Constituents from 1981. *Food and Chemical Toxicology*, 21, 503-507.

7. MAFF (1994). Ministry of Agriculture, Fisheries and Food. The British Diet: Finding the Facts. *Food Surveillance Paper* No. 40. The Stationary Office, London.

8. DWI (2008). Drinking Water 2007. Drinking water in England & Wales. A report by the Chief Inspector of Drinking Water. Drinking Water Inspectorate, June 2008, available at: http://www.dwi.gov.uk/pubs/annrep07/contents.shtm.

9. EVM (2003). Safe upper levels for vitamins and minerals. Report of the Expert Group on Vitamins and Minerals. Food Standards Agency, May 2003. ISBN 1-904026-11-7.

10. Mason, P. (2007). One is okay, more is better? Pharmacological aspects and safe limits of nutritional supplements. *Proc. Nutr. Soc.*, **66**(4), 493-507.

11. Ritchie, M.R. (2007). Use of herbal supplements and nutritional supplements in the UK: what do we know about their pattern of usage? *Proc. Nutr. Soc.*, **66**(4), 493-507.

12. Rock, C.L. (2007). Multivitamin-multimineral supplements: who uses them? *Am. J. Clin. nutr.*, **85**(1), 277S-279S.

13. Food Standards Agency (2006). Survey to assess the market for high dose vitamin and mineral supplements in the UK and to determine the use of voluntary advisory statement. *Food Survey Information Sheet* 12/06. Available at: http://www.food.gov.uk/multimedia/pdfs/fsis1206.pdf.

14. Food Standards Agency (2008). FSA funded survey 451602. Consumer consumption of vitamin and mineral food supplements. Conducted by GfK Social Research, available at: http://www.food.gov.uk/multimedia/pdfs/viminsupconsumer.pdf.

15. Henderson, L., Gregory, J. and Swan, G. (2002). The National Diet and Nutrition Survey: adults aged 19-64 years. Volume 1: Types and quantities of foods consumed. The Stationary Office, London.

16. Mintel Oxygen (2007). Vitamin and Mineral Supplements UK. Mintel International Group, London. May 2007.

17. WHO (2006). Elemental speciation in human health risk assessment. Environmental Health Criteria 234, International Programme on Chemical Safety. World Health Organization, Geneva.

18. Food Standards Agency (2008). Measurement of the concentrations of metals and other elements from the 2006 UK total diet study. Food Survey Information Sheet (to be published). Available at: http://www.food.gov.uk/science/surveillance/.

19. Finch, S., Doyle, W., Lowe, C., Bates, C.J., Prentice, A., Smithers, G. and Clarke, P.C. (1998). The National Diet and Nutrition Survey: People Aged 65 years and over. Volume 1: Report of the Diet and Nutrition Survey. HMSO, London.

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

21. Gregory, J., Collins, D.L., Davies, P.S.W., Hughes, J.M. and Clarke, P.C. (1995). The National Diet and Nutrition Survey: Children Aged 1½ to 4½ years. Volume 1: Report of the Diet and Nutrition Survey. HMSO, London.

22. Gregory, J., Lowe, S., Bates, C.J., Prentice, A., Jackson, L.V., Smithers, G., Wenlock, R. and Farron, M. (2000). The National Diet and Nutrition Survey: Young People Aged 4 to 18 years. Volume 1: Report of the Diet and Nutrition Survey. HMSO, London.

23. DEFRA (2003/04). Department for Environment, Food and Rural Affairs, Family Food - Expenditure & Food Survey; Consumption data from the 2003/04 Family Food report. Available at: http://statistics.defra.gov.uk/esg/publications/efs/2004/default.asp

24. MAFF (1996). Ministry of Agriculture, Fisheries and Food. *Dietary Survey of Vegetarians: Final Technical Report*. Research and Development Surveillance Report: 181.

25. Food Standards Agency (2008). COT discussion paper TOX/2008/29 Annex B. Available at: http://cot.food.gov.uk/pdfs/tox200829annexb.pdf.

26. SI (2000). Statutory Instrument No 2000/3184. The Water Supply (Water Quality) Regulations 2000 (SI2000/3184).

27. WHO (2006). Guidelines for Drinking-Water Quality. First addendum to third edition. Volume 1 Recommendations. Third edition. World Health Organization, Geneva.

28. WHO (2007). Safety evaluation of certain food additives and contaminants. WHO Food Additives Series 58. Prepared by the Expert Committee on Food Additives (JECFA). World Health Organization, Geneva.

29. EFSA (2008). Safety of aluminium from dietary intake. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC). The EFSA Journal, **754**, 1-34.

30. SI (1995). Statutory Instrument No 1995/3187. The Miscellaneous Food Additives Regulations 1995 (SI No 1995/3187), as amended.

31. SI (1995). Statutory Instrument No 1995/3124. The Colours in Food Regulations 1995 (SI No 1995/3124).

32. WHO (2001). Environmental Health Criteria 224: Arsenic and Arsenic Compounds. International Programme on Chemical Safety. World Health Organization, Geneva.

33. HSE (2005). Workplace Exposure Limits: Containing the list of workplace exposure limits for use with the Control of Substances Hazardous to Health Regulations 2002 (as amended). EH40/2005, Health & Safety Executive.

34. WHO (1989). Toxicological Evaluations of Certain Food Additives and Contaminants, 33rd Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Food Additive Series No. 24.

35. EFSA (2008). Open Calls for data: Request for data on arsenic levels in food and water. The European Food Safety Authority. Available at: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902010663.htm

36. WHO (2001). Concise International Chemical Assessment Document 33: Barium and barium compounds. World Health Organization, Geneva.

37. WHO (1993). World Health Organisation Guidelines for Drinking Water Quality, Volume 1: Recommendations. World Health Organization, Geneva.

38. Gavey, C.J., Szeto, M-L., Nwoloko, C.U., Sercombe, J. and Pounder, R.E. (1989). Bismuth accumulates in the body during treatment with tripotassium dicitrato bismuthate. *Aliment. Pharmacol.* Ther., 3(1), 21-28.

39. WHO (1988). Environmental Health Criteria 61: Chromium. International Programme on Chemical Safety. World Health Organization, Geneva.

40. US EPA (1998). Toxicological Review of Hexavalent Chromium. In support of summary information on the integrated risk information system (IRIS). US Environmental Protection Agecny, Washington DC.

41. Food Standards Agency (2007). FSA funded contract C02068. Development of a chromium speciation methodology. Final report FD 06/08. Conducted by J Lewis, CSL, Sand Hutton, York.

42. COMA (1991). Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on the Panel on Dietary Reference Values, Committee on Medical Aspects of Food and Nutrition Policy. HMSO, London.

43. WHO (1982). Toxicological Evaluation of Certain Food Additives. WHO Food Additive Series No. 17.

44. MAFF (1985). Ministry of Agriculture, Fisheries and Food. Steering Group on Chemical Aspects of Food Surveillance. Survey of aluminium, antimony, chromium, cobalt, indium, nickel, thallium and tin in food. Food Surveillance Paper No 15.

45. MAFF (1998). Ministry of Agriculture, Fisheries and Food. Metals and other elements in cows' milk and vegetables produced near industrial sites. Food Surveillance Information Sheet No 150.

46. Ungvary, G., Szakmary, E., Tatrai, E., Hudak, A., Naray, M. and Morvai, V. (2000). Embryotoxic and teratogenic effects of indium chloride in rats and rabbits. *J Toxicol. Environ. Health A.*, **59**(1), 27-42.

47. Nakajima, M., Takahashi, H., Sasaki, M., Kobayashi, Y., Awano, T., Irie, D., Sakemi, K., Ohno, Y. and Usami, M. (1998). Developmental toxicity of indium chloride by intravenous or oral administration in rats. T*eratog. Carcinog. mutagen.*, **18(**5), 231-238.

48. Schroeder, H.A. and Mitchener, M. (1971). Scandium, chromium (VI), gallium, yttrium, rhodium, palladium, indium in mice: effects on growth and life span. *Journal of Nutrition*, **101,** 1431-1438.

49. ATSDR (2007). Agency for Toxic Substances & Disease Registry. Toxicological Profile for Lead. US Department of Health and Human Services, Atlanta, GA.

50. WHO (1987). Safety evaluation of certain food additives and contaminants, WHO Food Additives Series 21. Lead.

51. WHO (2000). Safety evaluation of certain food additives and contaminants. WHO Food Additives Series **44**: Lead.

52. Koller, K., Brown, T., Spurgeon, A. and Levy, L. (2004). Recent developments in low-level lead exposure and intellectual impairment in children. *Environmental Health Perspectives*, 112(9), 987-994.

53. WHO (2007). Blood lead levels in children. Fact sheet no. 4.5, May 2007. European Environment and Health Information System, World Health Organization.

54. Bellinger, D.C. (2004). Lead. *Paediatrics,* **113** (supp 4), 1016-1022.

55. CDC (2005). Centres for Disease Control and Prevention Advisory Committee on Childhood Lead Poisoning Prevention. A review of evidence of adverse health effects associated with blood lead levels <10 µg/dL in children. In: Preventing Lead Poisoning in Children. US Department of Health and Human Services, Atlanta, GA.

56. WHO (1993). Evaluation of certain food additives and contaminants. forty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 837, World Health Organization, Geneva.

57. WHO (1995). Health Criteria 165: Inorganic lead. International Programme on Chemical Safety. World Health Organization, Geneva.

58. Davies, D.J.A., Thornton, I., Watt, J.M. *et al.* (1990). Lead intake and blood lead in two year old UK children. *Science of the Total Environment*. **90**: 13-29.

59. WHO (2004). Manganese in Drinking-water – Background document for development of WHO guidelines for drinking water quality. Available at: http://www.who.int/water_sanitation_health/dwq/chemicals/manganese.pdf.

60. Farago, M.E., Kavanagh, P., Blanks, R., Kelly, J., Kazantzis, G., Thornton, I., Simpson, P.R., Cook, J.M., Delves, H.T. and Hall, G.E. (1998). Platinum concentrations in urban road dust and soil, and in blood and urine in the United Kingdom. *Analyst*, **123**, 451-454.

61. Ravindra, K., Bencs, L. and van Grieken, R. (2004). Platinum group elements in the environment and their health risk. *The Science of the Total Environment*, **318**, 1-43.

62. WHO (2002). Environmental Health Criteria 226: Palladium. International Programme on Chemical Safety. World Health Organization, Geneva.

63. Johnson Matthey (1997). Tetraammine palladium hydrogen carbonate: Twenty-eight day repeated dose oral (gavage) toxicity study in the rat. Hertfordshire, Johnson Matthey plc (SPL Project No. 036/084; unpublished report reported in WHO, 2002, Health Criteria 226: Palladium. International Programme on Chemical Safety. World Health Organization, Geneva.)

64. COT (1996). Platinum-based fuel catalyst for diesel fuel. Annual Report 1996 Committees on Toxicity Mutagenicity Carcinogenicity of Chemicals in Food, Consumer Products and the Environment.

65. WHO (1991). Environmental Health Criteria 125: Platinum. International Programme on Chemical Safety. World Health Organization, Geneva.

66. Rossi, F., Incorvaia, C. and Mauro, M. (2004). Hypersensitivity reactions to chemotherapeutic antineoplastic agents. *Recent. Prog. Med.*, **95**(10), 476-481.

67. Touraine, F., Sainte-Laudy, J., Boumediene, A., Ndikumwenayo, F., Decroisette, C., Melloni, B., Vergnenegre, A. and Bonnaud, F. (2006). Investigation of allergic reactions to platinum salts. *Rev. Mal. Respir.*, **23**(5 part 1), 458-462.

68. Weidmann, B., Mullenseisen, N. Bojko, P. and Niederle, N. (1994). Hypersensitivity reactions to carboplatin. Report of two patients, review of the literature, and discussion of diagnostic procedures and management. *Cancer,* **73**(8), 2218-2222.

69. Galanski, M., Arion, V.B., Jakupec, M.A. and Keppler, B.K. (2003). Recent developments in the field of tumor-inhibiting metal complexes. *Curr. Pharm. Des.*, **9**(25), 2078-2089.

70. Kostova, I. (2006). Ruthenium complexes as anticancer agents. *Curr. Med. Chem.*, *13*(9), 1085-2107.

71. Ott, I. and Gust, R. (2007). Non platinum metal complexes as anti-cancer drugs. *Arch. Pharm. (Weinheim),* 340(3), 117-126.

72. MHRA (2007). Medicines and Healthcare products Regulatory Agency. Safety information on medicines for healthcare professionals sent in November 2007. Available at: http://www.mhra.gov.uk/Safetyinformation/Safetywarningsalertsandrecalls/Safetywarningsandmessagesformedicines/Monthlylistsofinformationforhealthcareprofessionalsonthesafetyofmedicines/CON2033230

73. WHO (1996). Environmental Health Criteria 182: Thallium. International Programme on Chemical Safety. World Health Organization, Geneva.

74. WHO (2003). Safety evaluation of certain food additives and contaminants. WHO Food Additive Series 52. Joint FAO/WHO Expert Committee on Food Additives. World Health Organization

Table 1: Comparison of the estimated dietary intakes of each element for each population group with the relevant health based guidance values

Estimated Dietary Exposure (µg/kg bw/day)[1, 2, 3]													
Element	Adults		Pre-school children (1.5 - 4.5 years)		Young people (4-18 years)		Elderly (free living)		Elderly (institutional)		Vegetarians[4]		Health Based Guidance Values[5]
	Mean	High level	Mean	High level	Mean	High level	Mean	High level	Mean	High level	Mean	High level	
Aluminium	71	144	187	345	123	246	59	135	58	167	87	151	JECFA PTWI equivalent to 143 µg/kg bw /day
Antimony	0.032 - 0.033	0.059 - 0.060	0.075 - 0.077	0.13 - 0.14	0.049 - 0.050	0.096 - 0.097	0.027	0.054	0.023 - 0.024	0.062	0.035 - 0.036	0.06	TDI of 6 µg/kg bw /day derived by WHO
Arsenic (Total)	1.7	6.8 - 6.9	2.7 -2.8	12	1.9 - 2.0	8.2	1.7 - 1.8	6.4	1.20	5.02	1. 6	8.70	COT has concluded that there are no appropriate health based guidance values.
Arsenic (Inorganic)	0.028 - 0.093	0.071 - 0.165	0.075 - 0.246	0.174 - 0.402	0.055 - 0.158	0.128 - 0.291	0.024 - 0.079	0.066 - 0.149	0.025 - 0.072	0.082 - 0.173	0.035 - 0.100	0.079 - 0.163	JECFA PTWI equivalent to 2.1 µg/kg bw /day COT concluded exposure should be ALARP.
Barium	9.40	45.3	22.2	85.0	14.4	64.8	6.4	24.5	4.64	11.7	14.2	63.3	TDI of 20 µg/kg bw/day derived by WHO
Bismuth	0.015 - 0.022	0.034 - 0.044	0.086 - 0.10	0.20 - 0.22	0.034 - 0.046	0.09 - 0.11	0.016 - 0.022	0.037 - 0.046	0.018 - 0.024	0.049 - 0.061	0.020 - 0.027	0.048 - 0.056	N/A
Cadmium	0.14 - 0.17	0.25 - 0.29	0.37 - 0.45	0.65 - 0.75	0.27 - 0.31	0.50 - 0.57	0.13 - 0.15	0.26 - 0.29	0.11 - 0.13	0.30 - 0.35	0.17 - 0.20	0.30 - 0.32	JECFA PTWI equivalent to 1 µg/kg bw /day
Chromium*	0.28 - 0.37	0.50 - 0.62	0.81 - 1.03	1.38 - 1.67	0.51 - 0.65	1.03 - 1.22	0.25 - 0.32	0.48 - 0.59	0.27 - 0.28	0.56 - 0.70	0.31 - 0.40	0.54 - 0.68	EVM guidance level of 150 µg/kg bw /day

Table 1: Comparison of the estimated dietary intakes of each element for each population group with the relevant health based guidance values *continued*

Estimated Dietary Exposure (µg/kg bw/day)[1, 2, 3]													
Element	**Adults**		**Pre-school children (1.5 - 4.5 years)**		**Young people (4-18 years)**		**Elderly (free living)**		**Elderly (institutional)**		**Vegetarians[4]**		**Health Based Guidance Values[5]**
	Mean	High level	Mean	High level	Mean	High level	Mean	High level	Mean	High level	Mean	High level	
Copper*	17.23	34.47	44.71	77.82	29.41	54.92	16.09	45.70	13.38	43.36	18.34	29.96	JECFA PMTDI of 500 µg/kg bw/day EVM safe upper limit of 160 µg/kg bw/day
Germanium	0.001 - 0.018	0.002 - 0.033	0.002 - 0.053	0.006 - 0.085	0.001 - 0.032	0.004 - 0.058	0.001 - 0.016	0.002 - 0.029	0.001 - 0.015	0.002 - 0.036	0 - 0.02	0 - 0.032	N/A
Indium	0.06 - 0.24	0.22 - 0.47	0.24 - 0.75	0.93 - 1.48	0.13 - 0.44	0.51 - 0.97	0.05 - 0.21	0.25 - 0.46	0.04 - 0.18	0.19 - 0.45	0.10 - 0.29	0.36 - 0.57	N/A
Lead	0.09 - 0.10	0.17 - 0.18	0.21 - 0.25	0.38 - 0.42	0.13 - 0.15	0.26 - 0.30	0.08 - 0.09	0.16 - 0.17	0.06 - 0.07	0.17 - 0.19	0.12 0.21	0.20 -	JECFA PTWI equivalent to 3.6 µg/kg bw /day
Manganese*	67	124	168	305	106	201	56	112	50	121	78	135	EVM guidance level of 200 or 150 (elderly) µg/kg bw /day
Mercury	0.02 - 0.05	0.10 - 0.13	0.04 - 0.12	0.17 - 0.26	0.03 - 0.08	0.11 - 0.18	0.02 - 0.05	0.09 - 0.12	0.02 - 0.04	0.07 - 0.12	0.02 - 0.05	0.12 - 0.15	JECFA PTWI for methyl mercury is equivalent to 0.23 µg/kg bw /day
Molybdenum*	1.6	3.0 - 3.1	4.8 - 4.9	7.5 - 8.3	3.0	5.8	1.4 - 1.5	3.0	1.3 - 1.4	3.5	2.0	3.3 - 3.4	N/A
Nickel	1.5 - 1.6	3.0 - 3.1	4.2 - 4.9	7.5 - 8.3	2.6 - 3.1	5.3 - 5.8	1.3 - 15	2.6 - 3.0	1.1 - 1.4	2.8 - 3.5	1.9 - 2.1	3.5 - 3.4	EVM guidance level of 4.3 µg/kg bw/day; TDI of 12 µg/kg bw/day derived by WHO
Palladium	0.009	0.015 - 0.016	0.027	0.055 - 0.056	0.016	0.032	0.008	0.015	0.007	0.018	0.010	0.018	N/A

Table 1: Comparison of the estimated dietary intakes of each element for each population group with the relevant health based guidance values *continued*

Estimated Dietary Exposure (µg/kg bw/day)[1, 2, 3]													
Element	**Adults**		**Pre-school children (1.5 - 4.5 years)**		**Young people (4-18 years)**		**Elderly (free living)**		**Elderly (institutional)**		**Vegetarians[4]**		**Health Based Guidance Values[5]**
	Mean	High level	Mean	High level	Mean	High level	Mean	High level	Mean	High level	Mean	High level	
Platinum	0 – 0.029	0 – 0.051	0 – 0.082	0 – 0.130	0 – 0.048	0 – 0.089	0 – 0.025	0 – 0.045	0 – 0.033	0 – 0.055	0 – 0.031	0 – 0.050	N/A
Rhodium	0 - 0.029	0 - 0.051	0 - 0.082	0 - 0.13	0 - 0.048	0 - 0.089	0 - 0.025	0 - 0.045	0 - 0.023	0 - 0.055	0 - 0.031	0 - 0.050	N/A
Ruthenium	0.0004-0.010	0.001-0.018	0.0008-0.029	0.0022-0.047	0.0005-0.017	0.0013-0.032	0.0003-0.0087	0.0009-0.016	0.0002-0.0081	0.001-0.02	0.0007-0.011	0.0015-0.018	N/A
Selenium*	0.83 - 0.95	1.65 - 1.79	1.97 - 2.27	3.77 - 4.10	1.27 - 1.44	2.60 - 2.84	0.73 - 0.82	1.48 - 1.60	0.59 - 0.68	1.58 - 1.74	0.64 - 0.76	1.43 - 1.54	EVM safe upper level of 7.5 µg/kg bw/day
Strontium	15.6	30.6	42.8	71.1	25.9	51.0	14.0	26.6	12.0	29.2	20.5	35.9	N/A
Thallium	0.011 - 0.012	0.020 - 0.021	0.024 - 0.027	0.043 - 0.046	0.016 - 0.018	0.032 - 0.035	0.009 - 0.01	0.017 - 0.018	0.007 - 0.008	0.017 - 0.019	0.010 - 0.011	0.018 - 0.019	N/A
Tin	23	82	89	341	48	191	20	93	13	68	35	132	EVM guidance level of 220 µg/kg bw/day
Zinc*	141	268	387	776	232	478	122	261	104	252	93	162	JECFA PTDI of 1000 µg/kg bw/day; EVM safe upper level of 700 µg/kg bw/day

Table 1 Notes

1 The method for calculating estimated exposures is described in the text.

2. Where an element was not detected in some food groups, the estimated exposures have been expressed as a range from a lower bound (in which it was assumed that all non-detectable concentrations were zero) to an upper bound (in which all non-detectable concentrations were assumed to be at the limit of detection). Where only one value is shown, this is either because all samples contained concentrations above the limit of detection (therefore the upper and lower bound mean values are equal) or because the difference between them is negligible. In the calculation of upper bound exposures for inorganic arsenic, the concentration in the food groups was assumed to be equal to the concentration of total arsenic (since this was lower than the LOD for inorganic arsenic) except in the case of the poultry food group where it was considered to be equal to the LOD.

3. All figures have been rounded off as appropriate.

4. Some of the respondents to the dietary survey of vegetarians were consumers of fish.

5. Health based guidance values taken from: (i) the Joint FAO/WHO Expert Committee on Food Additives (JECFA) which set Provisional Tolerable Weekly Intakes (PTWI) and Provisional (Maximum) Tolerable Daily Intakes (P(M)TDI); (ii) the World Health Organization (WHO) which set Tolerable Daily Intakes (TDI); and (iii) the Expert Group on Vitamins and Minerals (EVM) which set safe upper levels and guidance levels; N/A = none available.

* Essential trace elements.

Table 2a: Comparison of average population dietary exposures of aluminium (Al), antimony (Sb), arsenic (As), barium (Ba), bismuth (Bi), cadmium (Cd), chromium (Cr), copper (Cu), Germanium (Ge), Indium (In) and lead (Pb) from UK Total Diet Studies 1976 to 2006

Year	Population dietary exposure (mg/day)[1-4]											
	Al	Sb	Total As	Inorganic As	Ba	Bi	Cd	Cr	Cu	Ge	In	Pb
1976	n.d.	n.d.	0.075	n.d.	n.d.	n.d.	0.02	0.13	1.8	n.d.	n.d.	0.11
1977	n.d.	n.d.	0.1	n.d.	n.d.	n.d.	0.018	0.17	1.8	n.d.	n.d.	0.1
1978	n.d.	n.d.	0.081	n.d.	n.d.	n.d.	0.02	0.1	1.6	n.d.	n.d.	0.11
1979	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.017	n.d.	n.d.	n.d.	n.d.	0.09
1980	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.026	n.d.	n.d.	n.d.	n.d.	0.12
1981	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.019	n.d.	n.d.	n.d.	n.d.	0.08
1982	n.d.	n.d.	0.09	n.d.	n.d.	n.d.	0.018	n.d.	1.3	n.d.	n.d.	0.069
1983	n.d.	n.d.	0.07	n.d.	n.d.	n.d.	0.018	n.d.	1.2	n.d.	n.d.	0.067
1984	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.019	0.073	1.4	n.d.	n.d.	0.065
1985	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.018	n.d.	1.3	n.d.	n.d.	0.066
1986	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.017	n.d.	n.d.	n.d.	n.d.	0.06
1987	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.018	n.d.	n.d.	n.d.	n.d.	0.06
1988	3.9	n.d.	n.d.	n.d.	n.d.	n.d.	0.019	n.d.	n.d.	n.d.	n.d.	0.06
1991	10	n.d.	0.07	n.d.	n.d.	n.d.	0.018	0.25	1.4	n.d.	n.d.	0.028
1994	11	0.003	0.063	n.d.	0.58	0.0004	0.014	0.34	1.2	0.004	n.d.	0.024
1995	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1997	3.4	n.d.	0.065	n.d.	n.d.	n.d.	0.012	0.1	1.2	n.d.	n.d.	0.026
1999	n.d.	n.d.	0.05	0.0009 - 0.005	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2000	4.7	n.d.	0.055	n.d.	n.d.	n.d.	0.009	0.046	1.3	n.d.	n.d.	0.0073-0.0074
2006[5]	5.4	0.0025	0.061 - 0.064	0.0014 - 0.007	0.847 - 0.848	0.002	0.011 - 0.013	0.022 - 0.029	1.24	0.0001 - 0.0015	0.005 - 0.019	0.006 - 0.007

Table 2b: Comparison of average population dietary exposures of manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), palladium (Pd), platinum (Pt), rhodium (Rh), ruthenium (Ru), selenium (Se), strontium (Sr), thallium (Tl), tin (Sn) and zinc (Zn) from UK Total Diet Studies 1976 to 2006

Year	Population dietary exposure (mg/day)[1-4]												
	Mn	Hg	Mo	Ni	Pd	Pt	Rh	Ru	Se	Sr	Tl	Sn	Zn
1976	n.d.	0.005	n.d.	0.33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.4	10
1977	n.d.	0.005	n.d.	0.26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.2	10
1978	n.d.	0.005	n.d.	0.27	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.6	10
1979	n.d.	0.004	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.2	n.d.
1980	n.d.	0.005	n.d.	0.27	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1981	n.d.	n.d.	n.d.	0.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.4	n.d.
1982	n.d.	0.003	n.d.	0.15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.1	10
1983	4.6	n.d.	n.d.	0.15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.3	10
1984	5.3	n.d.	n.d.	0.16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.7	10
1985	5.0	n.d.	0.11	0.14	n.d.	n.d.	n.d.	n.d.	0.063	n.d.	n.d.	1.7	10
1986	n.d.	n.d.	n.d.	0.13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.2	n.d.
1987	n.d.	n.d.	n.d.	0.15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.0	n.d.
1988	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1991	6.2	0.002	0.11	0.17	n.d.	n.d.	n.d.	n.d.	0.060	n.d.	n.d.	5.3	10
1994	4.9	0.004	0.11	0.13	0.001	0.0002	0.0003	0.004	0.043	1.3	0.002	2.4	8.4
1995	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0395	n.d.	n.d.	n.d.	n.d.
1997	n.d.	0.003	n.d.	0.13	n.d.	n.d.	n.d.	n.d.	0.039	n.d.	n.d.	1.8	8.4
2000	4.9	0.0012-0.0015	n.d.	0.13	n.d.	n.d.	n.d.	n.d.	0.032 - 0.034	n.d.	n.d.	1.4	8.4
2006[5]	5.24	0.001 - 0.003	0.123 - 0.125	0.127 - 0.129	0.0007	0 - 0.0023	0 - 0.0023	0.00003 - 0.00081	0.048 - 0.058	1.20	0.0007 - 0.0008	1.80 - 1.81	8.8

Tables 2a and 2b Notes

1. Population dietary exposure (mg/day): the average consumption of the population, estimated by multiplying the amounts of food consumed (based on consumption data from the EFS) by the corresponding upper and lower bound mean elemental concentrations in each food group.
2. The population dietary exposures in the previous years were estimated using upper bound mean concentrations for each food group and consumption data taken from the National Food Survey 1997. The exception to this is the 2000 TDS where exposures have been estimated from the lower and upper bound mean concentrations and included as ranges where they apply.
3. Changes in the organisation of the TDS from 1981 onwards mean that exposures from TDSs before 1981 and from 1981 onwards are not directly comparable6.
4. For those years where no values are given, these elements were not included in TDSs for metals and other elements i.e. n.d.= not determined.
5. Dietary exposure estimates for the 2006 TDS and 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.

Table 3: Estimated tap water intakes for those elements for which information is available

Element[6]	Concentration (µg/L)	Estimated tap water exposure (µg/kg bw/day) [5]			
		Pre-school children	Young people	Adults	Elderly
Aluminium	158 [1]	25	9.4	6.2	4.5
Antimony	1.967 [1]	0.31	0.18	0.077	0.056
Arsenic	7.871 [1]	1.2	0.47	0.31	0.22
Barium	7004	111	41.7	27.3	20
Cadmium	1.0418 [1]	0.17	0.062	0.041	0.030
Chromium	11.774 [1]	1.9	0.70	0.46	0.33
Copper	2000 [2]	317	119	78	57
Lead	25 [3]	4.0	1.5	0.97	0.71
Manganese	31.75 [1]	5.0	1.9	1.2	0.90
Mercury	0.3404 [1]	0.054	0.020	0.013	0.0097
Molybdenum	704	11.1	4.2	2.73	2
Nickel	17.914 [1]	2.8	1.1	0.70	0.51
Selenium	9.634 [1]	1.5	0.57	0.38	0.27

Tables 3 Notes

1 Maximum (99th percentile) concentration reported for 2007. Taken from monitoring results at consumer's taps, undertaken annually by each water company[8].

2 Maximum concentration of 4250 µg/L exceeded The Water Supply (Water Quality) Regulations 2000 for England and Wales[26]. Exposure has been calculated using the regulatory limit as it is assumed that regulatory action is taken for exceedances and that such events are a one-off occurrence.

3 Maximum concentration of 101.659 µg/L exceeded The Water Supply (Water Quality) Regulations 2000 for England and Wales[26]. Exposure has been calculated using the regulatory limit as it is assumed that regulatory action is taken for exceedances and that such events are a one-off occurrence.

4 World Health Organization guideline value for drinking water quality[27].

5 Elemental intakes from tap water were calculated assuming chronic tap water (97.5th percentile) consumptions of 158.429 mL/kg bw/day (pre-school children), 59.558 mL/kg bw/day (young people), 38.943 mL/kg bw/day (adults), and 28.386 mL/kg bw/day (elderly)[15,19,21,22].

6 There are no regulations or guideline values for bismuth, germanium, indium, palladium, platinum, rhodium, ruthenium, strontium, thallium, tin, and zinc.

Statement on Food Standards Agency-funded research on health effects of mixtures of food additives (T01040/41)

Background

1. Traditionally risk assessment has been carried out on individual chemicals. However, this does not reflect the real-life situation as humans are seldom, if ever, exposed to single chemicals and all foods are mixtures of many different chemicals. Chemicals may exert combined effects related to either concomitant or sequential exposure, depending on their toxicokinetic and toxicological properties. In recent years concern about the possible "cocktail" effects of mixtures of chemicals, and in particular possible combination effects at low doses, has stimulated research. In a report in 2002 the COT made recommendations on approaches to risk assessment of mixtures of pesticides and similar substances[1,2], and in 2004 it considered whether these could be applied to mixtures of additives and contaminants[3] and outlined other approaches used for assessing mixtures of additives and contaminants. The COT also commented[4] on the Draft guidance document on "Chemical mixtures: a framework for assessing risks"[5] prepared by the Interdepartmental Group on Health Risks from Chemicals (IGHRC)

2. Table 1 shows the terminology used in describing the toxicology of mixtures.

3. In 2001 the Food Standards Agency commissioned a project (Research on health effects of mixtures of food additives) which was carried out jointly at Leatherhead Food International, Leatherhead, Surrey and TNO Quality of Life, Zeist, the Netherlands. As part of an Horizon Scanning exercise at its meeting on February 5th 2008, the COT was provided with a summary of the results of this project and agreed it would like to have an opportunity to comment on the full report. This was discussed by the committee at its meeting on April 1, 2008. To date this project has generated three publications[7, 8, 9] with at least two additional ones in preparation.

4. The project set out to build on existing information on the mode of action of a range of food additives compiled by the ILSI-Europe Acceptable Daily Intake Task Force[10, 11]. This had identified a number of additives where different types of combined action were plausible but it was not possible to predict which were more likely. Four additives that had been shown to cause liver enlargement were selected (Table 2).

5. Table 2 shows the acceptable daily intakes (ADIs) set by the European Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) for the four compounds, and the NOAELs identified by Groten *et al.*[10]. For all four compounds, these NOAELs were based on observations of liver enlargement associated with induction of hepatic enzymes at the next highest dose Data were collated from a series of different papers, not all of which measured the same enzymes. The ADIs set by the SCF are currently being reviewed by the European Food Safety Authority (EFSA), starting with the colours.

Table 1: Terminology used in describing possible combined actions of chemicals in a mixture (based on COT 2002, after Cassee *et al.*[6])

Concept of type of combined behaviour	Terms used in this report	Synonyms	Observed effects
non-interaction - components of a mixture do not affect each other's toxic response.	simple similar action	simple joint action summation	Concentration/dose addition Chemicals have the same effect on the body and differ only in potency. The combined effect can be estimated from the total dose of all agents together, after adjusting for potency.
.	simple similar action	simple independent action independent joint action	Either response addition or effect addition. The modes of action and often the nature and site of effect differ among the chemicals in the mixture. "Response" reflects incidence data and response addition is determined by summing the incidence data for each component in the mixture. "Effect" reflects continuous data and effect addition is determined by summing the effect of each component in the mixture. Note that response and effect are sometimes used interchangeably.
interaction	potentiation	synergy supra-additivity	The combined effect of agents is greater than would be expected on the basis of dose-addition (if the chemicals have the same mode of action) or response-addition (if they do not have the same mode of action).
	antagonism	sub-additivity	The combined effect of agents is less than would be predicted by dose or effect/response addition.

Outline of the study

6. Studies were carried out in the rat using dietary administration with individual food additives, binary mixtures of all six pairings of the four compounds and quaternary mixtures of all four compounds. In addition, *in vitro* studies were carried out with the individual additives and mixtures of additives in cultured rat and human hepatocytes, in order to provide a direct comparison of the effects in human and rat liver.

Table 2: Additives used in the mixtures research

Additive	E Number	Uses	ADI (mg/kg bw)	NOAEL10 (mg/kg bw/day)
Butylated hydroxytoluene (BHT)	E 321	Antioxidant	SCF: 0 -0.05 [12] JECFA: 0 -0.3 [13]	25
Propyl gallate	E 310	Antioxidant	SCF: 0 – 0.5 [14] JECFA: 0 -1.4 [15]	135
Curcumin	E 100	Colour	SCF: ADI not specified[16] JECFA: 0 -3 [17]	220
Thiabendazole as an additive	E 233	Previously used as fungicide mainly on a range of fruits. No longer permitted	SCF: was 0 - 0.3 [18] JECFA: 0 – 0.1 [19]	10
Thiabendazole as a pesticide and veterinary medicine	N/A	Pesticide and veterinary medicine	EC: 0.120 JECFA: 0.121	10

In vivo studies

7. In preliminary range-finding studies, the food additives were fed individually to male Sprague-Dawley rats for 28 days to determine dietary levels of the compounds for use in the mixtures study and to identify biomarkers of effect for the individual compounds. Five concentrations (plus a zero control) were selected for each compound, based on data from the literature and historical data collated from 28 day studies in rats carried out in accordance with good laboratory practice (GLP). Dietary concentrations were selected to provide target doses of 25 to 1000 mg/kg bw/day for BHT and curcumin, 20 to 600 mg/kg bw/day for propyl gallate and 10 to 500 mg/kg bw/day for thiabendazole. The dietary concentrations ranged from 254 to 10154 mg/kg for BHT, from 254 to 10154 mg/kg for curcumin, from 203 to 6092 mg/kg for propyl gallate and from 102 to 5077 mg/kg for thiabendazole.

8. The highest dose level to be used for each of the compounds in the mixtures study was selected on the basis of the results of the endpoints examined in the preliminary study, including effects on body weight, liver weight and the various biomarkers measured (e.g. enzyme activities, mRNA levels). These highest (100%) dose levels were described by the researchers as being around or at the minimum observed adverse effect levels in the preliminary study, which differ from the NOAELs in Table 2 cited

by Groten *et al* [10]. Based on the dietary concentrations and food consumption, the achieved 100% doses were 333 mg/kg bw/day for BHT, 408 mg/kg bw/day for curcumin, 290 mg/kg bw/day for propyl gallate and 153 mg/kg bw/day for thiabendazole. The main mixtures study included 27 treatment groups and a control group, with each compound administered at combinations of 0, 25, 50 and 100% of the highest dose

9. The rationale for this protocol was to ensure that the 100% dose levels produced clear effects on some of the parameters measured, so that any effects due to combinations of chemicals in a mixture might be detected at lower individual dose levels (e.g. 25% of the total) when included in a mixture. The compounds were administered at 25, 50 and 100% of their maximum dose when given individually. For binary mixtures the individual compounds were each administered at 25 and 50% of their maximum dose, such that the total fractional dose added up to 50 or 100%. For quaternary mixtures the individual compounds were each administered at 6.25, 12.5 and 25% of their maximum dose such that the total fractional dose was 25, 50 or 100%. This protocol was designed to test if the observed findings were most compatible with those predicted by effect addition, dose addition or interaction (synergy or antagonism) based on statistical analysis of the dose response relationship.

10. At the end of the 28 day dosing period the animals were killed, blood was sampled and livers removed. Clinical chemistry was carried out on the blood samples. The livers were divided to provide RNA samples for TaqMan® analysis, material for transcriptomics, subcellular fractions for measurement of enzyme activities and fixed samples for histological examination. Samples from animals of the same treatment group were pooled for transcriptomics.

11. Analysis of gene expression data on the effect of treatment with BHT, curcumin, propyl gallate and thiabendazole individually was carried out using a cDNA chip containing about 3000 different sequence verified rat cDNAs. Microarray analysis was carried out in accordance with the principles of Minimum Information About a Microarray Experiment (MIAME)[22]. Analysis of samples from the mixtures study was conducted using the Affimetrix GeneChip platform which provided a more robust system and a much larger number of probe sets (15923). Functional analysis of gene expression changes was performed using T-profiler[23], which is a TNO in-house (toxico)genomics database and analysis tool that allows comparison of systems toxicology/genomics datasets at the level of networks and pathways.

12. Predicted data for mixtures were derived from additivity surface equations obtained from response curve modelling for the individual additives. If measured data for mixtures were significantly different from predicted data, either on the assumption of dose or effect additivity, it was assumed the combined effects reflected interactions.

In vitro studies

13. Studies were performed to investigate the food additives and food additive mixtures in cultured rat and human hepatocytes using a 72 hour incubation period. Rat hepatocytes were treated with 40 concentrations of either individual food additives or mixtures of food additives. Chosen biomarkers of effect were CYP1A2 and CYP2B1 mRNA levels and 7-benzyloxy-4-trifluoromethylcoumarin (BFC)

O-debenzylase activity. (BFC is a substrate for CYP1A2 and CYP2B1). The human hepatocyte study consisted of a control and 27 concentrations of either individual food additives or mixtures of food additives and CYP1A2, CYP2B6 and CYP3A4 mRNA levels as biomarkers of effect. CYP1A2 mRNA was therefore the only parameter measured in both rat and human hepatocytes.

Brief Summary of the Results

14. The authors concluded that, for body weight and liver weight, most of the findings were consistent with dose addition. In most cases liver weights showed no substantial deviations from predicted values either in binary or quarternary mixtures. In the quarternary mixture with a total fractional concentration of 100% (25% of the maximum dose of each additive), the measured liver weight was 122% of control compared to the predicted value of 108% of control, indicating some deviation from the predicted value. However, as significant deviations from predicted values were not seen for liver weights in binary mixtures containing curcumin and propyl gallate, these results were considered by the authors to be primarily due to the relatively high dose levels of BHT and thiabendazole, both of which produced significant increases in relative liver weight without any evidence of hepatotoxicity.

15. The largest difference between predicted and measured values was for CYP1A2 expression, with all binary and quaternary mixtures showing antagonism. Apart from mixtures of BHT plus curcumin, this appeared to be a dose-dependent effect. Also, for all of the binary mixtures, at least one of the dose groups was not compatible with the concept of dose addition. Therefore, it is likely that antagonistic interactions occurred between these mixtures with respect to CYP1A2 mRNA expression.

16. Although curcumin alone did not induce the activity of glutathione S-transferase (GST), the induction observed with the combination of BHT and curcumin exceeded that seen with BHT alone, indicating an interaction. The induction was more pronounced for activity with 1,2-dichloro-4-nitrobenzene (DCNB), a marker of GSTmu forms, than with 1-dichloro-2,4-nitrobenzene (CDNB), which is a more general substrate for the different forms of GST.

17. Thiabendazole was found to have the most marked effects on the gene expression profile and also had a dominant effect in studies with binary and quaternary mixtures. Although curcumin and propyl gallate had only modest effects on gene expression and enzyme activity when administered individually, marked effects on gene expression were seen with binary combinations of these compounds.

18. In the studies conducted in rat and human hepatocytes, some quaternary mixtures produced antagonistic effects on CYP1A2 mRNA expression, as seen in the *in vivo* study. Both sub- and supra-additive deviations from predicted values were observed in the expression of CYP2B1 (rat), CYP3A4 (human) and CYP2B6 (human) with some binary and quaternary mixtures, but there was no consistent pattern across species or between the *in vitro* and *in vivo* studies.

19. In summary, no evidence of combination effects leading to overt toxicity was apparent with the conventional toxicological endpoints, whereas some interactions were observed for induction of GST activity and at the genomic level using transcriptomics.

Previous COT evaluations of relevance to mixtures

20. In 2004, the COT agreed general conclusions on mixtures of chemicals in food, extending the conclusions of its 2002 report2 to take account of the possibility that exposure to some food additives and ingredients of very low toxicity may be much higher than exposure to pesticides and veterinary medicines"[3]. These conclusions were:

    i. *"Because of the complexity and variability of chemical mixtures that may occur in the environment, risk assessment of any toxic effects of chemical mixtures is extremely difficult. Most experimental work has been directed at toxic effects due to combined actions on biological systems at relatively high levels of exposure in laboratory experiments in laboratory animals or using in vitro systems.*

    ii. *Direct chemical reactions can occur between the components of a mixture: there are relatively few studies of these substances that have investigated such reactions.*

    iii. *Several studies claim to have identified synergistic interactions of some mixtures. However, for the most part, these studies have been inadequately designed and based on an incomplete understanding of the concepts involved, but a few well-designed studies have demonstrated the occurrence of both synergistic and antagonistic interactions, as well as additive effects in mixtures. These effects have usually been demonstrated at high concentrations or high experimental exposure levels, which are probably unrepresentative of exposure doses to chemicals present at very low levels in food.*

    iv. *Some interactions may not be easy to predict, such as those that may occur at the transcriptional level of the genome or second messenger signalling pathways.*

    v. *The type of combined action or interaction found at clearly toxic effect levels may not predict what will happen at non-toxic levels, including levels only slightly lower than the lowest observed adverse effect levels (LOAELs).*

    vi. *In relation to most examples of possible human exposure to multiple residues, it will be important critically to evaluate whether any effects are likely to occur at low levels of exposure, such as those that will occur through food and water.*

    vii. *Studies in vivo with chemicals that exhibit the same mode of action in the same target organ have shown that the effects of mixtures of similarly acting toxicants show additivity (dose addition), which results from simple similar action. This is the case, over the whole dose range.*

    viii. *It is essential to know what happens at non toxic levels, including exposure levels just below the LOAEL, in order to assess the health risk for humans exposed to mixtures of pesticides, veterinary drugs and similar substances. Generally, when exposure levels of the chemicals within a mixture are in the range of the NOAELs, and the components of the mixture have different modes of toxic action, no additivity and no potentiating interactions are found, indicating the applicability of the basic concept of "simple dissimilar action", which suggests that adverse reactions would be unlikely.*

ix. *Some studies (acute and subacute toxicity, genetic toxicity, carcinogenicity) have addressed the combined effect of mixtures of pesticides and in a few studies clear cases of potentiation were observed in animals exposed to levels of toxic substances showing adverse effects of individual compounds. However, direct extrapolation of these findings to much lower dose levels is not valid. Thus the probability of any health hazard due to additivity or potentiating interaction of mixtures of pesticides at (low) non-toxic doses of the individual chemicals is likely to be small, since the dose of pesticides to which humans are exposed is generally much lower than the NOAEL, at least through food.*

x. *Some endpoints that have been studied in animals or in in vitro systems are relevant to groups in the population believed to be at higher risk than the general population. Such endpoints include developmental toxicity studies, endocrine and neurotoxic effects and genotoxicity studies. On the basis of limited information it seems likely that the default assumptions in relation to mixtures in children and pregnant and nursing mothers, would be the same as for the rest of the population."*

COT Assessment of the current research project

21. Important interactions between some toxicants are known to occur at relatively high doses (i.e. above their individual effect levels). Most of the evidence for such phenomena relates to pharmaceuticals. Exposures to chemical contaminants in food, consumer products and the environment are generally well below individual effect levels. Many of the mechanisms that underlie demonstrable interactions at high doses would not be expected to cause important interactions at the much lower doses used in this study. For example, major modification of metabolic activation or detoxification by enzyme induction or competitive inhibition is unlikely. The limited empirical evidence currently available provides no indication of important toxic interactions at low doses, although in some circumstances dose additivity can lead to effects from combinations of toxicants at doses of the individual compounds below their effect levels.

22. Testing for possible interactions between toxicants is complicated as often more than one measure of effect could be used, and additivity in relation to one effect measure will not necessarily imply additivity for another. For example, an effect on liver size could be characterised either by the average increase in liver weight at a given dose, or by the proportion of animals at that dose with more than a specified increase in liver weight. If individual doses of two compounds were just below those necessary to increase liver weight above the specified value, their combination could be additive in respect of average increase in liver weight (effect addition) but more than additive in respect of the proportion of animals with liver weights exceeding the specified increase (response addition). If another measure of effect were adopted, based on a linear measure of liver size (e.g. maximum "diameter" in mm), by definition this could not demonstrate additivity if effects on average liver weight (which varies as the cube of its linear dimensions) were additive.

23. In the new research project there was no evidence of interactions leading to overt toxicity with the "conventional" endpoints employed, and therefore the rationale for investigations at the genomic level was queried. Many of the endpoints studied have no clear role in toxicity and others may not be relevant to toxicity in humans. For instance the measurement of induction of xenobiotic-metabolising

enzymes was not focused on enzymes with a known role in toxicity, making it impossible to predict toxic effects that might result following induction of these enzymes. If the mode of action of induction for the chosen biomarkers was known to be a key event in the pathway leading to an adverse effect, there would be a far more plausible rationale for analysing the chosen gene. For example CYP2B induction could serve as a biomarker for activation of the constitutive androstane receptor (CAR), a key event in the hepatocarcinogenic effects of certain compounds. However, as analysis of the mode of action for the chosen biomarkers was not carried out, their role if any, in leading to adverse effects of the compounds (i.e. the four additives) is unknown.

24. The use of a very limited range of biomarkers, not necessarily reflecting adverse effects, meant that the dosing schedule used in this study might not have covered a sufficiently wide dose range to identify thresholds for adverse effects.

25. The study provided an opportunity to explore the use of transcriptomics in mixtures toxicology, but the design was not optimal and the rationale for the timing of these investigations at the genomic level was questioned. For transcriptomics analysis, samples are usually taken early in the study in order to identify direct effects of the treatment rather than secondary effects, for example resulting from pathological changes. In addition it is necessary to follow the time course of the changes from early on, and these should be anchored to some toxicological response to allow adequate interpretation ("phenotypic anchoring"). The timing of events will vary depending on the nature of the effects. At the present time, knowledge on the sequence of events in time is very limited, so that changes in the expression of certain genes could represent either a prelude to toxicity, homeostatic regulation or adaptation. Currently, it is often not possible to distinguish between these possibilities, and in the absence of any clear signs of toxicity, this becomes even more problematical. As time from the initial exposure increases, the possibility that any changes in gene expression may represent secondary effects, possibly due to homeostatic regulation, also increases. Hence, in-depth knowledge of the consequences of specific gene/pathway changes is required, not just qualitatively but also quantitatively. However, in the absence of any observable toxic response in the current study, secondary effects are unlikely.

26. For both propyl gallate and curcumin there were no changes in CYP levels on an individual basis, but small changes in gene expression were observed when these additives were tested in combination. In the absence of pathological changes, the significance of this is unclear.

27. The design allowed for testing of compatibility of the measured effects with predicted data for binary and quaternary mixtures, according to accepted principles in mixtures toxicology. A number of statistically significant deviations from combined effects predicted on the basis of effect additivity were observed. It was difficult from the way in which the data were reported to ascertain their biological significance. For example, information on the background variability in the responsive genes would have been of value, as would the extent to which allowance was made for the multiplicity of comparisons and whether the deviations observed were biologically coherent. However, as samples used for transcriptomics were pooled, such data could not have been obtained. Dose-dependency of a number of the changes was limited. This made them difficult to interpret in the context of mixtures toxicology.

Interpretation

28. Members did not consider it surprising that in this study on food additives, deviations from effect additivity differed for liver weight and the various molecular markers. The small deviations for some of the metrics were considered to be of limited importance. In practical terms when exposures are at low doses, what matters is whether large deviations from effect additivity could occur that could lead to important toxicity well above any that would be expected from the individual components of a mixture. There was no indication of such a phenomenon in these data. The deviations from effect additivity for the various molecular markers were generally less than two-fold, but with a few (one *in vivo* for CP1A2 mRNA and two *in vitro* for BFC O-debenzylase activity) up to about eightfold.

29. In assessing the toxicity of mixtures, the effects of combined exposure can be determined either by direct study of the mixture or by prediction, based on assumptions about how the components of the mixture might interact. The latter strategy requires fewer resources and fewer animals. The present study demonstrated little deviation from effect additivity for "conventional" endpoints and did not reflect a potential for adverse health effects. In addition some of the hepatic changes may not be relevant to humans and this needs to be taken into account.

30. Because of the absence of overt toxicity at the doses given, findings relating to mode of combined action of the four additives investigated were of very limited value in exploring the possibility that these data could be used to predict additive or greater than additive, changes in toxicity.

31. The choice of compounds studied in this research was based on possible effects on the liver. It was a logical approach for an initial grouping to be based on morphological or adverse effects in the liver but the rationale for grouping these compounds on the basis of effects on xenobiotic-metabolising enzymes is less clear. Whereas some effects other than changes in xenobiotic-metabolising enzymes were measured *in vivo*, the hepatocyte studies only looked at cytotoxicity and changes in CYP mRNA levels and enzyme activities. Hence, the applicability of studies in hepatocytes to explore potentially adverse effects of mixtures cannot be determined from this study. Synergistic effects were found in mRNA expression levels with some mixtures that had no measurable effect on any other parameter. The biological relevance of such effects is not known and no adverse or other effect was observed in a 28-day toxicity study. However, if the mixture had been tested and such changes had been seen, there might be some basis for investigating further.

33. These results demonstrate that transcriptomics analyses cannot be used routinely in risk assessment of mixtures, since interpretation of the data, and the relevance to risk assessment are unclear unless they can be linked to conventional toxicological endpoints.

34. Results obtained for the compounds tested in this project did not suggest that these mixtures would lead to adverse effects in humans, or suggest important toxic interactions at the low doses to which consumers would be exposed. However, due to limitations in the study, it was difficult to assess whether there might be a risk from low level combined exposures. This was because the choice of endpoints was often not informative of possible human health effects. No analysis of mode of action was undertaken and no toxicity data were generated *in vitro* with mixtures of the compounds.

Comparisons of human intake with the maximum combined exposure when no adverse effects were detected might provide useful information on the risk of mixtures of these compounds. In addition, rather than focus on biochemical changes it might have been more productive to look for effects, such as morphological changes that might have been predicted to occur based on available information on the four compounds and their effects in the liver.

35. The results of this study do not suggest important toxic interactions at low doses for the additives tested.

Priorities for future research

36. Priorities for future research remain to test the basic assumptions of mixture toxicology, including the application of modelling approaches. It might be possible to use hepatocytes, though this approach requires further consideration to identify relevant endpoints. Compounds could be included on the basis of target organ (and/or cell type), mode of action (e.g. cytotoxicity, cell proliferation), or mechanism of action (e.g. direct-acting or activated by specific P450 enzymes; constitutive androstane receptor or the peroxisome proliferator-activated receptor α (PPARα) activator).

37. Endpoint selection should be based on relevant key events and on toxicological effects of relevance to human health. At least one mixture should comprise compounds all at or below their respective NOAELs. The applicability of transcriptomics (and other "omics" technologies) to this problem needs further investigation. Factors that should be taken into account include sample timing, dose-response analysis, statistical robustness, and background variability in expression and biological coherence, e.g. through pathway analysis.

38. Additionally it may be useful to test the robustness and try to reproduce the finding on CP1A2 that suggested a larger deviation from effect additivity.

Committee Conclusions

39. We consider that this study, which was substantial and complex, was carried out to high technical standards. However, at the doses studied, no overt toxicity was observed with the four additives either individually or in combination. Without using dose levels that demonstrate relevant toxicity or being able to extrapolate to such levels it is not possible to interpret the results of the transcriptomics studies with respect to implications for risk assessment.

40. We conclude that the new research does not raise concerns that combined exposure to the four compounds tested would pose a risk to health at doses individually below the Acceptable Daily Intakes.

41. Further work is needed to determine the applicability of transcriptomics in the risk assessment of mixtures.

COT statement 2008/09
December 2008

References

1. COT (2000) Annual report 2000. Available at: http://cot.food.gov.uk/pdfs/cotmins5dec2000.pdf Paragraphs 1.89 - 1.91

2. COT (2002) Risk Assessment of Mixtures of Pesticides and Similar Substances Available at http://cot.food.gov.uk/pdfs/reportindexed.pdf

3. COT (2004) Mixtures of food contaminants and additives. In Annual Report 2004, paragraph 1.69. Available at: http://cot.food.gov.uk/pdfs/cotcomcocreport2004.pdf

4. COT(2007) Minutes of the meeting held on Tuesday 3rd July 2007, http://cot.food.gov.uk/pdfs/cotmins3july07incadditives

5. IGHRC (2007) Chemical Mixtures: a Framework for Assessing Risks. Available at:http://www.silsoe.cranfield.ac.uk/ieh/ighrc/mixtures_document.pdf

6. Cassee, FR, Sühnel, J, Groten, JP, Feron, VJ (1999) The toxicology of chemical mixtures. In: General and Applied Toxicology edited by Ballantyne B, Marrs TC, Syversen T. London, Macmillan Reference Limited. 303-320.

7. Price, RJ, Scott, MP, Walters, DG, Stierum, RH, Groten, JP, Meredith, C and Lake, BG (2004). Effect of thiabendazole on some rat hepatic xenobiotic metabolising enzymes. Food Chem. Toxicol. Jun;42(6):899-908.

8. Price, RJ, Scott MP, Giddings AM, Walters DG, Stierum RH, Meredith C, Lake BG (2008) Effect of butylated hydroxytoluene, curcumin, propyl gallate and thiabendazole on cytochrome P450 forms in cultured human hepatocytes. Xenobiotica. Jun;38(6):574-86.

9. Stierum, R, Conesa, A, Heijne W, Ommen, BV, Junker, K, Scott, MP, Price, RJ, Meredith, C, Lake, BG, Groten J (2008). Transcriptome analysis provides new insights into liver changes induced in the rat upon dietary administration of the food additives butylated hydroxytoluene, curcumin, propyl gallate and thiabendazole. Food Chem. Toxicol. Apr 25.; [Epub ahead of print doi:10.1016/j.fct.2008.04.019] Aug: 46(8): 2616 - 2628

10. Groten, JP, Butler, WH, Feron, VJ, Kozianowski, G, Renwick, AG and Walker, R, (2000.) An analysis of the possibility for health implications of joint actions and interactions between food additives. Regul. Toxicol. Pharmacol. 31, 77-91

11. Walton, K, Walker, R, van de Sandt, JJM, Castell, JV, Knapp, AG, Kozianowski, G, Roberfroid, M., Schilter, B, (1999). The application of *in vitro* data in the derivation of the acceptable daily intake of food additives. Food Chem. Toxicol. 37, 1175-1197

12. Reports of the Scientific Committee for Food. Twenty-second series; (1989) Commission of the European Communities, Report EUR 12535 EN. p 13 & p 22-23. available at: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_22.pdf

13. FAO/WHO (1999) Safety Evaluation of Certain Food Additives; WHO Food Additive Series 42; Evaluation of national intake assessments of butylated hydroxytoluene (BHT). IPCS, Geneva.

14. Reports of the Scientific Committee for Food Twenty-second series; (1999) Commission of the European Communities Report EUR 12535 EN. p 13 & p 19-20; available at: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_22.pdf

15. FAO/WHO (1993) Toxicological evaluation of certain food additives and contaminants; Gallates (propyl, octyl and dodecyl); WHO Food Additive Series 32, IPCS, Geneva.

16. Reports of the Scientific Committee for Food First series; (1975) Commission of the European Communities p26; available at: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_01.pdf

17. FAO/WHO (2004) Toxicological evaluation of certain food additives and contaminants; Curcumin (addendum) WHO Food Additive Series No 52. IPCS, Geneva.

18. Reports of the Scientific Committee for Food Sixth Series (1978) Commission of the European Communities; ISBN 92-825-6550-9. available at: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_06.pdf

19. FAO/WHO (1997) Toxicological evaluation of certain drug residues in food (1997) WHO Food Additive Series No 39; IPCS, Geneva.

20. European Commission (2001). Review report for the active substance thiabendazole. Finalised in the Standing Committee on Plant Health at its meeting on 12 December 2000 in view of the inclusion of thiabendazole in Annex I of Directive 91/414/EEC. Available at: http://ec.europa.eu/food/plant/protection/evaluation/existactive/list1-26_en.pdf

21. FAO/WHO (2002) Toxicological evaluation of certain drug residues in food (1997) WHO Food Additive Series No 49. IPCS, Geneva.

22. Brazma, A, Hingamp, P, Quackenbush, J, Sherlock, G, Spellman, P, Stoeckert, C, Aach, J, Ansorge, W, Ball, CA, Causton, HC, Gaasterland, T, Glenisson, P, Holstege, FC, Kim, IF, Markowitz, V, Matese, JC, Parkinson, H, Robinson, A, Sarkans, U, Schulze-Kremer, S, Stewart, J, Taylor, R, Vilo, J and Vingron, M (2001). Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat. Genet. 29(4), 365-371.

23. Boorsma, A, Foat, BC, Vis, D, Klis, F, and Bussemaker, HJ (2005). T-profiler: scoring the activity of predefined groups of genes using gene expression data. Nucleic Acids Res. 33(Web Server issue), W592-W595

2008 Membership of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

CHAIR

Professor I A Hughes MA MD FRCP FRCP(C) FRCPH F Med Sci (up to to 31st March 2008)
Professor and Head of Department of Paediatrics, University of Cambridge

Professor D Coggon OBE MA PhD DM FRCP FFOM FFPH FMedSci (from 1 April 2008)
Professor of Occupational & Environmental Medicine, University of Southampton

MEMBERS

Dr D Bell BSc (Hons) PhD
Reader in Molecular Toxicology, University of Nottingham

Professor A Boobis OBE BSc PhD CBiol FIBiol
Professor of Biochemical Pharmacology, Imperial College, London

Professor D Coggon OBE MA PhD DM FRCP FFOM FFPH FMedSci (up to to 31st March 2008)
Professor of Occupational & Environmental Medicine, University of Southampton

Professor C de Vries MA MSc PhD FRSM
Professor of Pharmacoepidemiology, University of Bath

Dr R Dearman BSc (Hons) PhD
Faculty of Life Sciences, University of Manchester

Dr C Elcombe BSc PhD FBTS (from 1st September 2008)
Co-founder and Research Director of CXR Biosciences,
Senior Lecturer, Biomedical Research Centre, University of Dundee Medical School

Dr J Foster BSc PhD FRCPath
Senior Principal Pathologist, AstraZeneca Pharmaceuticals
Chair of Panel of Examiners for the Toxicology Specialty, Royal College of Pathologists

Dr A Hansell MSc MB BCh MRCP MFPHM PhD (from 1st September 2008)
Senior Lecturer and Wellcome Intermediate Clinical Fellow, Imperial College London

Professor D Harrison BSc MB ChB FRCPath FRCPEd FRCSEd
Professor of Pathology, University of Edinburgh Medical School
Director, University of Edinburgh Cancer Research Centre

Dr J Hinson BSc (Hon) PhD DSc
Reader in Molecular and Cellular Endocrinology, Dean for Postgraduate Studies, Deputy Dean for Student Affair, Internal Lead QM Research Ethics Committee

Dr P Jackson BA (Oxon) MA (Oxon) MB ChB MRCP PhD FRCP
Reader in clinical pharmacology and therapeutics, University of Sheffield

Professor J Konje MBBS MD MRCOG
Head of Clinical Division of Obstetrics and Gynaecology, Leicester Royal Infirmary

Dr G McNeill MB ChB MSc PhD RPH Nutr
Senior Lecturer in Nutrition Epidemiology, Department of Environmental and Occupational Medicine, University of Aberdeen

Professor I Morris BSc (Hons) PhD
Associate Dean for Research, Hull York Medical School

Dr N Plant BSc PhD
Senior Lecturer in Molecular Toxicology, University of Surrey

Dr D Ray BSc PhD
Associate Professor of Neurotoxicology, University of Nottingham Medical School

Professor I R Rowland BSc (Hons) PhD
Professor of Human Nutrition and Director of Northern Ireland Centre for Diet and Health (NICHE), University of Ulster

Dr D Tuthill MB BCh MRCP MRCPCH
Consultant Paediatrician, Children's Hospital for Wales

Miss A Ward BA
Public Interest Representative

Mrs A Williams OBE BA (Hons)
Public Interest Representative

SECRETARIAT

Dr D Benford BSc(Hons) PhD	Scientific Secretary
Mrs J Shroff BA(Hons)	Administrative Secretary
Mr J Battershill BSc MSc	Scientific – HPA
Dr D Gott BSc(Hons) PhD	
Mr D Renshaw BSc EurBiol CBiol MIBiol	
Ms C A Mulholland BSc(Hons)	
Dr C Tahourdin BSc(Hons) PhD	
Dr D Mason BSc(Hons) PhD	(up to 9th July 2008)
Ms B Gadeberg BSc MSc	
Mrs F Hill BSc	
Ms R Harrison BSc MSc	
Dr N Thatcher BSc(Hons) PhD	
Mr B Maycock BSc(Hons) MSc	
Dr S Creton BSc(Hons) PhD	(up to 11th August 2008)
Dr D Parker BSc(Hons) MSc PhD	(from 30th April 2008)
Mr G Welsh BSc(Hons)	(from 29th September 2008)
Miss T Gray BA(Hons)	
Miss J Murphy BA(Hons)	

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

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor Ieuan Hughes (Chair ceased 31 March 2008)	BP Amoco	Shareholder	Archives of Disease in Childhood	Associate Editor
	BP Amoco	Daughter is an employee of this Company	Academy of Medical Sciences	Fellow
			Society for Endocrinology	Member
			Royal College of Paediatrics and Child Health	Fellow; Senior Examiner; Regional Academic Advisor
			Medical Research Council	Member of Advisory Board
			Pfizer Aventis NovoNordisk Diabetes UK Wellcome Trust Juvenile Diabetes Research Fund	Funds received from all these sources for Departmental research and education in medicine and health related topics
Professor David Coggon (member upto 31st March and Chair from 1 April 2008)	Halifax Standard Life	Shareholder	Colt Foundation British Occupational Health Research Foundation Faculty of Occupational Medicine Public Health Commission	Trustee Trustee Member President and Trustee

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Dr David Bell	Alliance & Leicester BAA BG Centrica HBOS Plc International Power National Grid RT Group Rolls Royce Scottish Power Thus Transco United Utilities University of Nottingham	Shareholder Employee	Food Standards Agency Central Science Laboratory Dow Aptuit Inc EFSA CEF Panel British Toxicology Society	Research Contract Part funded PhD studentship Research Grant Consultancy Member (with renumeration) Member of executive committee
Professor Alan Boobis OBE	Banco Santander SA Barclays BG Group BT Group Centrica Plc HBOS Iberdrola SA National Grid Scottish Power Thus Astellas Pharma Sumitomo Chemical (UK) Plc Proctor & Gamble Howrey LLP	Shareholder Consultancy	GlaxoSmithKline Food Standards Agency Department of Health Commission of the EU (FP6) ESRC ILSI HESI Elsevier JMPR JECFA (vet drugs) EFSA PPR Panel (Panel on Plant Protection Products and their Residues) ECETOC Task Force on Guidance for Classification of Carcinogens under GHS EFSA Scientific Committee Working Group on Risk-Benefit Assessment EFSA Scientific Committee Working Group on the Benchmark Dose	Support by Industry Research Contract PhD Studentship Unpaid chair of Board of Trustees Editor-in-Chief; Food and Chemical Toxicology Member

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Dr Rebecca Dearman	Syngenta CTL AstraZeneca Research Institute for Fragrance Materials, (RIFM) European Chemical Plasticizers Industry (ECPI)	Shareholder Consultancy Consultancy	Unilever Syngenta European Chemical Plasticizers Industry (ECPI) American Chemical Council (ECPI) BASF RIFM	Research Grant Research Grant Research Grant Research Grant Research Grant Research Grant
Dr Corrine de Vries	NONE	NONE	Schering AG Yamanouchi	Research Grant
Dr Clifford Elcombe (member as of 1 September 2008)	CXR Biosciences Ltd	CXR Biosciences Ltd Salaried Director Shareholder	Various Pharmaceutical and chemical companies	Contract Research at CXR
Dr John Foster	AstraZeneca	Shareholder	NONE	NONE
Dr Anna Hansell (member as of 1 September 2008)	Dept of Epidemiology & Public Health Imperial College London (includes Small Area Health Statistics Unit) Greenpeace Halifax	Employee Supporter (non-active) Shareholder	GlaxoSmithKline AstraZeneca	Research Grant Research Grant

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor David Harrison	University of Florida University of Canberra Response Genetics The Forensic Institute	Consultant Consultant Consultant Shareholder	EMMS Nazareth Melville Trust Medical Research Scotland Alma Diagnostics Yorkshire Cancer Research DoH GTAC HPA Committee on Carcingenicity CRUK Science Strategy Advisory Group Biomedical & Therapeutics Res Comm. (Scotland)	Trustee Trustee Trustee Research Collaboration Scientific advisory committee Vice chair Member Member Member
Dr Joy Hinson	GlaxoSmithKline	Shareholder	Society for Endocrinology Journal of Endocrinology Current Opinions in Endocrinology and Diabetes	Council member and Education Advisor Member of the editorial board
Dr Peter Jackson	Carillion Computacenter Ecofin Friends Provident Hochschild Mining Plc Informa Legal General Mapeley Melrose Senior St Ives St James Place Capital TT Electronics Venture Production	Shareholder	Bayer	Departmental Research Funding

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor Justin Konje				
Dr Geraldine McNeill	Smith & Nephew Diageo Café Direct BHP Billiton	Shareholder	World Cancer Research Fund	Grant panel member
Professor Ian Morris	Takada Pharmaceuticals Society for Endocrinology Society for Medicines Research Society for study of fertility British Society for Toxicology	Consultancy Membership		Son is a student fellow of British Heart Foundation
Dr Nicholas Plant	NONE	NONE	Xenobiotica British Toxicology Society Pfizer GlaxoSmithKline AstraZeneca	Associate Editor Member of Education sub-committee Research Funding
Dr David Ray	University of Nottingham ZLB Behring (Switzerland) Astellas pharmaceuticals CEFIC ESAP	Employee Consultancy Consultancy Independent advisor		

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor Ian Rowland (membership ceased as of 31 March 2008)	Alpro Foundation European Natural Soybean Association Glanbia Danone Clasado Woolwich Halifax	Consultancy Shareholder	ILSI Europe Kelloggs Cereal Partners Geest Vitacress Yakult UK Unilever Masterfoods Scottish Crops Research	Partner in EC funded project Funds received from these sources for Departmental research
Dr David Tuthill	Cardiff & Vale NHS Trust SMA Nutricia Milupa	Salary Consultancy	Royal College of Paediatrics and Child Health Welsh Paediatric Society British Society of Paediatric Gastroenterology, Hepatology and Nutrition Paediatric Research Society British Association of Parenteral and Enteral Nutrition Nutrition Society British Society of Clinical Allergy and Immunology	Fellowship
Miss Alison Ward	NONE	NONE	Farm Animal Welfare Council	Member
Mrs Alma Williams	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 2007, the Committee provided advice on a wide range of topics including genotoxicity of acrylamide, chemical mixtures, phenol and the assessment of mutagenic impurities in pesticides. A large proportion of COM business was devoted to the evaluation of acrylamide and its genotoxic metabolite glycidamide.

The COM initiated a revision of its guidance document (Guidance on a Strategy for Testing of Chemicals for Mutagenicity) which had been published in 2000, and initiated a review of the use of Toxicogenomics in genotoxicity evaluation.

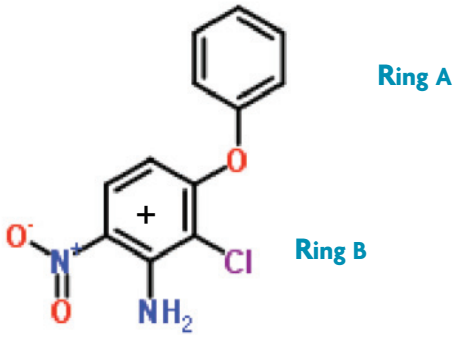
Professor P B Farmer Chair
MA DPhil CChem FRSC

COM evaluations

Aclonifen

2.1 The COM was asked for advice by the Pesticides Safety Directorate (PSD) on a pesticide active ingredient new to the U.K. which is undergoing evaluation through the independent Advisory Committee on Pesticides (ACP). The referral statement was as follows: 'ACP requested advice on the mutagenicity of Aclonifen and the genotoxicity risk assessment of the postulated metabolites hydroquinone and phenol. The referral does not include carcinogenicity data or the evaluation of mode of action for tumours in rodents observed in long-term carcinogenicity bioassays with Aclonifen'. 2. Aclonifen (2-chloro-6-nitro-3-phenoxyaniline) (figure 1.) is a selective systemic herbicide used for pre-emergence control of grass and broad leaved weeds in a range of crops.

Figure 1: Aclonifen



+ = position of uniformly radiolabelled phenoxyaniline ring

2.2 The COM considered a large amount of data on Aclonifen, which included an extract from the detailed record of ACP consideration of Aclonifen, extracts from draft EU assessment report on metabolism and genotoxicity of Aclonifen, which presented information on structure, use as a pesticide, ADME studies, toxicology, mutagenicity, carcinogenicity and reproduction, data from mutagenicity test reports on Aclonifen, copy of the report on the investigation of the potential for DNA-binding of Aclonifen and the revised position paper from the data holder on the cleavage of the diphenyl ether bond of Aclonifen. The data holder BayerCrop Science, submitted a presentation which was circulated to Members and in addition a revision to a report on cleavage of the diphenyl ether bond in the Aclonifen molecule. The COM considered Aclonifen at its 23 October 2008

2.3 The COM reached a number of conclusions as shown below;

i) The COM agreed that further data on Aclonifen metabolism was required. This could involve more *in vivo* tests with specific analysis for the formation of hydroquinone and phenol. Alternatively, it might be possible to undertake comparative *in vitro* studies using rodent and human tissues (with specific measurement of hydroquinone and phenol formation). It was considered this could provide evidence that exposure to Aclonifen was unlikely to be associated with significantly increased genotoxic risk, although this would not preclude the possible need for additional mutagenicity tests dependent on the outcome of the metabolism studies.

ii) The COM noted the approach to risk assessment had not been considered during the presentation, but that the data holder had included a proposed Margin of Exposure approach in the submission dated 13 August 2008. This would need to be considered further when appropriate metabolism data were available.

2.4 The COM agreed a statement which is reproduced at the end of this report.

Impurities

2.5 The COM had been informed of a published literature survey to evaluate the lowest detectable level of response in the Ames test for mutagens during the horizon scanning exercise for 2007. The approach adopted by the authors might have potential wider generic use which could be valuable for the review of the COM strategy and also for generic advice to Government Departments. (*Kenyon MO et al Regulatory Toxicol, Pharmacol, 48, 75-86, 2007.*) The COM reviewed this publication and also considered other recent publications which had considered a rationale for determining, testing, controlling specific impurities in pharmaceuticals that possess potential for genotoxicity.

Kenyon MO et al Regulatory Toxicol, Pharmacol, 48, 75-86, 2007

2.6 A literature survey of 454 mutagens tested in the Ames test was undertaken to estimate the lowest effective concentrations for a variety of classes of mutagens and to develop an understanding of the sensitivity of the test system. Overall for most representative classes, all compounds were detected at 2500 µg/plate. In a further analysis by class, the authors reported that only a small number of compounds had LECs that were greater than 250 µg/plate. Overall, the authors estimated that 85% of mutagenic impurities in an Active Pharmaceutical Ingredient (API) should be detected in Ames tests if present at ≥5% assuming the API is tested up to 5000 µg/plate. The literature review had been supported by a number of Ames tests of pharmaceutical agents undertaken in the presence of excess mannitol (to represent excess API) and verapamil and diltazem (two highly metabolised medicines). Members agreed that many impurities in APIs were present at less than 5% and it was likely such impurities would need to be isolated and tested separately in order to evaluate their potential mutagenic hazard. A negative result in Ames tests for a test material containing impurities below 5% would not provide reassurance that the impurity had been tested adequately.

*Muller L et al Regulatory Toxicology and Pharmacology, 44, 198-211, 2006. and EMEA guidance (CHMP/SWP/*5199/02, 28 June 2006)

2.7 Members acknowledged that the approach suggested was specific to pharmaceuticals and provided guidance on assessing genotoxic impurities in APIs particularly in relation to decisions on safety in respect of clinical trials. The TTC approach was based on assessment of likely intakes of impurities (i.e. a de *minimus* risk value (Threshold of Toxicological Concern (TTC) (1.5 µg/person/day)) could be identified for any chemical, including those of unknown toxicity, taking chemical structure into consideration). The TTC was originally applied to foodstuffs (e.g. impurities present in flavour materials and food contact materials) was introduced as a way of prioritising action on those most likely to cause the greatest risk and there had also been proposals that the TTC could be used to inform on decisions of acceptability.

2.8 The COM agreed the proposed approach had an advantage in aiding assessment of risk/benefits from clinical trials. Members agreed that it was not possible to conclude that scaling intakes resulted in the same mutagenic risk. Members noted that the EMEA guideline limit for genotoxic impurities in APIs could exceed the TTC for life-threatening illnesses.

Chemical Mixtures

2.9 The COM expressed an interest in the evaluation of the mutagenicity of chemical mixtures during the 2005 and 2006 horizon scanning exercises. One recommendation from COM was to consider the possible occurrence of synergistic interactions regarding mutagenic effects of chemical mixtures, the possible mechanisms for any synergistic effects and the implications of such a finding for risk assessment. It is possible that if synergistic effects between two or more *in vivo* mutagens occurred then co-exposure to mixtures containing these chemicals might result in a significant increase in the risk of mutagenicity and cancer compared to the risks associated with exposure to the individual chemicals alone. The COM evaluation outlined was intended to build on the work of the COT work on Risk Assessment of Mixtures of Pesticides and similar substances (WiGRAMP)[1] http://www.food.gov.uk/science/ouradvisors/toxicity/cotwg/wigramp/ which was subsequently extended to encompass other types of chemicals in food (see 2004 COT Annual report http://www.food.gov.uk/multimedia/pdfs/cotsection.pdf) and the ongoing work of the Interdepartmental Group on Health Risks from Chemicals (IGHRC) on the risk assessment of chemical mixtures http://www.silsoe.cranfield.ac.uk/ieh/ighrc/mixtures_document.pdf. Thus the definitions and nomenclature used to describe interactions regarding mutagenicity induced by chemicals in this statement were taken from these reviews.

2.10 A number of strategies have been considered for the evaluation of chemical mixtures. These include testing whole mixtures (integrative), fractionation of mixtures to determine mutagenic components (dissective, topdown approach), and investigations of interactions by testing simple combinations, recombined fractions, and spiking of mixtures/fractions (synthetic, bottom up approach). All of these approaches were identified from literature searches with regard to mutagenicity testing, although relatively few studies of whole mixtures were identified. Approximately 110 research papers with potentially relevant information were identified for consideration during the COM review

COM Discussion and Conclusions

Whole mixtures

2.11 The COM considered mutagenicity testing of whole mixtures, and approaches to dissection (fractionation/concentration) of mixtures. The primary purpose of such studies is to monitor mutagenic response in tests of a wide variety of mixtures (for example foods, samples of pollution (air and water) condensates or particles from pyrotechnic mixtures (e.g. cigarette smoke or mixtures of known compounds), hazardous wastes including industrial process effluents and municipal sludges. The COM noted that there were comparatively few data on mutagenicity testing of whole mixtures. The COM agreed that testing whole mixtures first using an *in vitro* screen (such as the Ames test or SOS chromotest) would have the advantage of picking up evidence of potential interactions, such as

synergy, that could be missed by testing individual fractions. However, the failure to detect mutagenicity when complex mixtures (e.g. fried foods) or fractions (e.g. catalytically cracked clarified oil) are tested either *in vitro* or *in vivo* did not prove the absence of potentially mutagenic compounds.

Approach to dissection of mixtures

2.12 The COM agreed an outline proposal for a strategy for monitoring mutagenicity of chemical mixtures (in particular occupational and environmental mixtures), based on proposals for evaluating the mutagenicity of mixtures in the published literature but noted that this was only general guidance and a case-by-case approach was needed.

Preliminary considerations

A. Collect information on chemical composition, and mutagenicity of chemicals in the mixture. Define the purpose of the monitoring approach (is this to monitor overall mutagenic hazard of the mixture, or to monitor the mutagenicity of selected levels of chemicals or groups of chemicals within the mixture?).

B. Review the literature for appropriate data on sampling, extraction and testing of similar mixtures. Review the mutagenicity test data on the mixture or similar mixtures or the chemicals within the mixture selected for monitoring.

Mutagenicity testing

C. Define *in vitro* testing strategy, focusing on optimising and standardising the approach.

D. Undertake *in vitro* monitoring to validate approach and identify sources of variation and their impact.

E. Consider, if necessary on a case-by-case basis, developing an *in vivo* segment to strategy. (This might include studies to test whether chemical(s) selected for monitoring had *in vivo* mutagenic potential if this was not known. It is unlikely that chemical(s) within a mixture which were known to be *in vivo* mutagens would need to be routinely tested.

Review of strategy

F. Implement the strategy and use data to inform on risk reduction strategies. It is important to periodically review the results of a monitoring strategy, particularly if there is any evidence for a change in the results being reported. There are many potential sources of variation which could affect the results and it would be important to differentiate between a change in results due to composition of the mixture from a change due to variation in fractionation and/or testing procedures. The inclusion of spiked samples in a strategy for mutagenicity testing of mixtures may be valuable.

Approach to evaluation of studies to investigate interactions

2.13 The COM agreed the concept of the 'envelope of additivity' was a helpful approach in the presentation of the results of studies and in the identification of non-interaction (e.g. dose-response and effect additive responses) and interaction responses (e.g. synergy and antagonism). The COM noted the proposed unifying approach of Gennings and colleagues (see Gennings C *et al* (2005). A unifying concept for assessing toxicological interactions: changes in slope. Tox Sci, 88 (2), 287-297.) for application of statistical methods in chemical mixture research which is based on the shape of the dose response curve and changes in the slope of the dose-response curve in studies using two or more chemicals, and agreed that this could be of potential use in evaluating genotoxicity.

Review of published studies on interaction between chemicals with regard to mutagenicity.

2.14 The COM noted that the available published literature presented a number of examples where interaction between chemicals with regard to mutagenicity had been reported. However, there was essentially no appropriate independent confirmation of the results in separate tests, or within an appropriate mutagenicity testing strategy for the identification of interactions and therefore no definite conclusions could be reached.

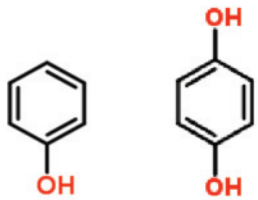
2.15 The COM agreed that the available studies had raised a number of potential hypotheses for interaction (see statement enclosed at the end of this annual report). There was a need for further research regarding such mechanisms, which if confirmed in an appropriate mutagenicity testing strategy might be of potential significance for public health.

2.16 The COM agreed a statement which is reproduced at the end of this report.

Phenol

2.17 HSE asked for advice from COM on phenol (along with hydroquinone) in 1994/95 and in 1999. A copy of the conclusions and the statement agreed in 1999 (published January 2000, COM/00S1). [Hydroquinone is a metabolite of phenol, see figure 1 below]

Figure 1:



Phenol Hydroquinone

2.18 In 1994, the COM concluded the *in vitro* mutagenicity data on phenol were of poor quality and results difficult to interpret, but *in vivo* data showed phenol to be a somatic cell mutagen at very high dose levels. (COM noted negative results in long term carcinogenicity bioassays in rats and mice). The COM noted the potential for rapid conjugation and detoxication via the glutathione pathway and that the mutagenicity of phenol appeared to be predominantly related to peroxidase activity and catalase could have a protective role. The COM agreed there was a potential for a threshold mechanism by the oral route of exposure but could not reach a similar conclusion with regard to dermal or inhalation exposure.

2.19 In 1995, the COM considered a submission from industry which provided some metabolism data. Overall the COM concluded that appropriate studies to determine the extent of pre-systemic metabolism following either inhalation or dermal exposure had not been undertaken. The COM provided guidance on the approaches which could be used including administration of hydroquinone or phenol via a bronchoscope with very early sampling for free and conjugated test substance in the blood.

2.20 In 1999, the COM considered a study on bioavailability and metabolism of hydroquinone after intratracheal instillation in male rats. The results showed free systemic hydroquinone in arterial blood 5-10 seconds after dosing. The COM considered the data suggested the potential for site of contact and systemic mutagenic effects after inhalation exposure. The COM considered a inhalation exposure transgenic Muta™mouse study but were unable to draw any conclusions in view of unacceptable levels of DNA packaging in many of the trials in the experiment. The COM noted a small but consistent positive result in bone marrow micronucleus studies in mice given intraperitoneal doses of around 100-160 mg/kg bw.

2.21 The COM agreed a statement (COM/00/S1; http://www.iacom.org.uk/statements/COM00S1.htm) in January 2000. The conclusions reached with regard to phenol were similar to those reached in 1994

2.22 In 2003, the COM considered a pre publication report from the Dow Chemical Company which provided results to suggest that the *in vivo* mutagenicity of phenol in the mouse bone marrow micronucleus assay originated from a transient hypothermia induced by high doses of phenol. The COM agreed the data supported a case for a threshold mechanism for the induction of MN in bone marrow of mice but considered publication of the study in a peer-review journal would be necessary before drawing any definite conclusions. A further COM statement was not published in 2003. The relevant study has now been published and was identified during the 2007 COM horizon scanning exercise. Members asked for a review of the paper during the COM horizon scanning exercise. In addition the HPA asked for advice on the genotoxicity of phenol and specifically whether a threshold approach can be used with regard to the risk assessment of genotoxicity of phenol.

Introduction to current COM review

2.23 The COM current consideration of phenol covered the period from 1994-2003. The objectives of the current review were i) produce an updated COM statement on phenol, ii) to evaluate the Spencer study on hypothermia and also iii) to consider if any *in vivo* mutagenic effect of phenol can be considered as related to a threshold effect

2.24 The COM agreed with the conclusions reached on phenol in its previous statement (COM/00/S1). (The COM agreed the overall conclusions reached in the draft EU Risk Assessment report which had been provided for members' information.)

2.25 The following overall conclusions were agreed.

a. Phenol is mutagenic *in vitro* in mammalian cells giving rise to gene mutation and chromosomal damage in the presence and absence of exogenous metabolic activation. The mode(s) of action had not been fully elucidated although there was evidence that effects were in part due to oxidative DNA damage

b. Phenol should be regarded as an *in vivo* somatic cell mutagen. The COM confirmed that there was consistent evidence for a small effect at doses below the i.p. LD50.

c. The COM agreed that the published study by Spencer *et al* 2007 had been well conducted but considered a dose level of 200mg/kg bw i.p would have been valuable. The dose level used in the study of 300 mg/kg bw clearly exceeded the maximum tolerated dose level. The committee considered that the degree and duration of hypothermia reported with phenol was severe and prolonged. Members concurred with the conclusion reached by the study authors and reported in the publication '.. overall, these studies suggest a role, but not necessarily a causality, for phenol-induced hypothermia in the formation of MN.'

d. The COM concluded that the additional 'in confidence' data on thermoregulatory support in phenol treated animals provided inconclusive evidence regarding the role of hypothermia in phenol-induced micronuclei in mice. Thus for phenol-treated animals there was evidence of impaired capacity to modulate temperature compared to controls and a transient hypothermia. It was possible that the application of thermoregulatory control could influence the formation of MN in control and phenol treated mice.

e. The COM concluded that all the available data on phenol suggested phenol should be regarded as a non-threshold *in vivo* systemic mutagen. There is insufficient evidence to support a threshold approach to risk assessment of systemic phenol.

2.26 The COM agreed a statement which is reproduced at the end of this report.

Horizon Scanning

2.27 The annual horizon scanning exercise was intended to provide an opportunity for members and advisers from Government Department/Agencies to discuss and suggest topics for further work. Considerable progress on the items identified in the 2006 horizon scanning exercise had been made, although it was noted that review on mutational spectra had not been initiated and this would be carried over to next years work programme. The primary objective of the 2008/9 horizon scanning exercise was to provide information to aid members' consideration of the scope and format of the revision of the COM guidance. The committee agreed that the following topics should be considered and could be included in the COM guidance; aneuploidy, mutational fingerprints/spectra, GADD 45 assay, and risk assessment.

2.28 Other suggestions for potential consideration included tissue concentrations in relation to lowest effect dose in carcinogenicity studies (the International Life Sciences Institute (ILSI) was doing some work on this), the Pig A assay (Bryce SM *et al* Envion Mol Mut, 49, 256-264, 2008), pesticide impurities and nanomaterials.

Test Strategies and Evaluation
Review of COM Guidance 2000

2.29 The current COM mutagenicity testing strategy (2000) was developed to update the strategy document published in 1989 (Report on Health and Social Subject No 35) which had been based on a strategy agreed in 1981. The COM guidance document published in 1989 contained a number of chapters on the basic science of mutations and their significance for human health as well as a testing strategy. The current COM strategy was a scientifically based approach to mutagenicity testing which updated the 1989 guidance, for example, with incorporation of the *in vitro* micronucleus assay as a test for clastogenicity/aneuploidy and the inclusion of newer approaches to *in vivo* testing such as use of transgenic animal models. The need to periodically reflect on developments was recognised by COM in 1981 and in 1989. The current COM guidance was not developed in response to a specific regulatory request but reflected the desire of COM members to update their guidance.

2.30 The guidance should produce a scientifically based strategy which can be used for screening compounds (not limited to one sector such as pharmaceuticals), evaluating genotoxicity of existing chemicals (such as contaminants) and providing case-by-case guidance in specific circumstances where specific questions regarding a compound arise (e.g. evaluating genotoxicity mode of action in rodent carcinogen target and non target tissues).

2.31 The Committee held two wide ranging scoping discussions during 2008 and during consideration of horizon scanning (see paragraph 2.24 for examples of areas to be considered during revision of the COM guidance). In addition several options for disseminating the COM review were explored including publication of a further booklet on a strategy for genotoxicity testing, a peer review publication and publication of a series of guidance documents on the COM Internet site. The advantage of a series of general guidance statements would be that these could be more readily updated when significant advances in genotoxicity testing and evaluation became available (e.g on identifying thresholds for genotoxicity or the assessment of *in vivo* mutagenic potency). It was agreed that all three options should be explored.

2.32 The COM agreed to consider the subject of potential thresholds for genotoxins at its February 2009 meeting.

Ongoing Reviews

Acrylamide

2.33 In 2007, the HSE requested a further evaluation from the COM regarding the information cited by the Polyelectrolyte Producers Group (PPG) in a letter to the chair of COM (dated 8 May 2007, COM statement 07/02). In view of the widespread dietary exposure to acrylamide, the Food Standards Agency requested that such a review should consider all available genotoxicity data on acrylamide. In 2007, the COM agreed that the EU risk assessment review completed by HSE (EU Risk Assessment report 2002) could be used as a basis for the review, and agreed a strategy for this to be extended with a systematic review of the scientific literature available subsequent to the EU report.

2.34 In 2008 Members reviewed the findings of the EU Risk Assessment Report and were presented with the systematic review of data relating to the genotoxicity of acrylamide and glycidamide published after 1995, and other pre 1995 references that had not been included in the EU risk assessment report. This systematic review, together with several presentations and submissions from the PPG, formed the basis of extensive discussions at each meeting in 2008. This has enabled a detailed statement to be drafted. The Committee expect to receive final comments on the fourth draft of the statement from the PPG in January 2009; with publication of the statement expected soon after, subject to any revisions in light of the submitted comments.

Toxicogenomics

2.35 The COT/COC/COM held a joint symposium on the issue of genomics and proteomics in October 2001 and published a joint statement in December 2004 on the use of toxicogenomics in toxicology. This was based on literature review of 50 studies and included information from the International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI) collaborative programme of research. This topic was identified during the 2006 horizon scanning exercise for an updated review. The DH Toxicology unit drafted a short overview of a number of new relevant *in vitro* studies, which included data on gene expression changes in studies on DNA adducts and mutagenicity for the October 2007 meeting. A large number of papers had been retrieved, but those selected for review were specifically chosen with the aim of identifying any advancement in the field, which may affect the conclusions drawn in the last statement. The COM considered a draft discussion paper at its October 2008 meeting. A further discussion paper is to be considered in 2009 reporting on the results of the ongoing ILSI/HESI trials.

Statements of the COM

Statement on mutagenicity assessment of chemical mixtures

COM/08/S1- March 2008

Introduction

1 The COM expressed an interest in the evaluation of the mutagenicity of chemical mixtures during the 2005 and 2006 horizon scanning exercises. One recommendation from COM was to consider the possible occurrence of synergistic interactions regarding mutagenic effects of chemical mixtures, the possible mechanisms for any synergistic effects and the implications of such a finding for risk assessment. It is possible that if synergistic effects between two or more *in vivo* mutagens occurred then co-exposure to mixtures containing these chemicals might result in a significant increase in the risk of mutagenicity and cancer compared to the risks associated with exposure to the individual chemicals alone. The COM evaluation outlined in this statement is intended to build on the work of the COT work on Risk Assessment of Mixtures of Pesticides and similar substances (WiGRAMP)[1] http://www.food.gov.uk/science/ouradvisors/toxicity/cotwg/wigramp/ which was subsequently extended to encompass other types of chemicals in food (see 2004 COT Annual report http://www.food.gov.uk/multimedia/pdfs/cotsection.pdf) and the ongoing work of the Interdepartmental Group on Health Risks from Chemicals (IGHRC) on the risk assessment of chemical mixtures http://www.silsoe.cranfield.ac.uk/ieh/ighrc/mixtures_document.pdf. Thus the definitions and nomenclature used to describe interactions regarding mutagenicity induced by chemicals in this statement have been taken from these reviews and are briefly commented on in paragraph 2 of this introduction.

2 The COT had noted that although there were a large number of studies on mixtures relatively few had appropriate data on the nature of the interactions between chemicals. The general principle reached from substantive consideration by the COT of data on pesticides across all toxicological end points was that in absence of data to the contrary, substances with similar modes of action could be assumed to act by dose-additivity, and substances with dissimilar modes of action could be assumed to act by effect additivity. The term interaction could imply a range of effects such as synergism, potentiation, supra-additivity, or sub-additivity. The COT had not specifically considered the most appropriate approaches to mutagenicity testing of mixtures or development of mutagenicity testing approaches to identify interactions with regard to mutagenicity.

Introduction to approaches to evaluation of mutagenicity of mixtures

3 A number of strategies have been considered for the evaluation of chemical mixtures.[3] These include testing whole mixtures (integrative), fractionation of mixtures to determine mutagenic components (dissective, top-down approach), and investigations of interactions by testing simple combinations, recombined fractions, and spiking of mixtures/fractions (synthetic, bottom up approach). All of these approaches have been identified from literature searches with regard to mutagenicity testing, although relatively few studies of whole mixtures were identified. Approximately 110 research papers with potentially relevant information were identified for consideration during the COM review.

4 A discussion paper on the mutagenicity testing of whole mixtures, approaches to dissection (fractionation/concentration) of mixtures regarding mutagenicity, and the presentation of a draft strategy for mutagenicity evaluation of mixtures was considered at the February 2007 meeting. http://www.iacom.org.uk/papers/documents/muto703.pdf

5 A discussion paper which presented a systematic review of published literature (up to the beginning of June 2007) of studies which had examined the potential interaction between chemicals regarding mutagenicity was considered at the October 2007 meeting. The Committee also briefly discussed approaches to design and evaluation of 'synthetic' studies investigating interaction between chemicals regarding genotoxicity. The COM considered the 'envelope of additivity' approach could be a useful approach to presenting data from studies designed to investigate potential interaction between chemicals with regard to mutagenicity and genotoxicity (outlined in paragraph 17). http://www.iacom.org.uk/papers/documents/muto715.pdf

6 This statement summarises the information contained in these discussion papers and the conclusions reached by COM.

Mutagenicity testing of whole mixtures, approaches to dissection (fractionation/concentration)

Whole mixtures

7 There were comparatively few studies where whole mixtures had been subjected to mutagenicity evaluation retrieved. An *in vivo* approach to the mutagenicity testing of cooked meats was considered.[4] The primary purpose for mutagenicity testing of whole mixtures outlined in the literature was the development of monitoring approaches to inform on risk reduction strategies. The studies need to be interpreted in terms of the overall mutagenic potency of the mixture and the sensitivity of the assay used to detect an effect, but it was noted that the data from such studies provided no information on the relative contribution of mutagenic chemicals present in the food or the interactions between chemicals regarding mutagenicity. A number of investigators have suggested that, where there is evidence that components of a mixture do interact and, in particular, where there is evidence of mutagenic synergy, then it might be prudent to evaluate whole mixtures as they exist to obtain appropriate information on mutagenic hazard.[5] Anwar (1993) proposed the term 'total mutagenic burden' for whole mixtures[6] However the failure to detect mutagenicity when complex mixtures (e.g. fried foods) or fractions (e.g. catalytically cracked clarified oil) are tested either *in vitro* or *in vivo* did not prove the absence of potentially mutagenic compounds.[7,8] The COM agreed that testing whole mixtures first using an *in vitro* screen (such as the Ames test or SOS chromotest) would have the advantage of picking up evidence of potential interactions, such as synergy that could be missed by testing individual fractions or chemicals isolated from a mixture.

Approaches to dissection (fractionation/concentration)

8 The key elements to approaches that might be potentially used are shown below in figure 1;

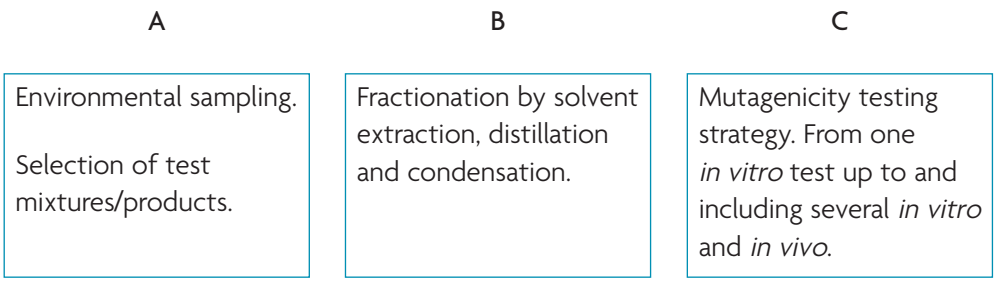


Figure 1; outline approach which could be used to evaluation of mutagenicity of chemical mixtures.

9 The COM considered published studies on the approach outlined in figure 1.[9-26] The COM agreed that a detailed review of environmental sampling for mutagenicity evaluation of mixtures was beyond the scope of the COM review. There were a wide range of factors which might affect the chemical mixture in samples recovered for mutagenicity testing including those affecting the emission of mixtures to the environment including variation in sources of release, distribution and degradation in the environment, the sampling procedure used (e.g. mass and volume of sample collected, the size distribution of particles in samples, the potential for reaction of sample with adsorbents/filters used in collection), and storage of samples prior to mutagenicity testing.[9] Overall, it was concluded sampling strategies can significantly influence the estimation of mutagenicity of chemical mixtures and there is thus a need for a careful case-by-case approach to a sampling strategy with consistency of sampling procedure attained in order to generate mutagenicity data that are comparable.

10 The COM reviewed fractionation procedures using solvent extraction, distillation and condensation for a number of mixtures samples (diesel particles collected occupationally or environmentally[13,14,15] or directly from exhausts[20,22] or from fumes e.g coke oven, roofing tar)[17], oil based liquids[16,23], condensates or particles from pyrotechnic mixtures (e.g cigarette smoke[9,12,19] or mixtures of known compounds[19]), hazardous wastes including industrial process effluents and municipal sludges18 and water samples taken from various points in the distribution system[21]). Most approaches used a single step extraction procedure. One particular difficulty in developing a strategy was optimising mutagenic response whilst avoiding excessive toxicity to the mutagenicity test indicator organisms used (e.g. bacteria). Multi step procedures can result in loss or modification of mutagenic components. In an WHO International Programme on Chemical Safety (IPCS) led collaborative study of the mutagenicity of mixtures (urban air samples, diesel particles and coal tar solution) significant interlaboratory and intralaboratory variance in the results of *Salmonella typhimurium* TA98 and TA100 with or without exogenous metabolic activation was noted, which was partly due to the method of extraction (either soxhlet or ultrasonication) using dichloromethane as a solvent as well as the mutagenicity test procedures used.[10,11] The final step in the fractionation procedure usually involved evaporation of extracts and resuspension in a solvent (usually DMSO) which is compatible with cell cultures used in mutagenicity tests and *in vivo* mutagenicity test systems. This final step may also introduce a potential source of variation regarding mutagenicity test data.

11 The COM considered that general guidance could not be provided regarding fractionation procedures, and that the testing strategy would need to be considered on a case by case basis. Both the top down and bottom up approaches to mutagenicity testing of mixtures were considered to have potential applications in different circumstances.

12 The primary objective of the mutagenicity testing strategy for chemical mixtures should be to identify hazard in the tested material or mixture. A comparison of the mutagenicity test data for test mixtures derived from the same sources and subject to the same extraction and fractionation procedures may provide information for monitoring hazard of environmental samples, commercial products, pyrolysis products and hazardous wastes. The IPCS collaborative study also reported considerable variation in results with regard to strain of *Salmonella* used, the activation conditions and between replicate mutagenicity tests within the same laboratory.[11,12] It is therefore likely that any successful approach to monitoring mutagenic hazard in chemical mixtures over a period of time would need to use well established sampling, extraction and fractionation procedures, and mutagenicity testing procedures with a high degree of quality control for each step. Additional procedures could include spiking mixtures with compounds of known structure and mutagenic potential to investigate procedures used (e.g. extraction[14] or pyrolysis[12]). Most studies are conducted to monitor the mutagenicity of chemical mixtures (e.g. serial samples from one potential source or batch to batch sampling of a product) but it is possible to use them in an investigative approach to study potential sources of mutagen release (e.g. the effect of agricultural run off on mutagenicity of water samples by timing and positioning sample collection from water courses[21]).

13 The majority of mutagenicity studies of chemical mixtures identified for the COM review used *Salmonella typhimurium* test strains as the only mutagenicity test.[3,9,13,14,18,20,23,25] These studies may include exogenous metabolic activation systems selected to increase the number of revertant colonies formed for a particular tested mixture or to test for the mutagenicity of particular groups of compounds within a mixture (e.g. use of hamster S-9)[23] or selection of particular *Salmonella* strains (e.g. use of nitroreductase (NR) deficient strains[20], and NR and O-acetyltransferase deficient strains[25]) or treatments (use of ROS scavengers such as α-tocopherol and/or ascorbate[15]) to monitor the mutagenicity of particular groups of mutagenic chemicals within the mixture. Additional *in vitro* tests (e.g. using mammalian cells) can extend the potential for monitoring mutagenic hazard over a wider range of chemicals present in the mixture.

14 Relatively fewer studies use additional *in vitro* and *in vivo* tests.[15-17] *in vivo* mutagenicity tests are usually incorporated into testing strategies for single chemicals to confirm the potential for a compound of unknown mutagenic potential to induce effects *in vivo*. The COM agreed that the inclusion of *in vivo* tests would have a confirmatory role only for monitoring of chemical mixtures, rather than being used routinely. This would be the case particularly when the environmental monitoring procedures were being carried out on mixtures containing known *in vivo* mutagens, but possibly at levels below the level of detection in *in vivo* assays. One potentially useful approach regarding the inclusion of *in vivo* tests in a strategy for monitoring complex mixtures was provided by Williams and Lewtas 1985[17] who correlated the mutagenic response (slope of the dose-response curve) to organic extracts from diesel, coke oven, roofing tar and cigarette smoke emissions in *in vitro* tests (*Salmonella typhimurium* TA98 +S-9 (rat or hamster), and mouse lymphoma mutagenicity) with the

response in mouse skin tumour initiation assays. Having correlated mutagenic potency *in vitro* and *in vivo* (in this case between different mixtures) it would therefore be possible to continue monitoring and undertake comparative ranking of different samples of these mixtures using an *in vitro* mutagenicity test strategy. It is possible to reach this conclusion as there was relatively good knowledge of the chemical composition of the mixtures included in the study, and a key hypothesis under test was the investigation of mixtures of PAHs which helped to define the *in vitro* and *in vivo* parts of the testing strategy.

15 The COM agreed an outline proposal for a strategy for monitoring mutagenicity of chemical mixtures (in particular occupational and environmental mixtures such as described in paragraph 10 of this statement), based on proposals for evaluating the mutagenicity of mixtures in the published literature[24,26] but noted that this was only general guidance and a case-by case approach was needed.

Preliminary considerations

A. Collect information on chemical composition, and mutagenicity of chemicals in the mixture. Define the purpose of the monitoring approach (is this to monitor overall mutagenic hazard of the mixture, or to monitor the mutagenicity of selected levels of chemicals or groups of chemicals within the mixture?).

B. Review the literature for appropriate data on sampling, extraction and testing of similar mixtures. Review the mutagenicity test data on the mixture or similar mixtures or the chemicals within the mixture selected for monitoring.

With regard to mutagenicity testing

C. Define *in vitro* testing strategy, focusing on optimising and standardising the approach.

D. Undertake *in vitro* monitoring to validate approach and identify sources of variation and their impact.

E. Consider, if necessary on a case-by-case basis, developing an *in vivo* segment to strategy. (This might include studies to test whether chemical(s) selected for monitoring had *in vivo* mutagenic potential if this was not known. It is unlikely that chemical(s) within a mixture which were known to be *in vivo* mutagens would need to be routinely tested.)

Review of strategy

F. Implement the strategy and use data to inform on risk reduction strategies. It is important to periodically review the results of a monitoring strategy, particularly if there is any evidence for a change in the results being reported. There are many potential sources of variation which could affect the results and it would be important to differentiate between a change in results due to composition of the mixture from a change due to variation in fractionation and/or testing procedures. The inclusion of spiked samples in a strategy for mutagenicity testing of mixtures may be valuable.

Approaches to evaluating mutagenic interaction between chemicals

16 The design of synthetic studies to investigate the potential for interaction between chemicals, fractions or after spiking mixtures with chemicals is particularly complex. A number of factors to include, illustrated in the studies identified for review (for example[3,12,25]) included the need for consideration of expected patterns of mutagenic response in bacterial tester strains used, the design of a testing strategy to limit the number of combinations tested to a minimum required to evaluate the nature of any interactions in mutagenicity tests (by selecting concentrations of test materials taking into account the dose-response of individual compounds or fractions in the tester strains, the consideration of the need for replicate experiments), and the consideration of the most appropriate approach to statistical analysis of data. The data could be analysed by a number of methods including the projections to latent structures (PLS) approach which overcomes many of the problems inherent in inter-correlated (dependent) predictor variables and produces results which are easily viewed.[13]

17 The COM agreed the concept of the envelope of additivity was potentially a helpful approach to graphically presenting the results of studies and to help identify non-interaction (e.g. dose-response and effect additive responses) and interaction responses (e.g. synergy and antagonism)[27]. The COM noted the proposed unifying approach for application of statistical methods in chemical mixture research based on the shape of the dose response curve and changes in the slope of the dose-response in studies using two or more chemicals.[28] The approach suggested by Gennings *et al* linked the traditional statistical models of interaction (as found in the general linear model / factorial ANOVA models) to the different concepts of joint toxic action. The unification of the approaches is achieved by showing that there is no interaction if the dose-response relationship of one chemical is not changed by the presence of other chemicals. An interaction exists if there is a change in the slope of the response. This concept of interaction related to underlying statistical models of additivity. Members agreed that the approach suggested by Gennings *et al* 2005 could be potentially helpful when assessing mutagenicity studies of interaction between chemicals.

Review of studies investigating the potential interaction between chemicals regarding mutagenicity

18 A total of 91 research papers were identified by literature searches up to June 2007. A quality scoring approach was used to select the best quality studies for further review by COM. The quality screening approach was based on Borgert *et al* 2001[29] for evaluating interaction studies in terms of the quality of design, data and interpretations. Reliable interaction studies were considered to be those that are interpretable without making assumptions about untested and unanalysed parameters. (An overview of the quality scoring criteria is given in Annex 1 to this statement.) Very few (n=15) published studies met all five of the criteria and these were considered in detail.[30-44] Brief summaries of other papers not meeting all of the quality screening criteria were also provided for the COM.

19 The COM agreed that the well-conducted studies of defined mixtures of mutagenic chemicals did not provide a consistent picture of combination effects being predictable on the basis of the single agent dose-response information. In the majority of cases, substances tested in these studies are mutagens with relatively well understood mechanisms of action (e.g. B[a]P, and the alkylating agents EMS, MMS, MNU). In only one instance was the same combination of chemicals tested (EMS and ENU) in two

different tests (Ames[36] and in an *in vivo* mouse micronucleus test[42]). Kawazoe and colleagues showed that in the Ames assay EMS and ENU induced linear dose-responses and that using dose addition it was possible to model the combined effect of these chemicals.[36] In the mouse micronucleus assay, these chemicals induced non-linear dose response curves, but mixture effects were consistent with dose addition predictions.[42] For other combinations of alkylating agents, however, it is not clear why additivity is not observed. In many of these cases, observed mixture effects appear to fall within the additivity envelope and as some investigators do not estimate confidence 'belts' for the additivity predictions, it is possible the observations are not truly statistically significantly different from the non-interaction predictions.

20 The COM considered that an important part of the assessment of genotoxicity studies of interaction between chemicals would be reproducing results seen in one test system with other appropriate genotoxicity tests (e.g. confirming results seen in bacterial gene mutation assays in mammalian cell gene mutation assays). This could be used in a weight of evidence assessment of interactions and would be particularly important for assessment of interactive effects such as synergy or antagonism. The strategy for assessment of interaction with regard to mutagenicity would also need to include *in vivo* tests with appropriate consideration of toxicokinetics and exposure of sampled tissues. Members commented that the available published literature presented a number of examples[35,37,38,40] where interaction had been reported, but there was essentially no appropriate independent confirmation of the results in separate tests, or within an appropriate mutagenicity testing strategy for the identification of interactions and no definite conclusions could be reached.

21 The COM considered the four available published studies which reported the best evidence for interaction in detail to provide advice on possible mechanisms of mutagenicity might be associated with interaction.

22 Homme M *et al* (2000)[35] had documented synergistic DNA damage using UDS assays in human fibroblasts between 4-nitroquinoline-1-oxide (4-NQO) and non-effective methyl methanesulfonate (MMS). The authors had proposed that the ultimate DNA reactive metabolites formed from 4-NQO resulted in unwinding of super helical DNA so that more molecules of MMS could reach the bases of DNA resulting in increased methylation and mutation. The COM considered that a viable hypothesis had been proposed. It would be necessary to undertake independent confirmation of the results and to include additional combinations of mutagens with and without 4-NQO to provide further data to investigate the proposed mechanism. At present no definite conclusions could be reached on this specific example of an interaction.

23 Kojima H *et al* (1992)[37] had investigated the potential for interaction between MMS and EMS in Chinese hamster V79 cells using cell killing, induction of 6-thioguanine mutants (6TG resistant mutants) and chromosome aberrations. These authors had reported evidence for synergistic interactions for both cell killing and 6TG mutation and evidence for additivity with regard to chromosome aberrations. The authors had suggested that the DNA damage produced by one alkylating agent could be increased in the presence of a small amount of another alkylating agent. The COM noted the predominant SN2 mechanism of MMS and the SN1 mechanism of EMS and considered that these differences could form the basis for a hypothesis of interactive effects with regard to genotoxicity.

However the COM considered there was a need for independent confirmation of these results and further investigations of other alkylating agents before any definite conclusions could be reached.

24 Lutz WK *et al* (2005)[38] had reported evidence for antagonism using a combination of N-methyl-N-nitrosourea (MNU) and the topoisomerase-II inhibitor genistein (GEN) in the mouse lymphoma assay in LY5178Y cells. In separate tests when MMS was combined with GEN an additive response (reported to be within the envelope of additivity) was reported. The authors hypothesised that the profile of DNA methylation and or epigenetic effects were responsible for the different responses reported for the binary combinations tested. The COM considered these investigations raised interesting hypotheses for further testing but no definite conclusions could be reached on these data.

25 Marrazzini A *et al* (1994)[40] had undertaken *in vivo* mouse bone marrow MN tests in mice using intraperitoneal administration of binary combinations of hydroquinone, catechol and phenol. Mixtures of hydroquinone and phenol and catechol and phenol were reported to result in synergistic induction of micronuclei. Members noted that it was not possible to discern a potential mechanism of interaction from these studies which could be used to support hypotheses for further testing.

26 The COM was aware of the different interpretations of the term synergy was used by the research groups and the limitations in the available data made it difficult to reach any definite conclusions. However, overall there was insufficient evidence to conclude that the studies reviewed provided conclusive evidence for interaction effects (either synergy or antagonism). However, a number of the studies provided evidence to suggest hypotheses for interaction (see paragraphs 22-25) which could be further examined in appropriately designed mutagenicity testing strategies. These included the interaction between ultimate DNA reactive chemicals and DNA structure, (e.g. different mechanisms of DNA alkylation), the effect of covalent binding to DNA of one chemical on the potential for other reactive metabolites and chemicals to bind to DNA, and possible epigenetic mechanisms which could potentially result in a mutagenic response that resulted from an interactive effect between chemicals (i.e. synergistic or antagonistic). The COM agreed that the potential for interactions between chemicals with regard to genotoxicity needed to be studied on a case-by-case basis.

COM Discussion and Conclusions

Whole mixtures

27 The COM considered mutagenicity testing of whole mixtures, and approaches to dissection (fractionation/concentration) of mixtures. The primary purpose of such studies is to monitor mutagenic response in tests of a wide variety of mixtures for example foods, samples of pollution (air and water) condensates or particles from pyrotechnic mixtures (e.g. cigarette smoke or mixtures of known compounds), hazardous wastes including industrial process effluents and municipal sludges. The COM noted that there were comparatively few data on mutagenicity testing of whole mixtures. The COM agreed that testing whole mixtures first using an *in vitro* screen (such as the Ames test or SOS chromotest) would have the advantage of picking up evidence of potential interactions, such as synergy, that could be missed by testing individual fractions. However, the failure to detect mutagenicity when complex mixtures (e.g. fried foods) or fractions (e.g. catalytically cracked clarified oil) are tested either *in vitro* or *in vivo* did not prove the absence of potentially mutagenic compounds.

Approach to dissection of mixtures

28 The COM agreed an outline proposal for a strategy for the fractionation and monitoring of the mutagenicity of chemical mixtures (as outlined in paragraphs 10-15 of this statement) but noted that this was only general guidance and a case-by case approach was needed.

Approach to evaluation of studies to investigate interactions

29 The COM agreed the concept of the 'envelope of additivity' was a helpful approach in the presentation of the results of studies and in the identification of non-interaction (e.g. dose-response and effect additive responses) and interaction responses (e.g. synergy and antagonism). The COM noted the proposed unifying approach of Gennings and colleagues (see reference 28) for application of statistical methods in chemical mixture research which is based on the shape of the dose response curve and changes in the slope of the dose-response curve in studies using two or more chemicals, and agreed that this could be of potential use in evaluating genotoxicity.

Review of published studies on interaction between chemicals with regard to mutagenicity.

30 The COM noted that the available published literature presented a number of examples where interaction between chemicals with regard to mutagenicity had been reported. However, there was essentially no appropriate independent confirmation of the results in separate tests, or within an appropriate mutagenicity testing strategy for the identification of interactions and therefore no definite conclusions could be reached.

31 The COM agreed that the available studies had raised a number of potential hypotheses for interaction (see paragraph 26). There was a need for further research regarding such mechanisms, which if confirmed in an appropriate mutagenicity testing strategy might be of potential significance for public health.

March 2007
COM/07/S1

Statement on Mutagenicity Evaluation of Mixtures Annex 1

Approach to quality screening of published papers on interaction studies: Sumerised from Borget CJ *et al* (2001) Hum Ecol Risk Assess, 7, 259-306

1 In 2001, Borgert and colleagues (*Hum Ecol Risk Assess* **7**(2): 259-306, 2001) proposed a set of criteria for evaluating interaction studies in terms of the quality of design, data and interpretations. Reliable interaction studies are those that are interpretable without making assumptions about untested and unanalysed parameters. Although there is debate among experts regarding which models of non-interaction, which methods of combination analysis, and which statistical tests are most appropriate, it was still possible to apply the principles outlined by Borgert *et al* to assist in data interpretation. The criteria proposed were designed to assist risk assessors in identifying studies that can be used in component-based mixture risk assessments as well as those studies that are less useful due to inadequacies in design or interpretation. The aim was for them to apply broadly to interaction data for all effects of drugs, pesticides, industrial chemicals, food additives and natural products.

2 These criteria appear to provide a useful basis on which to evaluate the studies identified on mutagenic interactions. The five criteria set out below have been refined where necessary to facilitate their specific application to genetic toxicology studies and then used to evaluate the 91 retrieved articles.

I. Dose-response relationship for the individual mixture components are adequately characterised

Without adequate dose-response relationship characterisation for the individual components, it is not possible to determine whether a biological effect of a mixture is due to interactions between the components.

Ideally, single agent dose-response characterisation should enable slope, inflection points, and maximum and minimum effects to be estimated. Most importantly, key to being able to decide the appropriate 'no interaction' hypothesis (Criterion II, below) is whether the individual components of the mixtures have linear or non-linear dose-response curves and whether they have similar slopes. Inadequate characterisation of the dose-response relationship can lead to erroneous conclusions of interactions and this might be compounded further if the mixture components have significantly different shaped dose-response relationships.

For the purposes of this COM review, it was decided to focus, in the first instance, on mixtures of chemicals where all components are mutagenic. That is, evidence of "potentiation" from mixtures of mutagens with co-mutagens has not been considered at this point. Therefore, it is assumed that each mixture component alone induces a measurable genotoxic effect and detailed dose-response data are available.

II. An appropriate 'non-interaction' or 'additivity' hypothesis should be, *a priori*, explicitly stated and used as the basis for assessing combination effects.

Interactions are inferred when a mixture of chemicals produces a biological response greater or less than expected based on mathematical concepts of additivity (non-interaction). Two models of non-interaction have been well-developed in the pharmacological and toxicological literature and are appropriate as the basis for non-interaction hypotheses. Dose addition is based on the concept that an agent cannot interact with itself, and predicts that two non-interacting compounds will behave as dilutions of one another when combined. The second model is response addition, and expresses probabilistic independence between two compounds. In this case, independence implies functional independence between two chemicals such that the incremental effect of one compound is unchanged in the presence of a second.

In the literature, dose addition largely assumes a strictly similar mechanism of action of all mixture components, while response addition is based on the idea of completely dissimilar mechanisms of action of the mixture components. Therefore, if mechanisms of action are well-enough understood, this may suggest the most appropriate non-interaction model to assume. However, in most cases adequately detailed understanding of the toxicological mechanisms of action for the individual mixture components is not available. Therefore it may be useful to compare observed combination responses with both models of non-interaction. In so doing, applying both models will generate a range of effects delineated by dose addition and response addition, referred to by some researchers as an 'additivity envelope', in which a non-interacting mixture would be expected to lie (**Figure 1**). This approach would be considered to meet this criterion. In addition, as the number of individual components in the mixtures of interest increases, it is likely that there will be a variety of chemicals with similar and dissimilar mechanisms of action and it may not be appropriate to use dose addition or response addition. In this regard, some groups are beginning to combine the two models, but as an interim, it is feasible to assume effects will lie in the additivity envelope if the mixture is non-interactive.

It should be noted, that dependent on the default non-interaction model applied, there are different demands made on the ideal single substance dose-response data (which has an impact on Criterion I). That is, for dose addition, single substance studies have to provide concentration-effect data for the same effect levels that will be assessed in the combination studies. For the application of response addition, it is necessary to have detailed resolution of the single substance dose-response relationships at effect levels below the region of interest for the mixtures.

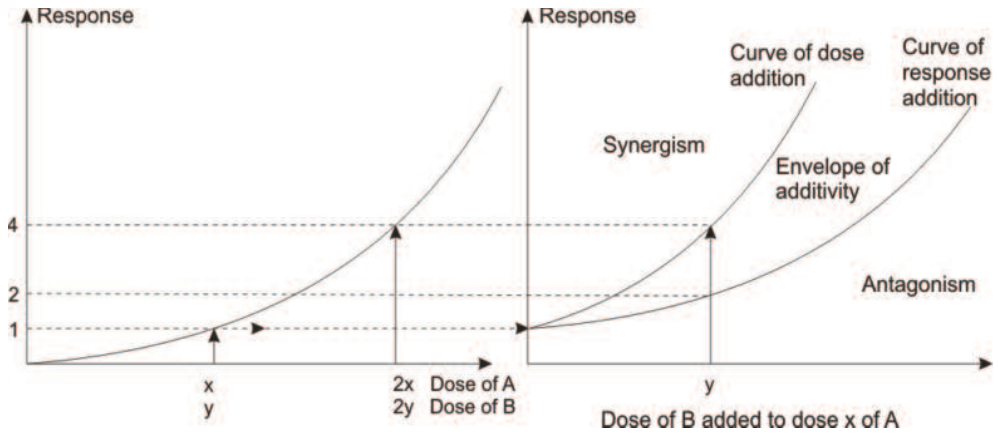


Figure 1. Schematic representation of a non-linear dose-response relationship (left hand side for two substances,A and B) **and classification possibilities for the response of a mixture of the two components** (right hand side; dose response for B added to dose x of A). Taken from Lutz *et al.* (2005). Dose x of chemical A produces a response of 1 effect unit, and dose y of chemical B has the same effect magnitude, in fact chemicals A and B have the same dose-response curves. A mixture of dose x of substance A plus dose y of substance B generated a response of effect level 4, one might postulate that A and B acted in a synergistic manner. This is interpretation is not correct when the shape of the chemicals' dose-response curves are considered. Therefore, the mixture of dose x of substance A plus dose y of substance B can be considered as dose 2x of chemical A or 2y of chemical B, and these doses generate a response of effect level 4, i.e. in agreement with dose addition. If the two chemicals acted independently of each other, the expectation would be the lower of the two curves in the right hand panel, i.e. response addition. This curve has exactly the same shape as the dose-response on the left hand panel, except that it is set off on the y-axis by response level 1 (the effect generated by dose x of A). On this basis, the mixture of dose x of substance A plus dose y of substance B would result in effect level 2 as shown by the lower dotted line on the right hand panel.

III. Combinations of mixture components should be assessed across a sufficient range of concentrations and mixture ratios to support the goals of the study

The characteristics of a mixture are clearly dependent on the components of the mixture and the concentration range of the mixture that is tested. However, there may also be considerable dependence on the ratios at which each component is present within the mixture. This is because different types of interactions can be exhibited by the same mixture of chemicals at different mixture ratios. Approaches to mixture testing routinely used include:

- full factorial design: tests a full complement of component ratios across the dose-response range of each mixture ratio.

- fractional factorial design: reduces the number of tests to a specified subset of mixture combinations while still maintaining a substantial proportion of the information that would be produced with a full factorial design.

- ray design: tests fixed-ratio mixtures, i.e. a constant ratio of the mixture components, across a range of concentrations.

There are no hard and fast rules as to the correct approach to take in all cases, but it is important to employ the design that will satisfy the goals of the study, and not to over-interpret the resulting data. Detailed descriptions of these different approaches have been published recently (IGHRC, US EPA etc.)

IV. Formal statistical tests should be used to determine whether the response produced by a combination is different from that predicted by the additive hypothesis.

Some researchers evaluate only whether responses differ statistically from controls and whether dose combination responses differ statistically from individual component responses. Such comparisons do not actually address the question of whether there is an interaction. As detailed in Criterion II, the appropriate non-interaction model will have been stated, and statistical tests should compare the observed mixture effect with that of the expected joint effect on the basis of the non-interaction hypothesis. Without a clearly stated non-interaction hypothesis, the results of any statistical test cannot be interpreted. Statistical methods that have been used to infer that mixture components interact include simple t-tests, linear models (including ANOVA and multiple regression) and multivariate regression. Ideally, the statistical approaches will allow confidence intervals to be placed on the observed mixture data and also on the predictions based on the mathematical models of dose addition or response addition. As the prediction is based on experimental (variable) data on the single substances, it is possible to estimate the variability associated with the predicted combined effect.

V. Interactions should be assessed at relevant levels of biological organisation.

Although the primary objective of the mutagenicity testing strategy for chemical mixtures should be to identify hazard in the tested material or mixtures, it is important to understand if the mixture poses a significantly greater hazard than the individual components. Identifying a potential interaction which might be of potential importance for public health, therefore requires not only a mechanistic rationale, *in vitro* evidence of interaction and *in vivo* evidence of interaction but also, the information must consistently point towards a synergistic interaction.

Interaction studies at the level of the whole organism or population can be difficult to interpret without information from underlying levels of biological organisation. Without knowledge of the mechanism of action of the mixture components it may not be possible to establish which non-interaction hypothesis is most appropriate. It may therefore be necessary to employ an additivity envelope approach (as detailed above in criterion II), consequently reducing the chance to detect true interactions. On the other hand, numerous interactions may be detected in studies carried out at the molecular, biochemical or cellular level, and these interactions may never manifest change in the organism. Ideally the systems used to assess combination effects should be fit for purpose, which implies use of accepted mutagenicity/genotoxicity tests.

References

1 COT (2002). Committee on Chemicals in Food Consumer Products and the Environment (COT). Risk Assessment of Mixtures of Pesticides and Similar Substances. Published Food Standards Agency September 2002. Crown Copyright. FSA/0691/0902.

2. Interdepartmental Group on Health Risks from Chemicals (IGHRC) (2007). Chemical Mixtures: a Framework for Assessing Risks (version 6-Arpil 2007) (Unpublished draft.)

3. Eide I (1996). Strategies for Toxicological Evalaution of Mixtures. Food Chem Toxicol, 34, 1147-1149.

4. Heddle JA *et al* (2001). A test of the mutagenicity of cooked meats *in vivo*. Mutagenesis, 16, 103-107.

5. Ma TH *et al* (1992). Synergistic and antagonistic effects on genotoxicity of chemicals commonly found in hazardous waste sites. Mutation Research, 270, 71-77.

6. Anwar WA (1993). Chemical Interaction: Enhancement and inhibition of clastogenicity. Environmental Health perspectives, 101 (suppl3), 203-206.

7. Lee H *et al* (1994). Bacterial mutagenicity, metabolism, and DNA adduct formation by binary mixtures of benzo(a)pyrene and 1-nitropyrene. Environ Mol Mutagen, 24, 229-234.

8. Przygoda RT *et al* (1999). Assessment of the utility of the micronucleus tests for petroleum-derived materials. Mutation Research, 438, 145-153.

9. Chrisp CE and Fisher GL (1980). Mutagenicity of airborne particles. Mutation Research, 76, 143-164.

10. Krewski D *et al* (1992). Sources of variation in the mutagenic potency of complex chemical mixtures based on the Salmonella/microsome assay. Mutation Research, 276, 33-59.

11. Claxton LD *et al* (1992). Results of the IPCS collaborative study on complex mixtures. Mutation Research, 276, 23-32.

12. Baker RB *et al* (2004). An overview of the effects ot tobacco ingredients on smoke chemistry and toxicity. Food Chemical Toxicology, 42S, S53-S83.

13. Østby L *et al*(1997). Mutagenicity of organic extracts of diesel exhaust particles after fractionation and recombination. Archives of Toxicology, 71, 314-319.

14. Bostrom E *et al* (1998). Mutagenicity testing of organic extracts of diesel exhaust particles after spiking with polycyclic aromatic hydrocarbons (PAH). Archives of Toxicology, 72, 645-649.

15. Cheng YW *et al* (2004). Genotoxicity of Motorcycle exhaust particles *in vivo* and *in vitro*. Toxicological Sciences, 81, 103-111.

16. Lockard JM *et al* (1982). Comparative study of the genotoxic properties of Eastern and Western U.S shale oils, crude petroleum, and coal-derived oil. Mutation Research, 102, 221-235.

17. Williams K and Lewtas J (1985). Metabolic activation of organic extracts from diesel, coke oven, roofing tar, and cigarette smoke emissions in the Ames assay. Environmental Mutagenesis, 7, 489-500.

18. Stewart-houk V and Claxton LD (1986). Screening complex hazardous wastes for mutagenic activity using a modified version of the TLC/Salmonella assay. Mutation Research. 169, 81-92.

19. Karlsson N *et al* (1991). Mutagenicity testing of condensates of smoke from titanium dioxide/hexachloroethane and zinc/hexachloroethane pyrotechnic mixtures. Mutation research, 260, 39-46.

20. Clark CR *et al* (1981). Mutagenicity of diesel exhaust particle extracts: Influence of cart type. Fundamental and Applied Toxicology, 1, 260-265.

21. DeMarini DM *et al* (1982). Use of four short –term tests to evlaute the mutagenicity of municipal water. Journal of toxicology and Environmental Health, 9, 127-140.

22. Brooks AL *et al* (1984). A comparison of genotoxicity of automotive exhaust particles from laboratory and environmental sources. Environmental Mutagenesis, 6, 651-668.

23. Blackburn GR *et al* (1986). Predicting carcinogenicity of petroleum distillation fractions using a modified Salmonella mutagenicity assay. Cell Biology and toxicology, 2, 63-84.

24. Waters MD *et al* (1990). Genetic activity profiles in the testing and evaluation of chemical mixtures, Teratogenesis, Carcinogenesis, and Mutagenesis, 10, 147-164.

25. Rivedal E *et al* (2003). Supplemental role of the Ames mutation assay and gap junction intercellular communication studies of possible carcinogenic compounds from diesel particles. Archives of Toxicology, 77, 533-542.

26. Schaeffer DJ (1987). A new approach for using short-term tests to screen complex mixtures.. Regulatory Toxicology and Pharmacology, 7, 417-421.

27. Lutz, W. K.,*et al.* (2005). Different types of combination effects for the induction of micronuclei in mouse lymphoma cells by binary mixtures of the genotoxic agents MMS, MNU, and genistein. Toxicol Sci 86, 318-323.

28. Gennings C *et al* (2005). A unifying concept for assessing toxicological interactions: changes in slope. Tox Sci, 88 (2), 287-297.

29. Borgert CJ, *et al* (2001). Evaluating interaction studies for mixture risk assessment. Hum Ecol Risk Assess 7:259-306.

30. Blasiak, J. *et al* (2000) Synergistic effect of vitamin C on DNA damage induced by cadmium. Gen.Physiol Biophys. 19, 373-379.

31. Cross, H. J. *et al* (1996) Effect of quercetin on the genotoxic potential of cisplatin. Int J Cancer 66, 404-408.

32. Dashwood, R. H. *et al* (1986). Mutagenicity to Salmonella of four derivatives of the azo mutagen 5I: some implications for structure--activity databases and the evaluation of combinations of mutagens. Mutagenesis 1, 261-265.

33. Hass, B. S. *et al* (1981) Synergistic, additive, and antagonistic mutagenic responses to binary mixtures of benzo(a)pyrene and benzo(e)pyrene as detected by strains TA98 and TA100 in the Salmonella/microsome assay. Environ Mutagen. 3, 159-166.

34. Hass, B. *et al* (1987) Binary mixtures containing isomers of nitrobenzo[a]pyrene induce greater-than-additive mutational responses in Salmonella typhimurium. I. Analysis by the total concentration-proportional mixture model. Mutat.Res 190, 247-252.

35. Homme, M. (*et al* 2000) Synergistic DNA damaging effects of 4-nitroquinoline-1-oxide and non-effective concentrations of methyl methanesulfonate in human fibroblasts. Mutat.Res 461, 211-219.

36. Kawazoe, Y. *et al* (1986) Studies on chemical carcinogens and mutagens. XXXIII. Mutation frequencies induced by combinations of methylating and/or ethylating mutagens. Chem Pharm Bull (Tokyo) 34, 250-255.

37. Kojima, H. *et al*, (1992) Combined mutagenicity of methyl methanesulfonate and ethyl methanesulfonate in Chinese hamster V79 cells. Mutat.Res 266, 171-180.

38. Lutz, W. *et al* (2005) Different types of combination effects for the induction of micronuclei in mouse lymphoma cells by binary mixtures of the genotoxic agents MMS, MNU, and genistein. Toxicol Sci 86, 318-323.

39. Maki-Paakkanen, J. *et al* (2004) Bacterial and mammalian-cell genotoxicity of mixtures of chlorohydroxyfuranones, by-products of water chlorination. Environ Mol.Mutagen. 43, 217-225.

40. Marrazzini, A. *et al* (1994) *in vivo* genotoxic interactions among three phenolic benzene metabolites. Mutat.Res 341, 29-46.

41. Schaumloffel, N. and Gebel, T. (1998) Heterogeneity of the DNA damage provoked by antimony and arsenic. Mutagenesis 13, 281-286.

42. Suzuki, T. *et al* (1995) Combination effects of clastogens in the mouse peripheral blood micronucleus assay. Mutagenesis 10, 31-36.

43. Taylor, M. S. *et al*, (1995) Examination of the additivity assumption using the spiral and standard Salmonella assays to evaluate binary combinations of mutagens. Mutat.Res 335, 1-14.

44. Thornton-Manning, J. R. *et al* (1989) Role of nitroreduction in the synergistic mutational response induced by mixtures of 1- and 3-nitrobenzo[a]pyrene in Salmonella typhimurium. Environ Mol.Mutagen. 13, 203-210.

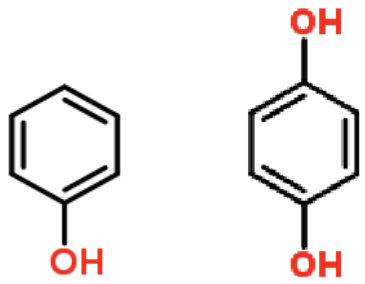
Update statement (2008) Mutagenicity of Phenol

COM/08/S2- November 2008

Introduction

Background to COM review

1 HSE asked for advice from COM on phenol (along with hydroquinone) in 1994/95 and in 1999. A copy of the conclusions and the statement agreed in 1999 (published January 2000, COM/00S1.[1] [Hydroquinone is a metabolite of phenol, see section on metabolism and figure 1 below]



Phenol Hydroquinone

Figure 1.

2 In brief, in 1994, the COM concluded the *in vitro* mutagenicity data on phenol were of poor quality and results difficult to interpret, but *in vivo* data showed phenol to be a somatic cell mutagen at very high dose levels. (COM noted negative results in long term carcinogenicity bioassays in rats and mice). The COM noted the potential for rapid conjugation and detoxication via the glutathione pathway and that the mutagenicity of phenol appeared to be predominantly related to peroxidase activity and catalase could have a protective role. The COM agreed there was a potential for a threshold mechanism by the oral route of exposure but could not reach a similar conclusion with regard to dermal or inhalation exposure.

3 In 1995, the COM considered a submission from industry which provided some metabolism data. Overall the COM concluded that appropriate studies to determine the extent of pre-systemic metabolism following either inhalation or dermal exposure had not been undertaken. The COM provided guidance on the approaches which could be used (including administration of hydroquinone or phenol via a bronchoscope with very early sampling for free and conjugated test substance in the blood.

4 In 1999, the COM considered a study on bioavailability and metabolism of hydroquinone after intratracheal instillation in male rats. The results showed free systemic hydroquinone in arterial blood 5-10 seconds after dosing. The COM considered the data suggested the potential for site of contact and systemic mutagenic effects after inhalation exposure. The COM considered a inhalation exposure

transgenic Muta™mouse study but were unable to draw any conclusions in view of unacceptable levels of DNA packaging in many of the trials in the experiment. The COM noted a small but consistent positive result in bone marrow micronucleus studies in mice given intraperitoneal doses of around 100-160 mg/kg bw (relevant BMMN studies are reviewed in para 14 below).

5 The COM agreed a statement (00/S1) in January 2000. The conclusions reached with regard to phenol were similar to those reached in 1994.

6 In 2003, the COM considered a pre publication report from the Dow Chemical Company which provided results to suggest that the *in vivo* mutagenicity of phenol in the mouse bone marrow micronucleus assay originated from a transient hypothermia induced by high doses of phenol. The COM agreed the data supported a case for a threshold mechanism for the induction of MN in bone marrow of mice but considered publication of the study in a peer-review journal would be necessary before drawing any definite conclusions. A further COM statement was not published in 2003. The relevant study has now been published and was identified during the 2007 COM horizon scanning exercise.[2] Members asked for a review of the paper during the COM horizon scanning exercise. In addition the HPA asked for advice on the genotoxicity of phenol and specifically whether a threshold approach can be used with regard to the risk assessment of genotoxicity of phenol.

Introduction to current COM review

7 The COM consideration of phenol covers a period from 1994-2003. The objectives of the current review is to i) produce an updated COM statement on phenol, ii) to evaluate the Spencer study on hypothermia and also iii) to consider if any *in vivo* mutagenic effect of phenol can be considered as related to a threshold effect.

8 The COM have considered many of the key studies on phenol in full in the past but over quite a period of time. Thus in order to provide a comprehensive overview of the mutagenicity of phenol, the secretariat have submitted a draft EU risk assessment review which has been provided by HSE (Germany acting as rapporteur) dated 1/09/2005.[3] In addition relevant information from important studies on phenol were provided to the COM.

Overview of phenol mutagenicity

in vitro mutagenicity studies

Bacterial tests

9 The COM agreed that phenol was not mutagenic in standard bacterial mutagenicity tests.[3]

Mammalian cell gene mutation tests

10 The Committee considered the available mammalian gene cell mutation studies. Phenol induced a dose-related increase in the frequency of *Hprt* mutants in V79 cells in the absence of exogenous metabolic activation (4-fold increase at the top dose). Cell survival at the top dose was 50%.[4] A positive result had also been documented in SHE cells using the Na+/K+ and Hprt loci in the absence

of exogenous metabolic activation at the highest dose tested.5 There was no evidence of cytotoxicity reported in this study. Evidence for a positive result had been documented in mouse lymphoma L5178Y cells in the presence and absence of exogenous metabolic activation at dose levels which induced cytotoxicity.[6] A similar results had also been documented in LY5178Y cells in the presence and absence of exogenous metabolic activation.[7] Overall it was prudent to conclude a positive response in gene mutation assays in mammalian cells in the presence and absence of exogenous metabolic activation, although the mechanism for the induced effects had not been resolved.

Mammalian cell chromosomal aberration tests

11 Phenol gave a positive result for chromosomal aberrations in CHO cells in the presence and absence of exogenous metabolic activation.[8] Members noted the increase in the absence of exogenous metabolic activation was approximately 3 fold and there was no evidence for a dose response in the presence of exogenous metabolic activation. Positive results were also reported in a number of micronucleus tests in CHO cells both in presence and absence of exogenous metabolic activation[9], in V79 cells and human PBLs (both in the absence of exogenous metabolic activation).[10,11] No evidence for an aneugenic effect of phenol was reported in a test where chromosome number in metaphase spreads were scored and reported (positive results were reported for benzene in the same experiment but a known aneugenic positive control was not used).[5] Evidence for a moderate increase in both kinetochore positive and negative micronuclei was reported in PBLs indicating some evidence for both clastogenic and aneugenic activity with phenol.[11] Overall members considered no definite conclusion regarding the potential for aneugenicity could be drawn from these data.

Studies investigating DNA damage

12 A number of *in vitro* studies investigating the potential for DNA damage were available. Members noted the evidence for UDS in the absence of exogenous metabolic activation in SHE cells.[5] Members noted the evidence for ssDNA breaks in mouse lymphoma cells in the presence of exogenous metabolic activation.[12] Members considered the evidence for formation of 8-hydroxy-deoxyguanosine (8-OHdG) 3 indicated some potential for oxidative DNA damage but commented that undifferentiated HL60 cells were likely to be more predisposed towards formation of free radicals and oxidative DNA damage than differentiating HL60 cells[12] or normal human peripheral blood lymphocytes[13] Members noted the evidence for formation of DNA adducts in calf thymus DNA in the presence of horseradish peroxidase and hydrogen peroxide.[15] The data reported provided some evidence for oxidative DNA damage with phenol but the test system was likely to be predisposed to formation of free radicals and oxidative DNA damage.[15]

Conclusion: *in vitro* mutagenicity data

13 Thus phenol was mutagenic *in vitro* in mammalian cells giving rise to gene mutation and chromosomal damage in the presence and absence of exogenous metabolic activation. The mode(s) of action had not been fully elucidated although there was evidence that effects were in part due to oxidative DNA damage.

in vivo mutagenicity studies

14 The results of available studies considered in the draft EU risk assessment report reported evidence for a 2-2.5 fold induction of BMMN using oral and i.p. doses which equate to or exceed the relevant LD50 in mice. An important conclusion reached by COM during its previous consideration of phenol related to the evidence for a small but consistent *in vivo* BM MN positive effect at dose levels below the i.p. LD50 in mice. Members reconsidered the three key studies supporting this conclusion. Chen and Eastmond used 3 doses of 160 mg/kg phenol i.p. followed by BM sampling 24h after the last dose. There was no discernable effect on the PCE/NCE ratio but signs of toxicity, if observed were not reported. FISH analysis indicated that the positive results were due to chromosome breakage.[16] Mazzarini A *et al* 1994 reported a significant positive effect following a single i.p. dose of 120 mg/kg bw to a group of 3 CD-1 mice followed by bone marrow sampling 18h after treatment.[17] There was no apparent effect on the PCE/NCE ratio but signs of toxicity, if observed were not reported. Shelby M *et al* Env Mol Mutagen, 21, 160-179, 1993 reported a positive trend test for BM MN induction in two separate studies where male B6C3F1 mice were given i.p. doses of 0, 45, 90 or 180 mg/kg bw phenol on three consecutive days with bone marrow sampling 48h after the last dose. All animals survived and there was no apparent effect on percent PCEs. However signs of toxicity, if observed , were not reported.[18]

15 The COM affirmed its previous assessment of these studies. The COM agreed the overall conclusions reached in the draft EU Risk Assessment report.[3] Thus phenol should be regarded as an *in vivo* somatic cell mutagen. The COM confirmed that there was consistent evidence for a small effect at doses below the i.p. LD50.

Evidence regarding mode of action for the *in vivo* mutagenicity of phenol.

Induction of micronuclei by phenol in mouse bone marrow. Association with chemically induced hypothermia. (Spencer et al Tox Sci, 97, 120-127, 2007)[2]

16 Groups of four male and four female CD-1 mice were dosed i.p. with 0, 50, 150, 200, 300, 400, or 500 mg/kg bw phenol (Hypothermia test). The relative Body Temperature was monitored subcutaneously using programmable transponders (also used for animal identification) prior to dosing, 5, 30, 60, 90 min and 2h, 3,4,5,6,24 and 48h after dosing. Clinical signs of toxicity were recorded. In the MN test groups of 6 animals/sex were dosed at 30, 100 or 300 mg/kg (separate group dosed p.o. with 120 mg/kg cyclophosphamide, 24h sampling). BT was measured prior to dosing, and 2,5,24 and 48h. Animals were killed at 24 or 48h post dose and bone marrow collected. For kinetochore evaluation a group of 6 males was dosed with 300 mg/kg bw phenol (CP (p.o 120 mg/kg bw) and vinblastine (4 mg/kg bw i.p) used as positive controls with 24h sampling). For MN evaluation 2000 PCEs were scored blind to dosing status. Data were transformed by adding one and taking natural log of adjusted number. Pairwise comparison of data used Dunett t-test. Kinetochore positive MN-PCEs were compared using Fisher exact test.

17 All mice dosed at 400 mg/kg bw or 500 mg/kg bw died within 24h of dosing. A single male and female in the 300 mg/kg bw group died prior to the 48h observation time point. No deaths occurred at 200 mg/kg bw and below. Signs of toxicity included reduced activity (200 mg/kg bw and above) and twitching and tremors (at 100 mg/kg bw and above) which were noted shortly after dosing. Surviving mice appeared normal 1h post dose. Males appeared to be more sensitive with a more rapid onset of signs of toxicity and shorter period to death. Predose mean body temperatures in males and females were 36.7°C and 37°C respectively. Thirty minutes post dose at 300 mg/kg bw mean BT reduced to 32°C and the mean BT as low as 28°C 5h post dose in both sexes. BT did not return to baseline within the 48h observation period and was depressed 4-5°C at the end of the experiment. BT reductions of up to 8°C were recorded at 400 and 500 mg/kg bw (at up to 6h post dose). Smaller transient reductions in BT were reported at 100, 150 and 200 mg/kg bw. From the information presented in figure1 of the published paper, the reduction at 100 mg/kg bw appears to be around 2°C with a return to baseline around 2-3h post dose. At 200 mg/kg bw the decrease in BT appears to be around 2-3°C with a return to baseline at around 4-6h. No evidence for an effect on BT was reported at 50 mg/kg bw.

18 In the MN test one animal dosed at 30 mg/kg bw died (not related to treatment). The authors report phenol related signs of toxicity in about one third of males and one half of females dosed at 300 mg/kg bw (table 1 of the published paper). Signs of toxicity appeared within minutes and had subsided about 1h post dose. There was evidence for very transient signs in animals dosed at 100 mg/kg bw (lasting only several minutes). No treatment related signs of toxicity were reported at 30 mg/kg bw. BT was reported at 24 and 48h post dose. A 4-5°C reduction was evident at 24h post dose in both males and females. By 48h the decrease was approximately 7°C in males and 6°C in females. BT at these time points was unaffected at 100 mg/kg bw and 30 mg/kg bw. BT was unaffected in CP positive control animals.

19 A statistically significant increase in MN-PCE/1000 PCE was recorded at 300 mg/kg bw at 24h sampling (male 10.8 c.f. 2.1 in control and 11.3 in females cf 2.5 in controls). At 48h the mean frequency of MN-PCE/1000PCEs was 18.3 in males and 17.8 in females. The mean percent PCE values was reduced at 24h (all doses) and 48h (in males/females at 300 mg/kg bw). The frequency of MN-PCEs/1000 PCEs was not increased at 30 and 100 mg/kg bw. CP gave the expected positive result.

20 The authors conclude that phenol induced MN formation occurred only in the presence of marked hypothermia.

21 In the kinetochore experiment, a statistically significant increase in the proportion of kinetochore positive MN was observed in phenol treated mice at 300 mg/kg bw. Vinblastine (VB) gave the expected positive result. The proportion of kinetochore positive MN was substantially higher in VB treated mice.

22 In their discussion the authors note the finding of phenol induced hypothermia at doses at or above the MTD was a novel finding. The induction of hypothermia was associated with a NOEL for MN formation and thus phenol induced MN by a secondary mechanism associated with regulation of BT in mice. It was noted that in part, it was possible to speculate that BT affected spindle function thus resulting in kinetochore positive MN. However a proportion of phenol induced MN were clastogenic

and might have been due to an effect of phenol, hydroquinone (a metabolite of phenol) or a combination of phenol/hydroquinone. It is noted that the available data on phenol suggest that any direct genotoxic activity is likely to be mediated by oxidative DNA damage and hence would be presumed to have a potential threshold for activity. Overall the authors suggested a role for hypothermia but did not prove causality. The authors suggest further studies to investigate the role of physically induced changes in BT on the induction of MN in phenol treated animals would be an appropriate way forward.

COM conclusions on Spencer et al 2007

23 Members agreed that the study had been well conducted but considered a dose level of 200 mg/kg bw i.p would have been valuable. The dose level of 300 mg/kg bw clearly exceeded the maximum tolerated dose level. The committee considered that the degree and duration of hypothermia reported with phenol was severe and prolonged. Members concurred with the conclusion reached by the study authors and reported in the publication '...overall, these studies suggest a role, but not necessarily a causality, for phenol-induced hypothermia in the formation of MN.'

Additional in-confidence data on thermoregulatory support study

24 Members considered the additional in confidence data on the thermoregulatory support study which had been provided by Dow Chemicals.

25 A full report of the studies undertaken by DOW has been submitted as an in-confidence document. Essentially phase 1 and phase 2 of the study were published in Spencer *et al* 2007.[2] Additional studies were undertaken to investigate the approach to thermoregulatory control induced (i.e. applying external heat to prevent hypothermia) in mice dosed with phenol (phase 3) and a rescue experiment was undertaken (phase 3). The objective of the rescue experiment was to oblate phenol induce MN formation in mice by appropriate thermoregulatory control. This was not achieved (a statistical increase in MN formation was reported at 24h post dose). The investigators also noted that the application of external heat to control mice also resulted in a statistically significant increase in MN formation at 24h post dose. Overall the results of the rescue study were considered to be inconclusive. A further Telemetry experiment (phase 4) was undertaken to monitor body temperature in phenol dosed and control animals under thermoregulatory control conditions at five minute intervals to provide more comprehensive data on the effectiveness of thermoregulatory support. Thermoregulatory control in control mice resulted in an overall elevation of body temperature compared to animals maintained under normal environmental conditions. For phenol-treated animals there was evidence of impaired capacity to modulate temperature compared to controls and a transient hypothermia. It was possible that the application of thermoregulatory control could influence the formation of MN in control and phenol-treated mice. In phase 5, the results of kinetochore staining experiments were reported (these data have been published in Spencer *et al* 2007[2]).

26 The COM accepted that thermoregulatory support was in practice very difficult to achieve. It was noted the effects resulting from dosing of phenol and also thermoregulatory support would have been stressful to the animals. Members observed that thermoregulatory support had not offset the

phenol induction of micronuclei in mice. The application of thermoregulatory support had resulted in evidence for a slight increase in micronuclei formation in control females. However overall the observed induction of micronuclei by phenol could not be discounted. Members were aware that the principal study author had written to the secretariat and had concluded that, at this time, it is tenuous to make a conclusion regarding the mutagenicity of phenol under conditions of altered thermoregulation in the mouse micronucleus test.

Additional published studies on hypo-and hyperthermic induction of micronuclei in rodents.

27 Members considered the generic paper on the role of hypo- and hyperthermia in the formation of micronuclei in rodents. [19-23] Of particular interest was the publication by Tweats DJ *et al* 2007.[23] These data support the observation that chemical induced hypothermia in mice and hyperthermia in rats and mice may be potential modes of induction of MN in bone marrow. Experimental evidence needed to support hypothermia or hyperthermia as a mode of action for an unknown chemical would include a time course showing the association between core body temperature and MN induction and evidence for reversibility of the chemical induced MN formation by adjusting core body temperature. The assessment of hypothermic induction of MN for a specific chemical also requires evaluation for evidence regarding other modes of genotoxicity. A clear negative *in vitro* package of genotoxicity tests would rule out other modes of genotoxicity when deriving conclusions regarding the role of hypothermia in any observed *in vivo* MN formation. Evidence for positive *in vitro* genotoxicity would suggest other potential modes of genotoxic action *in vivo* which need to be taken into account in the overall assessment.

COM conclusions

28 The COM agreed with the conclusions reached on phenol in its previous statement (COM/00/S1). The COM agreed the overall conclusions reached in the draft EU Risk Assessment report.[3] The following overall conclusions were agreed.

a. Phenol is mutagenic *in vitro* in mammalian cells giving rise to gene mutation and chromosomal damage in the presence and absence of exogenous metabolic activation. The mode(s) of action had not been fully elucidated although there was evidence that effects were in part due to oxidative DNA damage

b. Phenol should be regarded as an *in vivo* somatic cell mutagen. The COM confirmed that there was consistent evidence for a small effect at doses below the i.p. LD50.

c. The COM agreed that the published study by Spencer *et al* 2007 had been well conducted but considered a dose level of 200 mg/kg bw i.p would have been valuable. The dose level used in the study of 300 mg/kg bw clearly exceeded the maximum tolerated dose level. The committee considered that the degree and duration of hypothermia reported with phenol was severe and prolonged. Members concurred with the conclusion reached by the study authors and reported in the publication '...overall, these studies suggest a role, but not necessarily a causality, for phenol-induced hypothermia in the formation of MN.'

d. The COM concluded that the additional 'in confidence' data on thermoregulatory support in phenol treated animals provided inconclusive evidence regarding the role of hypothermia in phenol-induced micronuclei in mice. Thus for phenol-treated animals there was evidence of impaired capacity to modulate temperature compared to controls and a transient hypothermia. It was possible that the application of thermoregulatory control could influence the formation of MN in control and phenol-treated mice.

e. The COM concluded that all the available data on phenol suggested phenol should be regarded as a non-threshold *in vivo* systemic mutagen. There is insufficient evidence to support a threshold approach to risk assessment of systemic phenol.

November 2008

References

1. Committee on Mutagenicity. Mutagenicity of hydroquinone and phenol. COM statement COM/00/S1-January 2000.

2. Spencer PJ, Gollapudi BB and Waechter JM Jr (2007). Induction of Micronuclei by phenol in the mouse bone marrow:1. Association with chemically induced hypothermia. Tox Sci, 97, 120-127.

3. Draft EU Risk Assessment report; Phenol (CAS 108-95-2). Draft of 01.09.2005

4. Paschin Yu V and Bahitova LM (1982). Mutagenicity of benzo(a)pyrene and the antioxidant phenol at the HGPRT locus of V79 chinese hamster cells. Mutation Res, 104, 389-393.

5. Tsuitsui T, Hayashi N, Maizumi H, Hujj J and Barett JC (1997). Benzene-, catechol-, hydroquinone- and phenol-induced cell transformation, gene mutations, chromosome aberrations, aneuploidy, sister chromatid exchanges and unscheduled DNA synthesis in syrian hamster embryo cells. Mutation Res, 373, 113-123.

6. Wagenenheim J and Bolcsfoldi G (1988) Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. Mutagenesis, 3, 193-205.

7. McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Riach C, Caspray WJ (1988) Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay: III 72 coded chemicals. Environ Mol Mutagen, 12, 85-154.

8. Ivett JL, Brown BM, Rodgers C, Anderson BE, Resnick MA and Zeiger E. Chromosomal aberrations and sister chromatid exchange tests in Chinese hamster ovary cell *in vitro* IV. Results with 15 chemicals. Environ Mol Mut, 14, 165-187.

9. Miller BM, Pujadas E, and Gocke E (1995). Evaluation of the micronucleus test *in vitro* using Chinese hamster cells. Environ Mol Mutagen, 26, 240-247.

10. Glatt H, Padykula R, Berchtold GA, Ludewig G, Platt KL, Klein J and Oesch F (1989). Multiple activation pathways of benzene leading to products with varying genotoxic characteristics. Environ Health Perspective, 82, 81-89.

11. Yager JW , Eastmond DA, Robertson ML, Paradisin WM and Smith MT (1990). Characteristation of micronuclei induced in human lymphocytes by benzene metabolites. Cancer Research, 50, 393-399.

12. Covacci V, Torsello A, Palozzi P, Sgambato A, Romano G, Boninsagna A, Cittadini A, and Wolf F (2001). DNA Oxidative damage during differentiation of HL-60 Human Promyelocytic Leukaemia cells. Chem Res Tox, 14, 1492-7.

13. Bagchi D, Balmoori J, Benner FJ, Kuszynski CA, and Stoks SJ (1999). Cadmium and chromium induced oxidative stress, DNA damage and apoptotic cell death in cultured chronic myelogenous leukemic K562 cells, promyelocytic leukemic HL60 cells and normal human peripheral blood mononuclear cells. J Biochemical and Molecular Toxicology, 14, 33-41.

14. Garberg P, Akerblom EL and Bolcsfoldi G (1988). Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxapatite elution. Mutation Res, 203, 155-176.

15. Subrahmanyam V and O'Brien, PJO (1985). Peroxidase-catalysed binding of [U-14C]phenol to DNA. Xenobiotica, 15, 859-871.

16. Chen H and Eastmond DA (1995). Synergistic increase in chromosomal breakage within the euchromatin induced by an interaction of the benzene metabolites phenol and hydroquinone in mice. Carcinogenesis, 16, 1963-1969.

17. Mazzarini A, Chelotti L, Barrai I, Loprieno N and Barale R (1994). *in vivo* genotoxic interactions among three phenolic benzene metabolites. Mutat Res, 341, 29-46.

18. Shelby M, Erexson GL, Hook GJ and Tice RR (1993). Evalaution of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. Env Mol Mutagen, 21, 160-179.

19. Asanami S, Shimono K and Kaneda S (2001). Effect of temperature on the frequency of chromosome aberrations and micronuclei in cultured chinese hamster cells. The J of Tox Sci, 26, 323-326.

20. Asanami S and Shimono K (1997). High body temperature induces micronuclei in mouse bone marrow. Mutat Res, 390, 79-83.

21. Asanami S, Shimono K, Kaneda S (1998). Transient hypothermia induces micronuclei in mice. Mutat Res, 413, 7-14.

22. Shuey DL, Gudi R, Krsmanovic L and Gerson RJ (2007). Evidence that oxymorphone-induced increases in micronuclei occur secondary to hyperthermia. Tox Sci, 95, 369-375.

23. Tweats DJ, Blakey D, Heflich RH, Jacobs A, Jacobsen SD, Morita T, Nohmi T, O'Donovan MR, Sasaki YF, Sofuni T and Tice R. Report of the IWGT working group on strategies and interpretation of regulatory *in vivo* tests I. Increases in micronucleated bone marrow cells in rodents that do not indicate genotoxic hazards. Mutation Research, 627, 78-91.

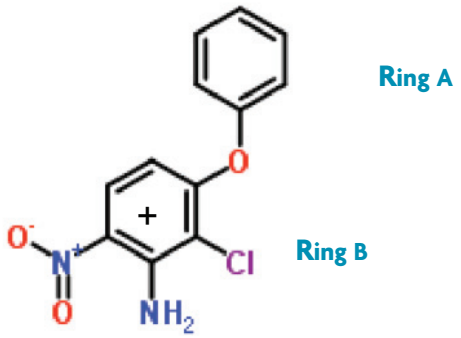
Statement on the review of Mutagenicity of Alconifen and risk assessment of its Postulated Metabolites Hydroquinone and Phenol)

COM/08/S3 - November 2008

Introduction

1. The COM has been asked for advice by the Pesticides Safety Directorate (PSD) on a pesticide active ingredient new to the U.K. which is undergoing evaluation through the independent Advisory Committee on Pesticides (ACP). The referral statement was as follows: 'ACP requested advice on the mutagenicity of Aclonifen and the genotoxicity risk assessment of the postulated metabolites hydroquinone and phenol. The referral does not include carcinogenicity data or the evaluation of mode of action for tumours in rodents observed in long-term carcinogenicity bioassays with Aclonifen'.

2. Aclonifen (2-chloro-6-nitro-3-phenoxyaniline) (figure 1.) is a selective systemic herbicide used for pre-emergence control of grass and broad leaved weeds in a range of crops.



+ = position of uniformly radiolabelled phenoxyaniline ring

Figure 1. Aclonifen

3. On 31 January 2008, at the request of the ACP Chair, an approach was made by PSD to the COM Chair for advice as to whether DNA adducts could be detected and measured in the existing stored tissues from animals dosed with Aclonifen. The COM Chair advised that it would not be advisable to undertake a retrospective analysis of stored tissues from Aclonifen treated animals for DNA adducts.

4. A teleconference was held between the data holder (Bayer CropScience), PSD and Health Protection Agency (HPA) (representing COM Secretariat) on the 13 June 2008. The comments raised by HPA during this teleconference outlined the particular need to address the metabolism of Aclonifen to hydroquinone and phenol and the assumption that these two metabolites were non-threshold *in vivo* mutagens.[1,2] Subsequent to the teleconference, the data holder submitted a revised position paper on 13 August 2008 on the relevance of phenol and hydroquinone formation following Aclonifen exposure which outlined their evaluation of the genotoxicity data on Aclonifen and metabolism of Aclonifen. ACP requested advice from COM on 30 July 2008.

5. The COM Secretariat held a teleconference with the data holder on 11 September 2008 to explain COM procedures, the referral for advice from ACP, data that COM would consider and to outline the procedures during committee with regard to a presentation from the data holder. Information on the possible areas of Aclonifen evaluation which COM Members might wish to raise questions was outlined, although it was noted that other aspects of Aclonifen might be raised.

6. The data holder submitted a presentation for the COM meeting on 13 October 2008 which was circulated to Members. In addition, on 21 October 2008 the data holder submitted a revision to the report dated 16 July 2004 on cleavage of the diphenyl ether bond in the Aclonifen molecule which had been circulated to Members.[3] The revised report was circulated to COM Members and replaced the aforementioned 2004 report.

7. The data holder attended the COM meeting of 23 October 2008 to make a short presentation and answer COM queries regarding the evaluation of the metabolism and mutagenicity of Aclonifen.

COM consideration of areas for discussion

8. The COM considered the submitted data, which included an extract from the detailed record of ACP consideration of Aclonifen at ACP meeting 329, extracts from draft EU assessment report on metabolism and genotoxicity of Aclonifen, which presented information on structure, use as a pesticide, ADME studies, toxicology, mutagenicity, carcinogenicity and reproduction, data from mutagenicity test reports on Aclonifen, copy of the report on the investigation of the potential for DNA-binding of Aclonifen and the revised position paper from the data holder on the cleavage of the diphenyl ether bond of Aclonifen.[3-13]

9. The Chair asked COM to consider the questions to ask the data holder and proposed Members should first consider the metabolism of Aclonifen followed by the mutagenicity data on Aclonifen. The discussion of mutagenicity data focussed on determining whether it was possible that the potential genotoxic effects of hydroquinone and phenol formed from Aclonifen could be assessed in these studies.

The areas for discussion related to:

10. Evaluation of data for the metabolism of Aclonifen and evidence for the systemic formation of hydroquinone and phenol from absorbed Aclonifen. It was noted that there were published papers in the peer reviewed scientific literature which provided examples of diphenyl ether breakage in a variety of species.[14-17]

11. Evaluation of the comparisons made by the data holder between mutagenicity data on hydroquinone and phenol with Aclonifen.

12. Evaluation of the mutagenicity testing strategy used by the data holder and specifically the reasons for undertaking an *in vitro* rather than an *in vivo* rat liver UDS study.

13. The COM noted the evaluation of carcinogenicity data was not included in the referral to COM, but agreed the data holder should be asked if there were data on tissue exposure from the carcinogenicity studies which might assist in evaluation of the mutagenicity data.

Data holder presentation

14. The data holder was asked to make a short presentation to the COM and to answer Members' queries.

15. The data holder presented an overview of Aclonifen rat metabolism studies, genotoxic potential of Aclonifen and their conclusions on the genotoxicity of Aclonifen.

16. Aclonifen had been 14C-labelled on the phenoxyaniline ring (B) but no radiolabelled studies had been undertaken with the phenyl ring (A) (figure 1.). The data holder noted that Aclonifen was rapidly absorbed via the oral route of administration and extensively metabolised with the majority of administered material (>90%) eliminated in the first 24 hrs via urine for both single dose and repeat dose studies (at 30 mg/kg bw). Approximately 40-48% of the absorbed dose was eliminated via the bile following an oral dose of 30 mg/kg bw. Tissue levels of radioactivity were very low. Aclonifen was metabolised by hydroxylation, methylation, reduction of the nitro group, N-acetylation, cleavage of the diphenyl ether bond and phase II conjugations. Potential diphenyl ether breakage had been inferred from the formation of glucuronide and sulphate metabolites from ring B. The data holder noted there were uncertainties in determining the total potential diphenyl ether bond breakage but overall this was estimated to be 9.2% in males and 7.3% in females. The data holder noted there was no evidence for cleavage metabolites in the repeat dose metabolism study and proposed that it was necessary for Aclonifen to be hydroxylated, and glucuronidated and sulphated before diphenyl ether breakage to form the conjugated forms of hydroquinone and phenol. This would provide an explanation for the negative findings in genotoxicity tests with Aclonifen.

17. With regard to the available mutagenicity studies on Aclonifen, negative results had been obtained in Ames tests, an *in vitro* chromosome aberration study in human lymphocytes, an *in vitro* gene mutation study in V79 cells (HPRT locus), and an *in vitro* rat liver UDS assay. Negative results had also been obtained in a mouse micronucleus test using the oral route of administration and no evidence for DNA binding in liver and urinary bladder had been reported in mice dosed orally with ^{14}C-labelled Aclonifen (labelled in ring B). The data holder considered the higher concentrations used and evidence for reduced toxicity in the presence of exogenous metabolic activation in *in vitro* mutagenicity studies in mammalian cells suggested that Aclonifen was being metabolised. The data holder noted that hydroquinone and phenol had given positive results in comparable studies for clastogenicity and gene mutation in V79 cells. In particular, Aclonifen was negative in an *in vitro* rat liver UDS study where metabolism would have been expected. In addition, phenol and hydroquinone were positive in *in vitro* UDS tests in Syrian Hamster Embryo (SHE) cells at dose levels almost 100-fold lower than tested with Aclonifen. The data holder noted the negative *in vivo* oral mouse bone marrow micronucleus test (high dose level 7260 mg/kg bw) with Aclonifen and compared this with evidence for positive results in studies with hydroquinone (80 mg/kg bw) and phenol (265 mg/kg bw). The data holder concluded that Aclonifen was not genotoxic and that, if hydroquinone and phenol

were formed during the metabolism of Aclonifen, then the results of the oral micronucleus test in mice should have been positive. The data holder drew the attention of COM to the detailed supporting slides in the presentation.

COM questions for data holder

18. A summary of the response given by the data holder on the areas for discussion is given below.

19. The data holder commented there were no specific data available on the formation of hydroquinone and phenol from Aclonifen. The data holder had considered the aspect of there being no evidence for diphenyl ether breakage of Aclonifen in the repeat dose study and suggested the breakage metabolites in the single dose studies being artefacts of mass spectrometry in these studies as one possibility for this observation. It was unlikely that there were individual animal data for the diphenyl ether breakage metabolism of Aclonifen as samples had been pooled prior to analysis and thus no assessment of the potential extent of inter-animal variation in metabolism could be made. With regard to the potential metabolism of Aclonifen to hydroquinone and phenol in exogenous metabolic fractions used in mutagenicity tests, the data holder considered the higher doses used and evidence for reduced toxicity in the presence of S-9 (compared to tests in the absence of S-9) in *in vitro* mutagenicity studies in mammalian cells with Aclonifen provided some reassurance that exogenous metabolism had occurred although there were no specific data on metabolites formed. Members considered that alternatively it was possible that protein binding occurred in the presence of exogenous metabolising fractions reducing the dose available to cells.

20. The data holder commented that the comparisons of mutagenicity data on Aclonifen and that available on hydroquinone and phenol were based on the best available data and acknowledged that there were uncertainties, for example comparing different cell lines, and historic data from different laboratories. The COM considered there were likely to be quite substantial differences in metabolic competency between SHE cells (used for tests with hydroquinone and phenol) and primary rat liver cells (used for the test with Aclonifen). In addition, differences in solubility of the test materials in vehicles used would also affect any comparison of the mutagenicity data. With regards to mutagenicity testing strategy, the data holder noted the rationale used for undertaking an *in vitro* rather than an *in vivo* rat liver UDS study was based on decisions on testing strategy reached at the time of testing rather than the specific question of *in vivo* metabolism of Aclonifen to hydroquinone and phenol.

21. The data holder considered there were no relevant data from the carcinogenicity studies with Aclonifen on tissue concentrations in carcinogen target tissues (brain female rat), urinary bladder (mouse) which might assist in the understanding of potential genotoxicity of Aclonifen.

22. The data holder considered the data on polyploidy in the chromosome aberration study with Aclonifen to be within historical control levels for the laboratory.

23. The data holder withdrew from the meeting so that the COM could derive its conclusions.

COM discussions

24. The COM noted peer-reviewed scientific literature which provided examples of diphenyl ether breakage in rats, mice and one bacterial strain (*Sphingomonas wittichii*) and considered it was therefore feasible that metabolism of systemic Aclonifen could result in the formation of free (unconjugated) hydroquinone and phenol, although there were no specific data on this aspect. The COM considered if exogenous metabolic activation systems such as Arochlor-1254 could metabolise Aclonifen to hydroquinone and phenol and agreed there were no specific data available.

25. The COM discussed the revised metabolism pathway for Aclonifen submitted by the data holder and agreed the proposal was feasible but not supported by appropriate data. Members were informed by the data holder that formation of phenol and hydroquinone prior to conjugation was equally unsupported as a second hypothesis in terms of available data. Members noted the proposal from the data holder that, if hydroquinone and phenol were formed from Aclonifen, then some positive results should have been recorded in the mutagenicity studies on Aclonifen.

26. The COM considered that the comparisons made between mutagenicity of Aclonifen and hydroquinone and phenol were useful but had reservations regarding whether definite conclusions could be reached. Thus it was possible that, when Aclonifen was orally administered to mice, hydroquinone and phenol were formed but failed to induce a detectable increase in micronucleus frequency in the polychromatic erythrocytes of the bone marrow.

COM conclusions

27. The COM agreed that further data on Aclonifen metabolism was required. This could involve more *in vivo* tests with specific analysis for the formation of hydroquinone and phenol. Alternatively, it might be possible to undertake comparative *in vitro* studies using rodent and human tissues (with specific measurement of hydroquinone and phenol formation). It was considered this could provide evidence that exposure to Aclonifen was unlikely to be associated with significantly increased genotoxic risk, although this would not preclude the possible need for additional mutagenicity tests dependent on the outcome of the metabolism studies.

28. The COM noted the approach to risk assessment had not been considered during the presentation, but that the data holder had included a proposed Margin of Exposure approach in the submission dated 13 August 2008. This would need to be considered further when appropriate metabolism data were available.

November 2008

References

1. COM statement on hydroquinone and phenol. COM/00/S1. January 2000.

2. COM statement on phenol (2008). http://www.iacom.org.uk/statements/StatementsChoronological.htm

3. Fisher P. Position paper on the metabolic fate of Aclonifen in the rat: cleavage of the diphenyl ether bond revisited. 21 October 2008. Bayer CropScience.

4. Extract from detailed record of ACP consideration of Aclonifen. ACP meeting 329.

5. Extracts from draft EU risk assessment report on metabolism and genotoxicology of Aclonifen.

6. Kramer PJ. *in vitro* assessment for the mutagenic potential in bacteria with and without addition of a metabolising system. Inst of Toxiciology, E. Merck, Darmstadt, 08.04.1982, KUB 3358.

7. Herbold B. Salmonella/microsome test. Plate incorporation and preincubation method. Report AT02825. AE F068300 (Aclonifen). Study T1076125. TXCLX014. Bayer Health Care AG, PH-GDD Toxicology, 42096 Wuppertal, Germany.

8. Dance CA. *in vitro* assessment of the clastogenic activity of Aclonifen in cultured human lymphopcytes. LSR Report 92/RHA477/0471. Life Science Research, Eye, Suffolk, England.

9. Anonymous. Mammalian cell (V79) mutagenicity test on Aclonifen. Report SP 579/VT-19, Institute of Toxicology, University of Mainz, Obero Zahlbacher, Straβ3, 67, Germany. 6 March 1984.

10. Anonymous. Unscheduled DNA synthesis (UDS) in primary rat hepatocytes (Autoradiographic method). Aclonifen Technical. RTC report 121009-M-03691. Research Toxicology Centre S.p.A. 31/10/91.

11. Anonymous. Cytogenetic Investiogations in NMRI Mice after single oral administration of CME 127 (Aclonifen). Micronucleus test. Project 26M0286/8332. Translation Celamerck Document 127AD-457-005. March 8, 1984.

12. Sagelsdorff P. Investigation of the potential for DNA-binding of Aclonifen. CIBA-Geigy Ltd. Toxicology/Cell Biology CH-4002 Basel. Project CB95/24, 15 August 1995.

13. Semino G, Mackenzie E and Leake C. Position paper on the relevance of phenol and hydroquinone formation following Aclonifen exposure. 13 August 2008. Bayer CropScience.

14. Qiu X, Mercado-Feliciano M, Bigsby RM, Hites RA. Measurement of polybrominated diphenyl ethers and metabolites in mouse plasma after exposure to a commercial pentabromodiphenyl ether mixture. Environ Health Perspect. 2007 Jul;115(7):1052-8.

15. Keum YS, Lee YJ, Kim JH. Metabolism of nitrodiphenyl ether herbicides by dioxin-degrading bacterium Sphingomonas wittichii RW1. J Agric Food Chem. 2008 Oct 8;56(19):9146-51.

16. Chen LJ, Lebetkin EH, Sanders JM, Burka LT. Metabolism and disposition of 2,2',4,4',5-pentabromodiphenyl ether (BDE99) following a single or repeated administration to rats or mice. Xenobiotica. 2006 Jun;36(6):515-34.

17. Balsam A, Sexton F, Borges M, Ingbar SH. Formation of diiodotyrosine from thyroxine. Ether-link cleavage, an alternate pathway of thyroxine metabolism. J Clin Invest. 1983 Oct;72(4):1234-45.

2008 Membership of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

CHAIR

Professor Peter B Farmer MA DPhil CChem FRSC
Professor of Biochemistry, Cancer Studies and Molecular Medicine,

MEMBERS

Dr Carolyn Allen BSc MSc PhD
Non-specialist Member

Dr Brian Burlinson CBiol MIBiol PhD
Director of Cellular and Molecular Toxicology, Huntingdon Life Sciences

Dr Gillian Clare BSc PhD
Cytogeneticist, Covance

Dr Julie Clements BSc PhD
Head of Genetic and Molecular Toxicology, Covance

Dr Barry M Elliott BSc MSc PhD
Senior Toxicologist, Syngenta Central Toxicology Laboratory

Dr David Gatehouse BSc PhD CIBiol FIBiol FRCPath
Consultant in Genetic Toxicology, Covance

Mrs Rosie Glazebrook MA
Non-specialist member

Professor Nigel J Gooderham BSc PhD CChem FRSC FBTS
Professor of Molecular Toxicology, Biomolecular Medicine, Imperial College London

Dr David P Lovell BSc PhD CStat FSS CBiol FIBiol
Reader in Medical Statistics, Postgraduate Medical School, University of Surrey

Dr Ian Mitchell BA PhD
Consultant in Genetic and Molecular Toxicology, Kelvin Associates and Chilfrome Enterprises Ltd

Dr Elizabeth M Parry BSc DPhil
Part-time Senior Research Fellow, School of Medicine, University of Wales

Professor David H Phillips BA PhD DSc FRCPath
Professor of Environmental Carcinogenesis, Institute of Cancer Research

SECRETARIAT

Mr J Battershill BSc MSc	Joint Scientific Secretary – Health Protection Agency
Dr D Benford BSc PhD	Joint Scientific Secretary – Food Standards Agency
Dr L Hetherington BSc PhD	Scientific – Health Protection Agency
Ms F Pollitt MA DipRCPath	Scientific – Health Protection Agency
Mr S Robjohns BSc MSc	Scientific – Health Protection Agency
Ms S Kennedy	Administrative Secretary – Health Protection Agency

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

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor P B Farmer (Chair)	Banco Santander Bradford & Bingley Foreign & Colonial Friends Provident Health Effects Institute Torotrak ILSI HESI	Shareholder Shareholder Shareholder Shareholder Research Committee Member Shareholder Committee Member	American Chemistry Council CEFIC	Research support and conference attendance expenses. Research Support
Dr C Allen	NONE	NONE	NONE	NONE
Dr B Burlinson	Huntingdon Life Sciences	Salary Employee Share Option Holder	NONE	NONE
Dr G Clare	Covance Allied Domecq AstraZeneca Diageo HBOS Marks & Spencer	Salary Shareholder Shareholder Shareholder Shareholder Shareholder	NONE	NONE
Dr J Clements	Covance	Salary Share Option Shareholder	NONE	NONE
Dr B M Elliott	Syngenta AstraZeneca	Salary Share Option Holder Shareholder	NONE	NONE
Dr D Gatehouse	Covance Friends Provident GlaxoSmithKline	Salary Consultant Shareholder Pension Share Option Holder	NONE	NONE

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Mrs R Glazebrook	BT Group Lloyds TSB National Grid	Shareholder Shareholder Shareholder	NONE	NONE
Professor N J Gooderham	Banco Santander CENES Silence Therapeutics Hargreaves Lansdown Proctor & Gamble	Shareholder Shareholder Shareholder Shareholder Consultant	FSA GlaxoSmithKline FEMA (USA)	Research contract CASE studentship Research support
Dr D P Lovell	National Grid Transco Pfizer	Shareholder Shareholder Share Options Pension	AstraZeneca National Grid Transco	Spouse Shareholder Spouse Shareholder
Dr I Mitchell	Kelvin Associates IM Enterprises Chilfrome Enterprises GlaxoSmithKline Allergy Therapeutics BG Cadbury Schweppes GEC GSK ICH Mitchell & Butler Pfizer Real Good Food Renishaw Royal Dutch Shell RTZ Unilever Vedanta BP Centrica Green King Scottish & Southern	Associate Consultant Director/Creditor Director Pensioner Option and Shareholder Consultant Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder PEP Holder PEP Holder PEP Holder PEP Holder	NONE	NONE

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Dr E M Parry	Invesco Fleming Legal & General Quintiles	PEP Holder PEP Holder PEP Holder Consultancy	NONE	NONE
Professor D H Phillips	Aviva Banco Santander BG Group Bradford & Bingley Centrica National Grid ECETOC Servier Butler Jeffries (solicitors)	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Honorarium Honorarium Honorarium	NONE	NONE

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

Preface

The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) evaluates chemicals for their carcinogenic potential in humans at the request of UK Government Departments and Agencies. The membership of the Committee, agendas and minutes of meetings, and statements are all published on the internet (http://www.iacoc.org.uk/).

During 2008 the Committee has considered a number of interesting items. We began our consideration of the complex problem of the assessment of mixtures of chemicals for carcinogenicity. This proved a difficult task due to the inevitable lack of data on carcinogenicity testing of mixtures and the need to attempt to draw conclusions from short-term studies and epidemiological findings. The Committee intends to produce a statement on this topic in 2009.

The committee was also asked to advise the UK National Coordinator for the Organisation for Economic Cooperation and Development (OECD) Test Guidelines on the planned revision of the guidelines for chronic toxicity and carcinogenicity studies and the associated Guidance Document. The Committee has agreed to draft a chapter on the investigations undertaken in studies of this type, which will include advice on histopathology.

I would like to thank the members and secretariat of the Committee for the work they have undertaken during the past year. We look forward to new challenges in 2009.

Professor David H Phillips
BA PhD DSc FRCPath

COC evaluations

Age as an independent risk factor for chemically-induced acute myelogenous leukaemia in children

3.1 In 2006 the COC discussed the question of whether there are age-related differences in susceptibility to carcinogenesis. At the November 2008 meeting, the Committee considered a recent review by Pyatt *et al* (2007; J Toxicol Env Health B, Volume 10(5): pp 379-400) which had tested the assumption that children are inherently up to 10-fold more sensitive than adults to genotoxic carcinogens. It had done this using data on the development of secondary or therapy-related acute myelogenous leukemia (t-AML) in children who had received treatment with high dose chemotherapy and/or radiation. This disease is well established as a potential long-term consequence of exposure to such treatment. The review had investigated the effect of age at treatment on a child's susceptibility to developing t-AML.

3.2 Members noted that there was little information, which had led the authors of the review to draw cautious conclusions. The Committee concluded the data presented did not give cause to think that children are more susceptible than adults, although the evidence was not strong enough to rule out such an effect.

3.3 Members also noted that the dose of chemotherapy administered to children is often scaled by body surface area, using an algorithm incorporating height and weight, whereas, in a risk assessment of chemicals which cause leukaemia, exposure would be scaled relative to metabolic rate (oxygen demand) on the basis of an exponent of body weight. It was acknowledged that, where susceptibility of the subpopulation is the result of increased exposure, this would normally be incorporated into the risk assessment by separate assessment of the exposure of the subpopulation, with emphasis on children's specific exposure assessment.

Betel Quid, Pan Masala and Areca Nut Chewing

3.4 Areca nut is an ingredient of betel quid or pan masala which is chewed as an aid to digestion and as a stimulant. Areca nut may have limited use as a food ingredient. The Committee reviewed the use of areca nut in betel quid and pan masala in 1993 and 1994 and advised that there was evidence of mutagenic and carcinogenic activity of areca nut extracts and derived compounds in experimental systems. Also, areca nut derived nitrosoamines, including the potent carcinogen MNPN, have been detected in the saliva of betel quid chewers. There were very limited data from epidemiological studies on the effect of betel or areca nut products without tobacco, but there was sufficient epidemiological evidence of a link between the chewing of betel quid containing tobacco and cancer in humans. The Committee concluded that the use of these products without tobacco was possibly carcinogenic in humans.

3.5 Following the publication of new information, the COC was asked to look at this subject again. Most of the epidemiological data available concerned the use of areca nut in betel quid or pan masala and the association with oral cancers and pre-cancerous lesions. Other epidemiological data associated the use of areca nut with other cancers such as liver cancers. There are also some limited animal studies and *in vitro* studies.

3.6 The Committee was informed at, in 2003, the International Agency for Research on Cancer (IARC) had categorised both the chewing of betel quid and of areca nut as carcinogenic to humans. It had also stated that there was sufficient evidence in humans to conclude that betel quid chewed without tobacco causes oral cancer.

3.7 The Committee considered the available data and concluded that there was sufficient epidemiological evidence to conclude that areca nut, when used in the form of betel quid or pan masala, is carcinogenic to humans. This relates primarily to an increased risk of oral cancers from retaining the areca nut or betel quid in the mouth for a significant length of time. Members also agreed that the use of areca nut as a food ingredient may result in an increased risk of cancer.

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

Carcinogen-DNA adducts as a biomarker for cancer risk

3.9 Researchers seeking to understand the mechanism by which a chemical causes cancer in a particular (target) tissue may measure the levels of adducts derived from the chemical which are bound to DNA. At the April meeting, the Committee considered a methodological paper by Rundle (2008; Mutation Research, Volume 600: pp 23-36) on the use of adducts as a biomarker for cancer risk. The paper suggests that epidemiological studies which seek to establish an association between carcinogen-DNA adduct levels and the risk of cancer often fail to incorporate fundamental epidemiological principles into their methods. The author described a number of studies which have investigated associations between DNA-carcinogen adduct levels and cancer, and he addressed a number of methodological issues common to these studies, such as the use of target tissue versus surrogate tissue and how this choice impacts on the selection of controls, the use of inappropriate statistical analyses, and small sample sizes. A number of suggestions were made to improve study designs to overcome these issues in the future.

3.10 The Committee considered that researchers are aware of the limitations of using surrogate tissues to measure DNA adducts. It also pointed out that, even if adduct levels were measured in samples of target tissue rather than in surrogate tissue, they may also lack relevance to the underlying pathological condition. This is because only certain cell types in the tissue will be targets, and only a limited number of adducts are causal, with the majority occurring at non-critical sites or in non-critical genes. The Committee considered that it was an over-simplification to argue that target tissue samples will overcome a major limitation of adduct determination and to dismiss the value of adducts in surrogate tissues.

3.11 The Committee noted that adducts measured at the time of diagnosis may not reflect exposure at the critical period and may be affected by the pathology of the condition suffered by the patient. It recommended that lymphocyte fractions of blood samples could be stored in biobanks and later used for biomarker analysis in outcome studies.

Chlorinated drinking water and cancer

3.12 Chlorination has long been an important part of water treatment, intended to ensure that drinking water contains no microbes hazardous to human health. Disinfection of drinking water is fundamental to preventing the spread of waterborne disease, such as cholera.

3.13 In the mid-1970s, refinements in techniques of chemical analysis resulted in the detection in drinking water of traces of chemicals formed when organic chemicals (such as those which may occur naturally in rivers, lakes, reservoirs and other water sources) are subjected to chlorination. In most supplies, the main chlorination byproducts (CBPs) are the four chlorinated and brominated trihalomethanes (THMs, ie chloroform, bromodichloromethane, chlorodibromomethane and bromoform). However, numerous other CBPs have been identified in drinking water, but many have yet to be characterised.

3.14 Some CBPs, including some of the THMs, are known to be carcinogenic in laboratory mammals and some are genotoxic in test systems. There have been many epidemiological investigations into the possible association between chlorination of drinking water and cancer in humans and experimental studies of the mutagenicity and carcinogenicity of CBPs. The COC reviewed the epidemiological studies in 1992 and 1999 and reviewed the animal carcinogenicity data in 1996. In 1996, it concluded that the levels of the four THMs considered by the Committee in drinking water in the UK were unlikely to provide a carcinogenic risk to humans and, in 1999, it concluded that the new epidemiological studies failed to provide persuasive evidence of a consistent relationship between chlorinated drinking water and cancer. The COC considered that efforts to minimise exposure to CBPs remain appropriate, providing that they do not compromise the efficiency of disinfection of drinking water.

3.15 Thirteen further relevant epidemiological papers have been published since the 1999 review. The COC reviewed these at the July 2007 meeting and published the results of its review in 2008. The committee commented that problems remained in the interpretation of published studies on CBPs, particularly because adequate exposure assessment continued to be a major problem. It also noted that none of the studies reviewed were carried out in the UK and that it is possible that disinfection practices and constituents of the raw water may be different in other countries, in which case the study results may not be directly applicable to the UK. The committee concluded that the new studies on bladder cancer provided limited evidence for an association between bladder cancer and exposure to CBPs in men but that the evidence for an association in women is conflicting. In the 1999 review, the COC had commented that the studies of colorectal cancer gave inconsistent findings. In the current review, it noted that one well-conducted study provided some evidence for an association with colon cancer, but not rectal cancer, in men only. Also, a well-conducted study indicated an association with brain cancer in men but not in women. There were no consistent findings for other cancer sites. Overall, it concluded that the evidence for a causal association between cancer and exposure to CBPs is limited and any such association is unlikely to be strong. Efforts to minimise CBPs in drinking water should continue but must be balanced against the need for effective disinfection of drinking water.

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

Mode of Action/Human Relevance Framework

3.17 The International Programme on Chemical Safety (IPCS) Mode of Action (MOA) Framework is a conceptual framework for considering data on the mode of action of chemical carcinogens. The COC considered aspects of the MOA Framework in 1999 and, in 2004, considered a related topic, the Human Relevance Framework (HRF), which had been developed by a working group sponsored by the US Environmental Protection Agency and the International Life Sciences Institute (ILSI) Risk Science Institute (RSI). The HRF systematically considers the weight of evidence of hypothesized modes of action in animals and their potential human relevance for cancer.

3.18 In 2008, the Committee discussed recent developments made by the IPCS in the continuing evolution of HRFs. The IPCS HRF entails answering a series of three questions followed by a statement of confidence, analysis and implications. The COC considered 3 case studies which had used the IPCS HRF as an approach to determine the sufficiency of evidence and the relevance of an animal MOA for humans. These case studies entailed 3 different MOAs: 1) sustained cytotoxicity and regenerative proliferation leading to nasal tumours following exposure to formaldehyde, 2) direct alkylation of DNA leading to tumours in multiple sites following exposure to 4-aminobiphenyl, 3) increased hepatic clearance of thyroxin leading to thyroid tumours following exposure to thiazopyr.

3.19 The COC considered that the IPCS HRF was a valuable evolution of the work on this concept and proposed that the IPCS HRF approach should be used on a case-by-case basis in its future evaluations of chemicals.

3.20 The Committee also reviewed a paper by Sielken *et al* (2005; Scand J Work Environ Health, Volume 31, Suppl 1: pp151-5). This paper described a dose-response modelling approach to provide statistical insight into the relative likelihood of different mechanisms of action in cancer dose-response studies. The paper provided two examples based on time-to-tumour data for mammary fibroadenoma and adenocarcinoma in female Sprague-Dawley rats exposed to a pesticide in the diet. The examples considered how 34 different dose metrics (i.e. a measure of exposure to the pesticide or a measure of the biological activity potentially generated by the exposure if a specific mechanism of action applies) related to the incidence of fibroadenoma and adenocarcinoma and demonstrated how maximum likelihood statistical methodology could be used to provide an indication of the mechanism of action of the pesticide.

3.21 The Committee considered that it was unclear how the dose metrics and the different variables were identified and chosen for inclusion as no references were cited in the paper. It questioned the statistical robustness of the approach and considered that, although the most likely mechanism of action for the unidentified pesticide in the above examples was found to be hormonal, no other data were provided to show that it acted through a hormonal mechanism and therefore the assumption made in the paper was unwarranted. Moreover, no data were provided on the other dose metrics used. The Committee concluded that there may well be potential value in the approach suggested, but that more work was required. Before applying this approach to a specific example, it would be necessary to have alternative endpoints linked to a MOA.

3.22 The Committee also heard a short presentation by a PhD student at Imperial College London on the weight of evidence in framework approaches to cancer hazard identification.

Preliminary report by the EU Scientific Committees on Consumer Products, on Health and Environmental Risks, and on Emerging and Newly-Identified Health Risks on "Risk assessment methodologies and appproaches for mutagenic and carcinogenic substances"

3.23 The Committee was invited to comment on a preliminary report by the EU Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR).

3.24 The Committee considered the report to be well considered and up-to-date. However, it expressed concern about the discussion of the T25 method, which has been proposed for use in risk assessment. Several organisations no longer support this methodology due to its reliance on the lowest tested dose and lack of consideration of dose response which makes the methodology inherently more variable than the Benchmark Dose Modelling (BMD) approach. Also, the T25 method does not incorporate uncertainty in the analysis of the data. Committee members were concerned that the report suggests that the T25 and BMDL10 are equivalent. Most organisations considered that the BMDL10 was considerably superior to the T25 and that, where it was not possible to determine a BMDL10, it would not be possible to derive an informative T25 . The Committee also criticised the fact that the *post hoc* justification of the uncertainty factor of 10,000 commonly used in the Margin of Exposure (MoE) approach. This justification had never been adopted by the European Food Safety Authority (EFSA) and it was not clear that there was any reference to this specific derivation.

3.25 The Committee considered that the report should refer to the International Programme on Chemical Safety (IPCS) mode of action (MoA) framework since it is critical to understand whether there is likely to be a genotoxic MoA underlying the carcinogenicity of a chemical. It also noted that the text does not reflect the more refined framework for application of the Threshold of Toxicological Concern (TTC) by Kroes *et al* (2004; Food and Chemical Toxicology, Volume 42: pp 65–83). This methodology should not be used indiscriminately and consideration should be given to whether the chemicals under consideration are adequately represented by the database used to develop the TTC approach.

Pyrrolizidine alkaloids in food

3.26 The COC was asked by the COT for advice on the carcinogenicity of pyrrolizidine alkaloids (see paragraphs 1.44 to 1.47). The COC considered data on the mutagenicity and carcinogenicity of seven pyrrolizidine alkaloids (riddelliine, lasiocarpine, clivorine, petastitenine, senkirkine, symphytine and monocrotaline) and on the chemicals dehydroheliotridine and dehydroretronecine, which are the metabolites of many pyrrolizidine alkaloids. The Committee assessed whether the evidence was sufficient to conclude that each of the pyrrolizidine alkaloids had carcinogenic activity. As a general comment, the Committee noted that some of the data was rather old.

3.27 The most data were available for riddelline and lasiocarpine. Riddelliine is positive in a range of *in vitro* and *in vivo* assays for genotoxicity. In a carcinogenicity study conducted by the US National Toxicology Programme (NTP), it induced an increased incidence of liver haemangiosarcomas in both rats and mice, and of alveolar and bronchiolar neoplasms in female mice. The COC concluded that there was good evidence that riddelliine was genotoxic and carcinogenic and that it would be prudent to assume that at least part of its carcinogenic effect was due to a genotoxic mechanism. Lasiocarpine is positive in *in vitro* assays for genotoxicity but has not been tested in *in vivo* studies. In an NTP carcinogenicity study in rats, it induced an increased incidence of liver angiosarcoma in both sexes. In more limited studies in rats by either dietary or parenteral administration, treatment-related angiosarcomas of the liver and liver cell carcinomas were seen. The Committee decided that the database was less extensive than that for riddelliine but, due to the similarities in tumour profiles, concluded that it was also carcinogenic and likely to have a genotoxic mechanism

3.28 Clivorine shows conflicting results in *in vitro* assays for genotoxicity and has not been tested in *in vivo* assays. In a limited rat study in which clivorine was administered in drinking water, it induced an increased incidence of haemangioendothelial sarcoma of the liver. The COC considered that there were not enough *in vivo* data to reach a definite conclusion but clivorine was likely to have carcinogenic properties, based on its structure and limited evidence that it induced the same tumour type as riddelliine and lasiocarpine. Petasitenine is positive in *in vitro* assays for genotoxicity but has not been tested in *in vivo* studies. In a limited rat study in which petasitenine was administered in drinking water, there was a treatment-related increased incidence of liver haemangioendothelial sarcomas and liver cell adenomas. The Committee concluded that petasitenine would be likely to have carcinogenic properties, based on the structure and tumour type induced in the rat study.

3.29 Senkirkine has shown largely positive results in *in vitro* assays for genotoxicity but has not been tested in standard *in vivo* studies. In a limited study in male rats using parenteral administration, there was a treatment-related increased incidence of liver cell adenomas. The Committee concluded that there was insufficient evidence to conclude that senkirkine had carcinogenic activity. No genotoxicity data have been found on symphytine. In a limited study in male rats using parenteral administration, there was a treatment-related increase in liver haemangioendothelial sarcoma and a small treatment-related increase in liver cell adenomas. The COC concluded that it was probable that symphytine had carcinogenic activity based on the structure and the limited evidence that it induced angiosarcomas.

3.30 Monocrotaline has shown conflicting results in *in vitro* assays for genotoxicity and a positive result in one *in vivo* assay. In a limited study in male rats using parenteral administration, there were treatment-related increases in a number of tumours, principally liver cell carcinomas and pulmonary adenocarcinoma. From the available evidence and commonality of structure, the Committee concluded that the data were sufficient to conclude that monocrotaline had carcinogenic activity but with a different tumour profile to the other pyrrolizidine alkaloids. The mode of action was unclear. The metabolite dehydroretronecine has shown positive results in *in vitro* assays for mutagenicity but no genotoxicity data were found for dehydroheliotridine. The Committee found no convincing evidence of carcinogenicity for these metabolites.

3.31 The Committee decided that benchmark dose modelling on riddelliine and lasiocarpine, which had been carried out by the Secretariat, could be used as a basis for a Margin of Exposure approach to the risk assessment of pyrrolizidine alkaloids. A $BMDL_{10}$[7] of 0.073 mg/kg bw/day for lasiocarpine, based on the angiosarcoma incidence in the NTP study, should be used for any Margin of Exposure approach to the risk assessment. The Committee further agreed that a "Cumulative Assessment Group" approach, as described in the opinion of an EFSA Scientific Panel on methodologies for the assessment of cumulative and synergistic risks from pesticides, would be appropriate for pyrrolizidine alkaloids in view of the evidence for a common tumour pattern for several of these comounds.

Revision of OECD Test Guidelines for carcinogenicity studies

3.32 The Organisation for Economic Cooperation and Development (OECD) is currently revising its Test Guidelines for carcinogenicity studies and chronic toxicity studies. The purpose of these guidelines is to enable mutual acceptance of data by different regulatory authorities around the world and hence to reduce costs and use of animals. The OECD is also preparing a Guidance Document to accompany the revised guidelines. The first chapter, on dose selection, was discussed by the Committee at the April meeting when it was also asked whether the UK should offer to draft any of the other planned chapters. Members considered that, although much guidance already exists on histopathology, it would be important to bring it into an OECD context, and recommended that the UK should propose leading on this chapter.

3.33 The COC was also asked for advice on the guidelines in July, when it was informed that one of the principal issues under consideration for the revision of the Test Guideline for carcinogenicity testing (no. 451) is the required duration of the studies, in particular, how to deal with high levels of mortality before scheduled termination of the study. Specifically:

- if there is excess mortality in the high dose group and other treated groups. The Committee commented that this scenario would indicate a seriously flawed study and would recommend abandoning it at that point.

- if there is excess mortality in treated groups other than the high dose group. The Committee advised that there would be concern about study design and technical handling since the deaths would probably not be compound related. They considered that, on balance, it would be better to run the study to completion

- if there is excess mortality in the controls only, or in controls and one or more treated groups. It was noted that what action was taken would depend on how much survival is reduced. The COC recommended the continuation of the study only if the number of surviving animals is similar across the groups. It would be important to establish that the study still had sufficient power to detect effects at the level of concern.

[7] The lower 95% confidence limit on the benchmark dose associated with a 10% response.

3.34 The COC was also asked for comments on a paper by Roth *et al* (2007; Toxicologic Pathology, Volume 35: pp 1040-1043) which discussed excess mortality in two-year rodent carcinogenicity studies. The committee considered that the paper was suited more to testing of pharmaceuticals, where a risk/benefit analysis was required, than to other chemicals for which a hazard identification is needed. The paper was a reasonable qualitative description of potential strategies but failed to justify the details and included many "rules of thumb" of unknown origin. Many relevant issues had not been discussed in the paper.

3.35 The COC also considered whether the wording in the "Duration of Study" section of the 1981 Test Guideline 451 should be revised, and if so, how. The current text states that overall survival should be 50% for a negative study to be acceptable. The COC advised that the wording should be revised but the proposals set out in the Roth paper were not acceptable. The Committee also agreed with the proposal that the normal duration of carcinogenicity studies in mice should be revised to 2 years.

3.36 The Committee considered that the method of analysis to be used should be explicit at the outset and both data analysis and study design should be clearly linked to the primary objective of the study. Therefore, it was important that the key requirements for study design and data analysis were included in the Test Guideline, so that they become obligatory under the Mutual Acceptance of Data agreement and thus avoided rejection of completed studies or the need for duplication.

Horizon scanning

3.37 The COC undertakes "horizon scanning" exercises at regular intervals to identify new and emerging issues which have the potential to impact on public health. A number of topics were identified by the secretariat for consideration by the Committee at the 2008 exercise. From these and Committee members' own proposals, the COC considered that the following topics should be taken forward:

- RNA related effects as mechanism of carcinogenicity
- Endogenous DNA adducts
- Carcinogenic risk of carbon nanotubes
- Carcinogenic risk of exposure to environmental tobacco smoke in childhood
- Possible carcinogenic hazard from dietary insulin-like growth factor 1 (IGF-1)

Ongoing topics

Carcinogenicity of mixtures

3.38 The COC is discussing current developments in the assessment of chemical mixtures with regard to carcinogens and their modes of action. A statement is expected in 2009.

Chemical aetiology of Non-Hodgkin's lymphoma

3.39 Non-Hodgkin's lymphoma is the seventh most common cancer in men and the sixth most common cancer in women in the UK and statistics indicate that the incidence has increased since the 1970s. The COC is reviewing the scientific literature to assess whether there is any convincing evidence that environmental chemicals are responsible for the reported increase in the incidence of Non-Hodgkin's lymphoma.

3.40 A statement is expected in early 2009.

Update review of epidemiological studies on cancer incidence near municipal solid waste incinerators

3.41 The COC published a statement on municipal solid waste incinerators and cancer in 2000. In 2008, the Committee reviewed the results of new epidemiological studies published in the scientific literature since that date.

3.42 A statement will be published in 2009.

Statements of the COC

Second Statement on Chlorinated Drinking Water and Cancer

Introduction

1. In the United Kingdom, North America, and many other places, chlorination has long been an important part of water treatment, intended to ensure that drinking water contains no microbes hazardous to human health. Disinfection of drinking water is fundamental to preventing the spread of waterborne disease, such as cholera.

2. In the mid-1970s, refinements in techniques of chemical analysis resulted in the detection in drinking water of traces of chemicals formed when organic chemicals (such as those which may occur naturally in rivers, lakes, reservoirs and other water sources) are subjected to chlorination. Each of these chlorination byproducts (CBPs) is typically present in drinking water at a concentration below 1 microgram per litre (µg/l). In most supplies, the main CBPs are the four chlorinated and brominated trihalomethanes (THMs, ie chloroform, bromodichloromethane, chlorodibromomethane and bromoform), which may be present at concentrations up to 100 µg/l. However, numerous other CBPs have been identified in drinking water, but many have yet to be characterised.

3. Some CBPs, including some of the THMs, are known to be carcinogenic in laboratory mammals and some are genotoxic in test systems. There have been many epidemiological investigations into the possible association between chlorination of drinking water and cancer in humans and experimental studies of the mutagenicity and carcinogenicity of CBPs. In 1986, the Department of Health Committee on Medical Aspects of Contamination of Air, Soil and Water (CASW) reviewed the data which were then available and concluded that there was no sound reason to conclude that the consumption of the byproducts of chlorination, in drinking water that has been treated and chlorinated according to current practices, increases the risk of cancer in humans. The COC considered further epidemiological studies in 1992 and 1999 and reviewed the animal carcinogenicity data in 1996. In 1996 it concluded that "The ratio between the lowest dose level giving rise to a carcinogenic effect in animals and the likely human exposure level from drinking water for each of the four THMs considered by the Committee was in excess of 10,000. Thus the levels of these THMs in drinking water in the UK are unlikely to provide a carcinogenic risk to humans." In 1999, it concluded that the new epidemiological studies failed to provide persuasive evidence of a consistent relationship between chlorinated drinking water and cancer. The Committee stated: "It remains possible that there may be an association between chlorinated drinking water and cancer which is obscured by problems such as the difficulty of obtaining an adequate estimate of exposure to chlorination by-products, misclassification of source of drinking water (including the use of bottled water), failure to take adequate account of confounding factors (such as smoking status), and errors arising from non-participation of subjects" (1). The COC considered that efforts to minimise exposure to CBPs remain appropriate, providing that they do not compromise the efficiency of disinfection of drinking water.

4. Thirteen further relevant epidemiological papers have been published since the 1999 review. At our July 2007 meeting, we were asked to review these and to advise whether revision of the 1999 statement was required.

New epidemiological studies

5. The 13 new studies were on a range of cancers:

Type of cancer	Reference
Bladder cancer	3, 9, 10, 11, 12, 13
Colorectal cancer	8
Childhood acute lymphoblastic leukaemia (ALL)	5, 6
Adult leukaemia	7
Brain cancer	2
Pancreatic cancer	4

One study (14) examined mortality from a wide range of cancers.

6. Of the original studies, most were either hospital-based or population-based case-control studies. One was a prospective cohort study (9) and one a retrospective ecological study (14). There were one meta-analysis and two pooled analyses of overlapping sets of papers on bladder cancer. Four of the 13 studies were from Canada, with others from the US, France, Italy, Spain and Australia. None was from the UK. We recognise that the levels of and, therefore, exposure to, CBPs may not be the same in other countries as in the UK. Nevertheless, it is important to review these studies to determine whether there is a carcinogenic hazard from CBPs in drinking water.

7. As the Committee noted in 1999, those animal carcinogenicity studies which have been performed on CBPs do not identify any CBP, or group of CBPs, which appears likely to cause cancer at these sites at the concentrations found in drinking water. A number of different surrogates of exposure have been employed in epidemiological studies. In the recent studies, they include:

- Duration of time exposed to chlorinated water
- THM levels (usually total THMs)
- Chlorinated vs. non-chlorinated water source
- Source of water

n some papers, several exposure measures were used, resulting in multiple comparisons, which can influence the number of positive associations reported. Frequently, no historical measurements of THMs were available and estimates had to be made, for example, from information on water sources and history of chlorination treatment. There is also uncertainty about the lifetime estimates of water consumption made in some studies. Different exposure ranges were used, rendering comparisons between studies difficult. Overall, adequate exposure assessment continues to be a major problem with these studies.

8. Most of the new studies have attempted to control for known or suspected risk factors although the extent of control varied from study to study and was in part dependent on the degree to which there are known or suspected risk factors for the cancer under study. Nevertheless, as noted in 1999, where there are positive associations between cancer risk and measures of exposure, they are usually weak and the elevated risks may be within the range of uncertainty arising from possible confounding factors.

Bladder cancer

9. Previous epidemiological studies have suggested associations between bladder cancer and CBPs although the studies reviewed in 1999 were not considered to show any consistent dose-response relationship with estimated exposures to CBPs or THMs. Of the 6 new papers concerning bladder cancer, 3 were pooled analyses or meta-analyses of overlapping sets of papers, most of which we have already considered. The meta-analysis compared individual consumption of chlorinated drinking water and bladder cancer and reported small but statistically significantly elevated combined odds ratios (ORs) for men but not for women (10) [combined OR for ever consumption in men = 1.4, 95% confidence interval (CI) 1.1-1.9; OR for women = 1.2, 95% CI 0.7-1.8]. In the first pooled analysis of 6 case-control studies (3 of which were included in the meta-analysis), the adjusted OR for bladder cancer in men exposed to an average of more than 1 μg/l THM compared to those who had lower or no exposure was 1.24 (95% CI 1.09-1.41). Estimated ORs in men increased with increasing exposure up to 1.50 (95% CI 1.22-1.85) (11). No association was found among women. Additional results from the pooled analysis using different measures of exposure to THMs (total fluid consumption and intake of tap water) found that total fluid consumption was associated with a slightly increased risk of bladder cancer [adjusted OR = 1.08, 95% CI 1.03-1.13 overall for men and women] (12). Tap water consumption was also associated with a slightly increased risk of bladder cancer [adjusted OR/l/day increase overall = 1.10, 95% CI 1.04-1.17], with higher ORs reported in men than women.

10. Using data from a case-control study whose main objective was to assess the carcinogenic risk of ozonation of drinking water, no statistically significant association of bladder cancer was found with various measures of THM exposure (3). When adjusted for duration of exposure to ozonated water, a statistically significant association was found at the highest average levels of THM concentration [OR = 2.99, 95% CI 1.1-8.5] and with cumulative exposure to THM [OR = 3.39, 95% CI 1.2-9.6] but there was no statistically significant trend with exposure levels. A large case-control study reported a statistically significantly increased risk of bladder cancer in men associated with various estimates of CBP exposure including average residential THM level [adjusted OR up to 2.53, 95% CI 1.23-5.20], ingestion of THMs [adjusted OR up to 1.61, 95% CI 1.06-2.44], exposure from showering and bathing [adjusted OR up to 2.01, 95% CI 1.23-3.28] and swimming in pools [ever swimming vs. never swimming: OR =1.62, 95% CI 1.20-2.19] (13). In women, there were no statistically significantly raised risks from showering and bathing [2.26, 95% CI 0.58-8.90] nor from swimming in pools [ever swimming vs. never swimming: OR=1.19, 95% CI 0.30-4.72].

11. Conflicting results were found in two studies which examined the association between frequency of micronuclei in either urinary bladder epithelial cells (9) or exfoliated urothelial cells (13) and measures of THM exposure.

12. We consider that the additional studies provide limited evidence for an association between bladder cancer and exposure to CBPs.

Colon and rectal cancers

13. A number of studies have examined the association between cancer of the colon or rectum and exposure to chlorinated drinking water. A new, well conducted case-control study has been published on these endpoints (8). It found increased risks of colon cancer among males with a number of measures of exposure to THMs. The highest adjusted OR was 2.10 (95% CI 1.21-3.66) for >35 years exposure to >75 µg THM/l compared to <10 years. No significantly increased risks were found for colon cancer in females nor for rectal cancer.

Other sites

14. A study of exposure to drinking water contaminants and childhood ALL found no statistically significant increases in risk with a number of measures of exposure to THMs (5). However, an additional study of a subset of cases found significant interactions between pre- and post-natal exposure to THMs and polymorphisms in the *GSTT1* and *CYP2E1* genes (6). The OR for children with the GSTT1 null genotype exposed to an average total THM level in the postnatal period above 95th percentile was 9.13 (95% CI 1.44-57.82), and that for children with one or more CYP2E*1 alleles and average total THM level in the prenatal period at or above the 75th percentile was 9.75 (95% CI 1.10-86.01). We note that there were only 12 children with one or more CYP2E*1 alleles, most of whom would probably have been heterozygotes, and question the plausibility of such an association being causal. Nevertheless, the finding is of interest. A large case-control study of adult leukaemia cases found an increased risk of chronic myelocytic leukaemia (CML) with increasing years of exposure to several CBP indices but the risk of other leukaemia subtypes was found to decrease with increasing years of exposure to CBP (7).

15. A well-conducted case-control study found a positive, dose-related association in men between measures of exposure to CBPs and brain cancer (glioma) with a significant trend with estimated lifetime average THM concentration [OR for exposure to chlorinated surface water of >40 years = 2.5 (95% CI 1.2-5.0] (2). In contrast, no significant trend was found in women [OR for exposure to chlorinated surface water of >40 years = 0.7 (95% CI 0.3-1.6)].

16. No association was found between pancreatic cancer and increasing CBP levels in a population-based case-control study [OR for the highest THM concentration = 0.90 for men and women combined, 95% CI 0.62-1.33] (4).

17. In a retrospective ecological study which compared the mortality from a wide range of cancers in an area supplied with tap water with high THM levels with rates in an area with low THM levels, overall cancer mortality rates were slightly raised in the high THM area [men: SMR[8] = 1.2, 95% CI 1.1-1.4; women: SMR = 1.1, 95% CI 1.0-1.3] (14). In men, there were raised SMRs for cancers of the stomach [1.7 (1.2-2.5)], lung [1.3 (1.0-1.6)], melanoma [3.8 (1.0-10.5)] and breast [18.4 (1.0- 98.6)]. No individual cancer showed a raised rate in women.

[8] Standardised Mortality Ratio.

Conclusion

18. We have reviewed the new epidemiological studies on chlorinated drinking water and cancer published since 1999. In 1999, the COC concluded that the studies which were reviewed on bladder cancer did not show any consistent dose-response relationship with estimated exposures to CBPs or THMs. We consider that the new studies on bladder cancer, which include a meta-analysis and two pooled analyses by the same group, provide limited evidence for an association between bladder cancer and exposure to CBPs in men. The evidence for an association in women is conflicting.

19. In the 1999 review, the COC commented that the studies of colorectal cancer gave inconsistent findings. In the current review, one well-conducted study provides some evidence for an association with colon cancer, but not rectal cancer, in men only. In 1999, the COC did not consider the studies of other sites to be of good quality or to produce consistent associations. One new, well-conducted study has indicated an association with brain cancer in men but not in women.

20. Problems remain in the interpretation of published studies on CBPs. These include the small relative risks recorded, the possibility of residual confounding, and the problems with exposure assessment described above. There is no obvious reason why positive associations should be seen so frequently in men but not in women. There is always concern that publication may be biased in favour of positive results, as it may in any field of science. Moreover, as previously stated, none of the studies we have reviewed were carried out in the UK and it is possible that disinfection practices and constituents of the raw water may be different in other countries, in which case the study results may not be directly applicable to the UK.

21. We conclude that the evidence for a causal association between cancer and exposure to CBPs is limited and any such association is unlikely to be strong. Efforts to minimise CBPs in drinking water should continue but must be balanced against the need for effective disinfection of drinking water.

COC/08/S1
May 2008

References

1. Chlorinated drinking water and cancer. COC statement COC/99/S2 - May 1999. (http://www.iacoc.org.uk/statements/chlorinateddrinkingwaterandcancercoc99s2May1992.htm)

2. Cantor KP, Lynch CF, Hildesheim ME, Dosemeci M, Lubin J, Alavanja M, Craun G. Drinking water source and chlorination byproducts in Iowa. III. Risk of brain cancer. Am J Epidemiol 1999; 150(6):552-560.

3. Chevrier C, Junod B, Cordier S. Does ozonation of drinking water reduce the risk of bladder cancer? Epidemiology 2004; 15(5):605-614.

4. Do MT, Birkett NJ, Johnson KC, Krewski D, Villeneuve P; Canadian Cancer Registries Epidemiology Research Group. Chlorination disinfection by-products and pancreatic cancer risk. Environ Health Perspect 2005; 113(4):418-424.

5. Infante-Rivard C, Olson E, Jacques L, Ayotte P. Drinking water contaminants and childhood leukemia. Epidemiology 2001; 12(1):13-19.

6. Infante-Rivard C, Amre D, Sinnett D. GSTT1 and CYP2E1 polymorphisms and trihalomethanes in drinking water: effect on childhood leukemia. Environ Health Perspect 2002 Jun;110(6):591-593.

7. Kasim K, Levallois P, Johnson KC, Abdous B, Auger P. Canadian Cancer Registries Epidemiology Research Group. Chlorination disinfection by-products in drinking water and the risk of adult leukemia in Canada. Am J Epidemiol 2006; 163(2):116-126.

8. King WD, Marrett LD, Woolcott CG. Case-control study of colon and rectal cancers and chlorination by-products in treated water. Cancer Epidemiol Biomarkers Prev 2000 9(8):813-818.

9. Ranmuthugala G, Pilotto L, Smith W, Vimalasiri T, Dear K, Douglas R. Chlorinated drinking water and micronuclei in urinary bladder epithelial cells. Epidemiology 2003 14(5):617-22.

10. Villanueva CM, Fernandez F, Malats N, Grimalt JO, Kogevinas M. Meta-analysis of studies on individual consumption of chlorinated drinking water and bladder cancer. J Epidemiol Community Health 2003; 57(3):166-173. Correction at J Epidemiol Community Health 2005; 59: 87.

11. Villanueva CM, Cantor KP, Cordier S, Jaakkola JJ, King WD, Lynch CF, Porru S, Kogevinas M. Disinfection byproducts and bladder cancer: a pooled analysis. Epidemiology 2004; 15(3):357-367.

12. Villanueva CM, Cantor KP, King WD, Jaakkola JJ, Cordier S, Lynch CF, Porru S, Kogevinas M. Total and specific fluid consumption as determinants of bladder cancer risk. Int J Cancer 2006; 118(8):2040-2047.

13. Villanueva CM, Cantor KP, Grimalt JO, Malats N, Silverman D, Tardon A, Garcia-Closas R, Serra C, Carrato A, Castano-Vinyals G, Marcos R, Rothman N, Real FX, Dosemeci M, Kogevinas M. Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering, and swimming in pools. Am J Epidemiol 2007; 165(2):148-156.

14. Vinceti M, Fantuzzi G, Monici L, Cassinadri M, Predieri G, Aggazzotti G. A retrospective cohort study of trihalomethane exposure through drinking water and cancer mortality in northern Italy. Sci Total Environ 2004; 330(1-3):47-53.

Statement on Betel Quid, Pan Masala and Areca Nut Chewing

Introduction

1. Areca nut is an ingredient of betel quid or pan masala which is chewed as an aid to digestion and as a stimulant. Areca nut may have limited use as a food ingredient. The COC first considered the carcinogenicity of areca nut in 1993 and 1994. Following the publication of a number of new, relevant papers, the COC was asked to look at this subject again in November 2006.

Background

2. Areca nut is widely used in Asian immigrant populations in the UK and other countries in Europe (Warnakulasuriya, 2002). Areca nut is primarily used as an ingredient of betel quid, which is made up of areca nut mixed with slaked lime (calcium hydroxide) and catechu[9], wrapped in a betel leaf (Piper betel). The betel quid is usually chewed for between five minutes and an hour or maybe more. Traditional use varies between countries; sometimes tobacco is added to the quid and sometimes spices such as cardamom or ginger. Recently, pan masala has become a popular alternative to betel quid. This is a pre-prepared mixture containing the same ingredients as the betel quid, but is not wrapped in a betel leaf. Betel quid and pan masala are chewed to aid digestion and for their stimulatory effects. The juice is often spat out but sometimes it is swallowed. Once chewed, the fibrous remains of the quid are also normally spat out (Zain *et al*, 1999).

3. Whole areca nuts can be bought in some supermarkets in the UK but more commonly they are bought in shops stocking traditional Asian foods. It is thought that these are generally used to prepare betel quid at home, but it has also been suggested that they may be used to add flavour when cooking by adding grated or sliced nut to food. However, it has not been possible to substantiate this use.

4. Under the terms of The Medicines (Retail Sale or Supply of Herbal Remedies) Order 1977 part 1, Areca catechu (the botanical source of areca nut) is considered to be a medicinal plant and any substance derived from it should only be sold on licensed premises (a registered pharmacy or where a pharmacist is present) (HMSO, 1977). However betel quid is not considered medicinal by the Medicines and Healthcare Products Regulatory Agency (MHRA) and is therefore covered by food law.

Constituents and metabolism of betel quid ingredients

5. Areca nuts may be used ripe or unripe and may be processed by being sun-dried and/or cured. Uncured areca nut is reported to contain 11.4- 26% tannins and among the polyphenols identified are leucocyanadins, catechins, 3,4-flavandiols and hexahydroxyflavan. The main pharmacological action of areca nuts is attributed to the alkaloids arecoline, arecaidine, guvacine, guvacoline and arecolidine, which make up 0.15-0.67% of uncured nuts (Awang, 1986). *in vitro* experiments suggest that nitrosation of arecoline occurs readily, giving rise to at least four N-nitrosocompounds: N-nitrosoguvacoline

[9] An extract of wood from a variety of acacia species but can also be obtained from the leaves and bark of other plants.

(NGCO), N-nitrosoguvacine (NGCI), 3-(methylnitrosamino)proprionitrile (MNPN) and 3-(methylnitrosamino)proprionaldehyde (MNPA). Several of these N-nitroso compounds have been detected in the saliva of betel quid chewers (Wenke and Hoffmann, (1983), Nair *et al*, (1985), Nair *et al* (1987)).

6. Among their other components, the mature green leaves of Piper betel contain volatile oils including eugenol, chavicol, terpenes, and tannins.

7. Catechu is the residue of a hot water-extraction of the heartwood of Acacia catechu (also see note 1). It contains mainly tannin and polyphenols, including catechutannic acid, catechin, catechu red, quercetin, kaempferol, dihydroxycaempferol, taxifolin, isorhamnetin, (+) afzechin and dimeric procyanidin (IARC, 1985).

8. Metabolic studies suggest that arecoline is de-esterified in the liver and both arecoline and arecaidine are excreted in the urine as the mercapturic acid N-acetyl-S-(3-carboxyl-1-methylpiperid-4-yl)-L-cysteine. NGCO and NGCI are metabolised in the liver to N-nitrosonipectoic acid. This is largely excreted in the urine, though faecal excretion also occurs (IARC, 1985).

Previous COC advice

9. The Committee reviewed the use of areca nut in betel quid and pan masala in 1993 and 1994 and considered a range of human epidemiology studies, animal carcinogenicity studies and *in vivo* and *in vitro* mutagenicity studies[10]. On the basis of this evidence, the COC concluded the following:

   - There was evidence of mutagenic and carcinogenic activity of areca nut extracts and derived compounds in experimental systems. In particular, the potent carcinogenic activity of the areca-derived nitrosamine, MNPN had been confirmed, and methyl and cyanoethyl adducts had been detected in the DNA of the target tissues in which the tumours developed. There was evidence that endogenous nitrosation of areca nut alkaloids can occur in animals and humans; and areca nut derived nitrosoamines, including MNPN, have been detected in the saliva of betel quid chewers.

   - There were very limited data from epidemiological studies on the effect of betel or areca nut products without tobacco, which did not allow any conclusion to be drawn. There was, however, sufficient epidemiological evidence of a link between the chewing of betel quid containing tobacco and cancer in humans.

   - The Committee concluded that the use of these products without tobacco was possibly carcinogenic in humans.

Considerations by other expert bodies

10. The International Agency for Research on Cancer (IARC) assessed the use of betel quid in 2003 and concluded that both chewing of betel quid and areca nut should be categorised as Group 1 (known) human carcinogens. In its conclusions it stated that there is sufficient evidence in humans to conclude

[10] The conclusions can be found in the 1994 report of the Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (HMSO, 1994).

that betel quid chewed without tobacco causes oral cancer. IARC also concluded that there was sufficient evidence in animals to confirm the carcinogenicity of betel quid and areca nut without tobacco (IARC, 2003). The 2003 report followed up a previous review from 1985 in which it was concluded that there was inadequate evidence for the carcinogenicity of betel quid chewed without tobacco.

Studies published since 1993

11. Most of the epidemiological data available concerns the use of areca nut in betel quid or pan masala and the link with oral cancers and pre-cancerous lesions. The epidemiological studies are summarised in Table 1.

12. Other epidemiological data have linked the use of areca nut with other cancers such as liver cancers. There are also some limited animal studies and *in vitro* studies. The studies linking betel quid use with liver cancer have been summarised in Table 2.

Discussion

13. The Committee considered the data that had been published since its previous discussion and agreed with the recent IARC conclusions, as given above. The Committee considered that most of the evidence suggested that areca nut is a site of contact carcinogen acting by a genotoxic mechanism, and noted that there was some evidence that a non-genotoxic mechanism could also be involved. It seems likely that the carcinogenic mechanism involved nitrosation of the alkaloids present in the areca nut, which form N-nitroso products that can be detected in saliva. The Committee noted that these have been demonstrated to have mutagenic activity *in vitro*. Most of the available data indicates that areca nut and betel quid cause cancer of the oral cavity where the quid can be held for significant amounts of time.

14. The Committee concluded that, although there was insufficient evidence to definitively link the use of areca nut as a food ingredient with an increased incidence of cancer, this use should be regarded as potentially carcinogenic. The studies reviewed did not appear to show variations in cancer incidence in populations using differently prepared areca nut products; this and the possible genotoxic mode of activity led the Committee to conclude that areca nut may be carcinogenic in all forms.

Summary

15. The Committee was satisfied that there was sufficient epidemiological evidence to conclude that areca nut, when used in the form of betel quid or pan masala, is carcinogenic to humans. This relates primarily to an increased risk of oral cancers from the keeping the areca nut or betel quid in the mouth for a significant length of time. Members also considered that the use of areca nut as a food ingredient may also result in an increased risk of cancer.

COC/08/S2
July 2008

References

Awang MN (1986) Estimation of arecoline contents in commercial areca (betel) nuts and its relation to oral precancerous lesions, Singapore MJ 27 (4): 317-320

Chitra S, Ashok L, Anand L, Srinivasan V, Jayanthi V. Risk factors for esophageal cancer in Coimbatore, southern India : a hospital-based case-control study. Indian J Gastroenterol [serial online] 2004 [cited 2006 Aug 29];23:19-21. Available from: http://www.indianjgastro.com/article.asp?issn=0254-8860;year=2004;volume=23;issue=1;spage=19;epage=21;aulast=Chitra

Hasibe M., Mathew B., Kuruvilla B., Thomas G., Sankaranarayanan R., Maxwell Parkin D., Zhang Z.-F., (2000) Chewing Tobacco, Alcohol and the Risk of Erythroplakia. Cancer Epidemiology, Biomarkers and Prevention 9: 639-645

HMSO (1994) Annual report from the Committees on Toxicity, Mutagenicity, Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO, London, 1995

HMSO The Medicines (Retail Sale or Supply of Herbal Remedies) Order 1977 (SI 2130)

IARC (2003) http://monographs.iarc.fr/ENG/Monographs/vol85/volume85.pdf

IARC (2003) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Volume 85 Betel Quid and Areca-nut Chewing and Some Areca-nut derived Nitrosamines. Lyon, France. WHO Publication.

IARC (1985) Monographs on the Carcinogenic risk of Chemicals to Humans. Vol 7. Tobacco habits other than Smoking; Betel Quid and Areca Nut Chewing and some related Nitrosamines. IARC Lyon.

Ko Y.C., Huang Y.L., Lee C.H., Chen M.J., Lin L.M., Tsai C.C. (1995) Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan J Oral Pathol Med 24: 450-3

Lee C.-H., Ko Y.-C., Huang H.-L., Chao Y.-Y., Tsai C.-C., Shieh T.-Y. and Lin L.-M. (2003) The precancer risk of betel quid chewing, tobacco use and alcohol consumption in oral leukoplakia and oral submucous fibrosis in souther Taiwan. British Journal of Cancer 88 : 366-372

Merchant A., Husain S. S. M., Hosain M., Fikree F. F., Pitiphat W., Siddiqui A. R., Hayder S. J., Haider S. M., Ikram M., Chuang S-K., Saeed S. A., (2000) Paan without tobacco: an independent risk factor for oral cancer Int J Cancer 86 128-131

Nair J., Nair U.J., Ohshima H., Bhide S.V., Bartsch H. (1987) Endogenous nitrosation in the oral cavity of chewers while chewing betel quid with or without tobacco. IARC Sci Publ. (84) : 465-9.

Nair J., Ohshima H., Friesen M., Croisy A., Bhide SV., Bartsch H. (1985) Tobacco-specific and betel nut-specific N-nitroso compounds: occurrence in saliva and urine of betel quid chewers and formation *in vitro* by nitrosation of betel quid. Carcinogenesis. 6 (2) : 295-303.

Ranganathan K., Uma Devi M., Joshua K., Kirankumar K., Saraswathi T.R., (2004) Oral Submucous fibrosis: a case controlled study in Chennai, South India. J Oral Pathol Med 33:274-7

Sankaranarayanan R., Suffy S.W., Day N.E., Nair K., Padmakumary G. (1989) A case control investigation of cancer of the oral tongue and the floor of the mouth in Southern India. Int J Cancer 44 : 617-621.

Srivatanakul P., Parkin D.M., Khlat M., Chenvidhya D., Chotiwan P., Insiripong S., L'Abbe K.A. and Wild C.P. (1991) Liver Cancer in Thailand. II. A case control study of hepatocellular carcinoma. Int J Cancer 48, 329-332

Tsai J.F., Chuang L.Y., Jeng J.E., Ho M.S., Hsieh M.Y., Lin Z.Y., Wang L.Y., (2001) Betel quid chewing as a risk factor for hepatocellular carcinoma: a case control study 84, 709-713

Tsai J-F., Jeng J-E., Chuang L-Y., Ho M-S., Ko Y-C., Lin Z-Y, Hsieh M-Y., Chen S-C., Chuang W-L., Wang L-Y., Yu M-L., Dai C-Y., (2004) Habitual betel quid chewing and risk for hepatocellular carcinoma complicating cirrhosis. Medicine 83: 3 176-187.

Warnakulasuriya K.A.A.S., Johnson N.W., Linklater K.M., Bell, J. (1999) Cancer of mouth, pharynx and nasopharynx in Asians and Chinese immigrants resident in the Thames region. Oral Oncology 35, 471-475.

Warnakulasuriya S., Trivedy C., Peters T., (2002) Areca nut use: an independent risk factor for oral cancer 324: 799-800

Wenke G. and Hoffmann D. (1983) A study of betel quid carcinogenesis. 1. On the *in vitro* N-nitrosation of arecoline, Carcinogenesis 4 (2) 169-172

Zain R.B., Ikeda N., Gupta P.C., Warnakulasuriya, K.A.A.S., van Wyk C.W., Shrestha P., Axell T., (1999) Oral mucosal lesions associated with betel quid, areca nut and tobacco chewing habits: consensus from a workshop held in Kuala Lumpur, Malaysia, November 25-27. J Oral Pathol Med 28: 1-4.

Znaor A., Brennan P., Gajalakshmi V., Mathem A., Shanta V., Varghese C. and Boffetta P., (2003) Independent and combined effects of tobacco smoking, chewing and alcohol drinking on the risk of oral, pharyngeal and esophageal cancers in Indian men. Int. J Cancer 105 : 681-686.

Table 1: Human epidemiological studies on areca nut and betel quid usage and the link to oral cancers.

Study	Cases (No.)	Controls (No.)	Cancer types	Published results
Sankaranarayanan *et al* (1989)	228	456 (age and sex matched)	Carcinoma: Tongue (188) Buccal floor (40)	Regular use of betel quid with tobacco was associated with an increase in the incidence of the two types of cancer studied.
Ko *et al* (1995)	107 (m=104, f=3)	194 (age and sex matched)	Oral carcinoma	Compared with abstainers, betel quid chewers had an OR of 8.5, CI of 4.4-16.2 of developing oral cancer.
Warnakulasuriya *et al* (1999)	7521 (m=5072, f=2449)	-	Lip, mouth, pharynx, nasal cavity, larynx, bronchus	Age of onset was significantly lower in Chinese and Asian populations than in other groups
Merchant *et al* (2000)	79	149 (age, sex, hospital and time of attendance matched)	Oral squamous cell carcinoma, OSF1	After adjustment individuals with OSF were 19.1 times more likely to develop oral cancer than those without and individuals using betel quid without tobacco were 9.9 times more likely to develop oral cancer than non-users. Risk of oral cancer increased with higher intakes of betel quid with and without tobacco.
Hasibe *et al* (2000)	100	47773	Erythroplakia	Current chewing habits were 74.5% and 83.7% amongst male and female cases respectively. The adjusted odds ratio for regular chewers was 19.8 (95% confidence interval 9.8-40) after adjustment.
Znaor *et al* (2003)	1563 oral, 636 pharyngeal 566 oesophageal. All male.	1711 male patients with non-tobacco related cancers and 1927 healthy male hospital visitors.	Oral (lip, tongue mouth), pharyngeal (oropharynx, hypopharynx and pharynx), oesophageal	An increased risk for oral cancers of over 2 fold and a 60% increased risk for oesophageal cancers were observed among chewers without tobacco. Among chewers with tobacco, the increase in risk was 5-fold.for oral cancers and 2-fold for pharyngeal and oesophageal cancers.

Table 1: Human epidemiological studies on areca nut and betel quid usage and the link to oral cancers. *continued*

Study	Cases (No.)	Controls (No.)	Cancer types	Published results
Lee *et al* (2003)	219	876 (age and sex matched)	OL[2] (125 patients) and OSF1 (94 patients)	The risk of developing the two preneoplastic lesions studied was 22.3-40.7 fold higher in regular chewers of betel quid than in those who had never chewed. Ex-chewers were 7.1-12.1 times more likely to develop these lesions than non-chewers. Of the factors investigated, betel quid was found to be the strongest risk factor for OSF and OL.
Ranganathan *et al* (2004)	185	185 (age and sex matched)	OSF[1]	All OSF patients in this study had a history of chewing betel quid. Only low levels of areca nut use (in all forms) was seen in the control group, but higher use of alcohol and tobacco smoking was observed.
Chitra *et al* (2004)	90	90 (age and sex matched)	Squamous cell carcinoma of the oesophagus	Odds ratio between cases and controls for areca nut usage was 2.8 with a CI of 1.3-5.9.

1 Oral sub-mucous fibrosis – a pre-cancerous condition shown to precede oral cancer and manifests itself through discolouration of the oral mucosa and decreased flexibility of the cheeks.

2 Oral leukoplakia – A pre-cancerous lesion

Table 2: Human epidemiological studies on areca nut and betel quid usage and the link to other cancers.

Study	Cases (No.)	Controls (No.) (if applicable)	Cancer types	Published results
Srivatanakul *et al* (1991)	65 (47 M & 18 F)	65 (age and sex matched)	HCC[a]	The study suggests that regular use of betel quid conferred a higher risk of HCC although this was not statistically significant.
Tsai *et al* (2001)	263	263 (age and sex matched)	HCC[a]	The authors concluded that there was an association between regular betel quid use and HCC after controlling for confounding factors such as cirrhosis. A weak synergistic relationship was proposed between betel quid use and hepatitis B and C virus and the development of HCC.
Tsai *et al* (2004)	210	420 (210 patients with cirrhosis and 210 healthy controls) (age and sex matched)	HCC[a] complicating cirrhosis	Betel quid chewing was found to be an independent risk factor for HCC in patients with chronic viral infections. Evidence of a link in otherwise healthy patients was very weak and the authors concluded that betel quid was not a risk factor for HCC complicating cirrhosis in subjects without chronic viral hepatitis.

[a] Hepatocellular Carcinoma

2008 Membership of the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

CHAIR

Professor David H Phillips BA PhD DSc FRCPath
Professor of Environmental Carcinogenesis, Institute of Cancer Research

MEMBERS

Dr Carolyn Allen BSc MSc PhD
Non-specialist Member

Professor Alan Boobis OBE BSc PhD CBiol FIBiol
Section of Experimental Medicine and Toxicology, Division of Medicine, Imperial College London

Dr Philip Carthew BSc MSc PhD FRCPath
Senior Pathologist, SEAC Toxicology Unit, Unilever

Professor Peter B Farmer MA DPhil CChem FRSC
Professor of Biochemistry, Cancer Studies and Molecular Medicine, Cancer Biomarkers and Prevention Group, Biocentre, University of Leicester

Mrs Rosie Glazebrook MA
Non-specialist Member

Professor David Harrison BSc MB ChB MD FRCPath FRCP(Edin) FRCS(Edin)
Professor and Head of Department of Pathology, Director, University of Edinburgh Cancer Research Centre

Ms Denise M Howel BSc MSc CStat FIS
Senior Lecturer in Epidemiological Statistics, Institute of Health and Society, Newcastle University

Dr Brian G Miller BSc PhD CStat
Director of Research Operations, Institute of Occupational Medicine

Professor Ruth A Roberts BSc PhD ATS FBTS
Director of Toxicology, Safety Assessment UK, AstraZeneca

Professor David E G Shuker BSc ARCS PhD DIC CChem FRSC
Professor of Organic Chemistry, The Open University

Professor Paolo Vineis MD PhD
Professor of Environmental Epidemiology, Department of Epidemiology and Public Health, Imperial College London

Dr Nicola Wallis BSc MBChB FRCPath MFPM
Safety and Risk Management, Pfizer Global Research & Development

SECRETARIAT

Ms F Pollitt MA DipRCPath	*Joint Scientific Secretary – Health Protection Agency*
Dr D Benford BSc PhD	*Joint Scientific Secretary – Food Standards Agency*
Mr J Battershill BSc MSc	*Scientific – Health Protection Agency*
Dr L Hetherington BSc PhD	*Scientific – Health Protection Agency*
Mr S Robjohns BSc MSc	*Scientific – Health Protection Agency*
Ms S Kennedy	*Administrative Secretary – Health Protection Agency*

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

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor D H Phillips (Chair)	Aviva Banco Santander BG Group Bradford & Bingley Centrica National Grid Servier Buller Jeffries (Solicitors)	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Honorarium Honorarium	NONE	NONE
Dr C Allen	NONE	NONE	NONE	NONE
Professor A Boobis OBE	Banco Santander Barclays Bank BG Group BT Group Centrica Halifax National Grid Transco Scottish Power Astellas Pharma Sumitomo Chemical (UK) PLC Howrey LLP	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Consultancy	GlaxoSmithKline FSA Department of Health ILSI HESI Elsevier JMPR JECFA (vet drugs) EFSA PPR	Support by Industry Research Contract Unpaid member of Board of Trustees Editor-in-Chief Food & Chemical Toxicology Member
Dr P Carthew	Unilever	Salary	NONE	NONE
Professor P B Farmer	Banco Santander Bradford & Bingley Foreign & Colonial Friends Provident Health Effects Institute Torotrak ILSI HESI	Shareholder Shareholder Shareholder Shareholder Research Committee Member Shareholder Committee Member	American Chemistry Council CEFIC	Research support and Conference attendance expenses. Research Support

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Mrs R Glazebrook	BT Group Lloyds TSB National Grid	Shareholder Shareholder Shareholder	NONE	NONE
Professor D Harrison	The Forensic Institute University of Edinburgh Lothian NHS Response Genetics (University consultancy no fee payable.) University of Florida (University consultancy) University of Canberra (University consultancy)	Shareholder	PI Medical Research (Scotland) Chair EMMS Nazareth Member Scientific Advisory Committee, Yorkshire Cancer Research	Non specific research funding from Cancer Research UK. Breakthrough, CSO & other grant agencies. Trusteee Trustee (Healthcare Charity) Trustee
Ms D Howel	NONE	NONE	NONE	NONE
Dr B G Miller	Scottish Power	Shareholder	NONE	NONE
Professor R A Roberts	AstraZeneca HBOS P & O	Salary Shareholder Shareholder	NONE	NONE
Professor D E G Shuker	NONE	NONE	NONE	NONE
Dr P Vineis	NONE	NONE	NONE	NONE
Dr N Wallis	Pfizer	Salary Shareholder	NONE	NONE

ANNEX 1 - Terms of Reference

To advise at the request of:

Food Standards Agency
Health Protection Agency
Department of Health
Department for Business, Enterprise & Regulatory Reform
Department of Transport, Local Government and the Regions
Department of Trade and Industry
Health and Safety Executive
Pesticide Safety Directorate
Veterinary Medicines Directorate
Medicines and Healthcare Products Regulatory Agency
Home Office
Scottish Executive
National Assembly for Wales
Northern Ireland Assembly
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 and 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 307);
- 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 Chairman 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.

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 included below.

(i) Declaration of Interests to the Secretariat

Members of the Committee should inform the Secretariat in writing of their current personal and non-personal interests, when they are appointed, including the principal position(s) held. Only the name of the company and the nature of the interest are required; the amount of any salary etc. need not be disclosed. An interest is current if the member has an on-going financial involvement with industry, e.g. 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 in Discussions at Meetings and by Correspondence

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, and if items are taken by correspondence between meetings. The declaration should note whether the interest is personal or non-personal, whether it is specific to the item under discussion, or non-specific (see below) and whether it is current or lapsed. Having fully explained the nature of their interest the Chairman will, decide whether and to what extent the member should participate in the discussion and determination of the issue. If it is decided that the member should leave the meeting, the Chairman may first allow them to make a statement on the item under discussion.

Personal liability of Committee members

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

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

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. **Members' interests must be declared/confirmed annually on the declaration of interests form to the Committee Secretariat**.

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;
- **Membership or Affiliation**: any membership role or affiliation that you or a close family member has to clubs or organisations with an interest or involvement in the work of the Department.

Non-Personal Interests

A non-personal interest involves payment which benefits the organisation in which the member works, 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 organisation, e.g.

  i) a grant for the running of a unit or department;

  ii) a grant or fellowship or other payment to sponsor a post or a member of staff or a post graduate research programme. 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 in which they work, 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.

Specific Interests

A member must declare a personal, specific interest if they have at any time worked on a matter, product or substance under consideration and have personally received payment for that work, in any form.

A member must declare a non-personal, specific interest if they are aware that the organisation in which they work has at any time worked on the matter, product or substance under consideration but they have not personally received payment for that work, in any form.

Non-specific Interests

A member must declare a personal non-specific interest if they have a current personal interest in a company concerned with a matter, product or substance under consideration, which does not relate specifically to the matter, product or substance under discussion.

A member must declare a non-personal non-specific interest if they are aware that the organisation in which they work is currently receiving payment from the company concerned which does not relate specifically to the matter, product or substance under discussion.

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

Definitions

In this Code, 'the industry' means:

- Companies, partnerships or individuals who are involved with the production, manufacture, sale or supply of products subject to the following legislation;

  The Food Safety Act 1990

  The Medicines Acts 1968 and 1971

  The Food and Environmental Protection Act 1985

  The Consumer Protection Act 1987

  The Cosmetic (Safety) (Amendment) Regulations 1987

The Notification of New Substances Regulations 1982

- Trade associations representing companies involved with such products;
- Companies, partnerships or individuals who are directly concerned with research, development or marketing of a product which is being considered by the Committees on Toxicity, Mutagenicity, or Carcinogenicity of Chemicals in Food, Consumer Products and the Environment.

In this Code 'the Secretariat' means the Secretariat of the COC, COM and 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 page 314.

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, i.e. 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 (e.g. papers and minutes) confidential until an agreed opinion (e.g. statement) is available.

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

* Procedures for handling confidential information are outlined overleaf.

Summary of proposals for committee openness

Procedures for handling confidential information

Background

1 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 assessment.

2 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.

3 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

4 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 (e.g. 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 (e.g. 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 (e.g. 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 the paragraphs 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 – Good Practice Agreement for Scientific Advisory Committees

Introduction

1 *Guidelines 2000: Scientific Advice and Policy Making*[1] set out the basic principles which government departments should follow in assembling and using scientific advice, thus:

- think ahead, identifying the issues where scientific advice is needed at an early stage;
- get a wide range of advice from the best sources, particularly where there is scientific uncertainty; and
- publish the scientific advice they receive and all the relevant papers.

2 The *Code of Practice for Scientific Advisory Committees*[2] (revised in December 2007) provided more detailed guidance specifically focused on the operation of scientific advisory committees (SACs). The Agency subsequently commissioned a *Report on the Review of Scientific Committees*[3] to ensure that the operation of its various advisory committees was consistent with the remit and values of the Agency, as well as the Code of Practice.

3 The Food Standards Agency's Board has adopted a **Science Checklist** to make explicit the points to be considered in the preparation of papers dealing with science-based issues which are either assembled by the Executive or which draw on advice from the Scientific Advisory Committees.

4 Scientists who serve on a scientific advisory committee which advises the Agency are expected to comply with the **Universal Ethical Code for Scientist**, launched by the Government's Chief Scientific Adviser in March 2007.

5 The Board welcomed a proposal from the Chairs of the independent SACs to draw up Good Practice Guidelines based on, and complementing, the Science Checklist.

6 These Guidelines have been developed by nine advisory committees:

Advisory Committee on Animal Feedingstuffs[4]
Advisory Committee on Microbiological Safety of Foods
Advisory Committee on Novel Foods and Processes
Advisory Committee on Research (disbanded in 2007)
Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment[5]
Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment[6]
Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment[7]
Scientific Advisory Committee on Nutrition[8]
Spongiform Encephalopathy Advisory Committee[9]

[1] Guidelines on Scientific Analysis in Policy Making, OST, October 2005. Guidelines 2000: Scientific advice and policy-making. OST July 2000
[2] Code of Practice for Scientific Advisory Committees, OST December 2001
[3] Report on the Review of Scientific Committees, FSA, March 2002
[4] Joint FSA/Defra Secretariat, FSA lead
[5] Joint FSA/HPA Secretariat, HPA lead
[6] Joint FSA/HPA Secretariat, HPA lead
[7] Joint FSA/HPA, FSA lead
[8] Joint FSA/DH Secretariat
[9] Joint Defra/FSA/DH Secretariat

7 These committees share important characteristics. They:

- are independent;
- work in an open and transparent way; and
- are concerned with risk assessment not risk management.

8 The Guidelines relate primarily to the risk assessment process since this is the committees' purpose. However, the Agency may wish on occasion to ask the independent scientific advisory committees whether a particular risk management option is consistent with their risk assessment.

9 Twenty seven principles of good practice have been developed. However, the different committees have different duties and discharge those duties in different ways. Therefore, not all of the principles set out below will be applicable to all of the committees, all of the time.

10 This list of principles will be reconsidered by each committee annually as part of the preparation of its Annual report, and will be attached as an Annex to it.

Principles

Defining the issue

1 The FSA will ensure that the issue to be addressed is clearly defined and takes account of stakeholder expectations. The committee Chair will refer back to the Agency if discussion suggests that a re-definition is necessary.

Seeking input

2 The Secretariat will ensure that stakeholders are consulted at appropriate points in the committee's considerations and, wherever possible, SAC discussions should be held in public.

3 The scope of literature searches made on behalf of the committee will be clearly set out.

4 Steps will be taken to ensure that all available and relevant scientific evidence is rigorously considered by the committee, including consulting external/additional scientific experts who may know of relevant unpublished or pre-publication data.

5 Data from stakeholders will be considered and weighted according to quality by the committee.

6 Consideration by the secretariat and the Chair will be given to whether expertise in other disciplines will be needed.

7 Consideration will be given by the Secretariat or by the committee to whether other scientific advisory committees need to be consulted.

Validation

8 Study design, methods of measurement and the way that analysis of data has been carried out will be assessed by the committee.

9 If qualitative data have been used, they will be assessed by the committee in accordance with the principles of good practice, e.g. set out in guidance from the Government's Chief Social Researcher[10].

10 Formal statistical analyses will be included wherever possible. To support this, each committee will have access to advice on quantitative analysis and modelling as needed.

11 When considering what evidence needs to be collected for assessment, the following points will be considered:

- the potential for the need for different data for different parts of the UK or
- the relevance to the UK situation for any data originating outside the UK; and
- whether stakeholders can provide unpublished data.

12 The list of references will make it clear which references have either not been subject to peer review or where evaluation by the committee itself has conducted the peer review.

Uncertainty

13 When reporting outcomes, committees will make explicit the level and type of uncertainty (both limitations on the quality of the available data and lack of knowledge) associated with their advice.

14 Any assumptions made by the committee will be clearly spelled out, and, in reviews, previous assumptions will be challenged.

15 Data gaps will be identified and their impact on uncertainty assessed by the committee.

16 An indication will be given by the committee about whether the database is changing or static.

Drawing conclusions

17 The committee will be broad-minded, acknowledging where conflicting views exist and considering whether alternative hypotheses fit the same evidence.

18 Where both risks and benefits have been considered, the committee will address each with the same rigour.

19 Committee decisions will include an explanation of where differences of opinion have arisen during discussions, specifically where there are unresolved issues and why conclusions have been reached.

[10] There is of guidance issued under the auspices of the Government's Social Research Unit and the Chief Social Researcher's Office (Quality in Qualitative Evaluation: A Framework for assessing research evidence. August 2003. www.strategy.gov.uk/downloads/su/qual/downloads/qqe-rep.pdf and The Magenta Book. www.gsr.gov.uk/professional_guidance/magenta_book/guidance.asp).

20 The committee's interpretation of results, recommended actions or advice will be consistent with the quantitative and/or qualitative evidence and the degree of uncertainty associated with it.

21 Committees will make recommendations about general issues that may have relevance for other committees.

Communicating committees' conclusions

22 Conclusions will be expressed by the committee in clear, simple terms and use the minimum caveats consistent with accuracy.

23 It will be made clear by the committee where assessments have been based on the work of other bodies and where the committee has started afresh, and there will be a clear statement of how the current conclusions compare with previous assessments.

24 The conclusions will be supported by a statement about their robustness and the extent to which judgement has had to be used.

25 As standard practice, the committee secretariat will publish a full set of references (including the data used as the basis for risk assessment and other committee opinions) at as early a stage as possible to support openness and transparency of decision-making. Where this is not possible, reasons will be clearly set out, explained and a commitment made to future publication wherever possible.

26 The amount of material withheld by the committee or FSA as being confidential will be kept to a minimum. Where it is not possible to release material, the reasons will be clearly set out, explained and a commitment made to future publication wherever possible.

27 Where proposals or papers being considered by the Board rest on scientific evidence, the Chair of the relevant scientific advisory committee (or a nominated expert member) will be invited to the table at Open Board meetings to provide this assurance and to answer Members' questions on the science. To maintain appropriate separation of risk assessment and risk management processes, the role of the Chairs will be limited to providing an independent view on how their committee's advice has been reflected in the relevant policy proposals. The Chairs may also, where appropriate, be invited to provide factual briefing to Board members about particular issues within their committees' remits, in advance of discussion at open Board meetings.

Universal Ethical Code for Scientists

The Universal Ethical Code for Scientists, developed by the Government Chief Scientific Adviser, is a public statement of the values and responsibilities of scientists. The term 'scientists' means anyone whose work uses scientific methods, including social, natural, medical and veterinary sciences, engineering and mathematics.

Rigour, respect and responsibility: A universal ethical code for scientists

Rigour, honesty and integrity

- Act with skill and care in all scientific work. Maintain up to date skills and assist their development in others.
- Take steps to prevent corrupt practices and professional misconduct. Declare conflicts of interest.
- Be alert to the ways in which research derives from and affects the work of other people, and respect the rights and reputations of others.

Respect for life, the law and the public good

- Ensure that your work is lawful and justified.
- Minimise and justify any adverse effect your work may have on people, animals and the natural environment.

Responsible communication: listening and informing

- Seek to discuss the issues that science raises for society. Listen to the aspirations and concerns of others.
- Do not knowingly mislead, or allow others to be misled, about scientific matters. Present and review scientific evidence, theory or interpretation honestly and accurately.

You can read the full version of the Code (launched by the UK Government's Chief Scientific Adviser in March 2007) at www.dti.gov.uk/science/science-andsociety/public_engagement/code/page28030.html

Annex 5 – Glossary of Terms

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

Absorption (biological): Process of active or passive transport of a substance into an organism, in humans this is usually through the lungs, gastrointestinal tract or skin

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.

Acceptable Risk: Probability of suffering disease or injury which is considered to be sufficiently small to be "negligible"

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

Acute reference dose (ARfD): Estimate of the amount of a substance in food or drink, expressed on a body weight basis, that can be ingested in a period of 24 hours or less without appreciable health risk.

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.

Androgen: The generic term for any natural or synthetic compound that can interact with and activate the androgen receptor. In mammals, androgens (for example, androstenedione and testosterone) are synthesised by the adrenal glands and the testes and promote development and maintenance of male secondary sexual characteristics.

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.

Benchmark dose (BMD) modelling: An approach to dose-response assessment that aims to be more quantitative than the NOAEL process. This approach constructs mathematical models to fit all data points in the dose-response study and uses the best fitting model to interpolate an estimate of the dose that corresponds to a particular level of response (a benchmark response), often 10%. A measure of uncertainty is also calculated, and the lower confidence limit on the benchmark dose is called the BMDL. The BMDL accounts for the uncertainty in the estimate of the dose-response that is due to characteristics of the experimental design such as sample size. The BMDL can be used as the point of departure for derivation of a health-based guidance value or a margin of exposure.

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.

Enterohepatic circulation: Cyclical process involving intestinal re-absorption of a substance that has been excreted through bile followed by transfer back to the liver, making it available for biliary excretion again.

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.

Exposure Assessment: Process of measuring or estimating concentration or intensity, duration and frequency of exposure to an agent present in the environment.

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.

Horizon Scanning: The systematic examination of potential threats, opportunities and likely future developments, which are at the margins of current thinking and planning. Horizon scanning may explore novel and unexpected issues, as well as persistent problems and trends. Overall, horizon scanning is intended to improve the robustness of policies and the evidence base

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 leukaemia's which develop from lymphoid cells and the myeloid leukaemia's 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'.

Margin of exposure (MOE) approach: A methodology that allows the comparison of the risks posed by different genotoxic and carcinogenic substances. The MOE approach uses a reference point, often taken from an animal study and corresponding to a dose that causes a low but measurable response in animals. This reference point is then compared with various dietary intake estimates in humans, taking into account differences in consumption patterns.

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.

Risk Assessment: process of evaluating a potential hazard, likelihood of suffering, or any adverse effects from certain human activities

Risk Management: process designed to identify, contain, reduce, or eliminate the potential for harm to the human population; usually concerned with the delivery system and site rather than performance.

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 AAGGCTAA to ATGGCTAA. 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.

Stakeholder: A person or organisation representing the interests and opinions of a group with an interest in the outcome of (for example) a review or policy decision.

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.

Surfactant: Also called: surface-active agent. A substance, such as a detergent, that can reduce the surface tension of a liquid and thus allow it to foam or penetrate solids; a wetting agent.

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.

Testicular Dysgenesis Syndrome (TDS): The hypothesis that maldevelopment (dysgenesis) of the fetal testis results in hormonal or other malfunctions of the testicular somatic cells which in turn predispose a male to the disorders that comprise the TDS, i.e. congenital malformations (cryptorchidism and hypospadias) in babies and testis cancer and low sperm counts in young men.

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.

Total Toxic Equivalent (TEQ): Is a method of comparing the total relative toxicological potency within a sample. It is calculated as the sum of the products of the concentration of each congener multiplied by the toxic equivalency factor (TEF).

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

Toxicogenic: producing or capable of producing a toxin.

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 ribosome's, 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 (e.g. 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.

WHO-TEQs: The system of Toxic Equivalency Factors (TEFs) used in the UK and a number of other countries to express the concentrations of the less toxic dioxin-like compounds (16 PCDDs/PCDFs and 12 PCBs) as a concentration equivalent to the most toxic dioxin 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is that set by the World Health Organisation (WHO), and the resulting overall concentrations are referred to as WHO-TEQs (Total toxic equivalents).

Xenobiotic: A chemical foreign to the biologic system.

Xenoestrogen: A 'foreign' compound with estrogenic activity (see estrogen).

Annex 6 - 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 7 – Previous Publications

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.++

2003 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/0900/0504.++

2004 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/0992/0804.++

2005 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/1098/0906.++

2006 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/1184/0707++

2007 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/1260/0608++

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 (2002).++

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment: Variability and Uncertainty in Toxicology of Chemicals in Food, Consumer Products and the Environment, FSA/1150/0307 (2007).++

Guidance on a Strategy for the Risk Assessment of Chemical Carcinogens. Department of Health (2004)+

* Available on the COM website at http://www.iacom.org.uk/index.htm

** Available on the COT archive at http://archive.food.gov.uk/dept_health/archive/cot.htm

\+ Available on the COC website at http://www.iacoc.org.uk/index.htm

++ http://cot.food.gov.uk/cotreports/

If you require any further information about the work of the committees, or the contents of this report, please write to the committee's administrative secretary at the following address:

Julie Shroff
COT Secretariat
Food Standards Agency
Room 4C, Aviation House
Kingsway
London WC2B 6NH

Tel: 020 7276 8522
Fax: 020 7276 8513
Email: COT@foodstandards.gsi.gov.uk
http://cot.food.gov.uk

COC/COM Secretariat
Health Protection Agency
Centre for Radiation and Chemical Hazards
Chemical Hazards and Poisons Division (Head Office)
Chilton Didcot
Oxon OX11 0RQ

Tel: +44 (0)1235 841475
Fax: +44 (0)1235 841478
Email: Sue.Kennedy@hpa.org.uk
http://www.iacoc.org.uk/index.htm
http://www.iacom.org.uk/index.htm

Published by Food Standards Agency/Department of Health
July 2009
FSA/1410/0709