Committee on 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 at the request of the Food Standards Agency, Department of Health and other Government Departments including the Regulatory Authorities. All details concerning membership, agendas, minutes and statements are published on the Internet.

In 2005 the Committee has provided advice on a range of chemicals, including tryptophan, furan, kava kava, brominated organic contaminants, food additives and developmental toxicology and a hydrogel filler used in breast implants. Discussions commenced on fluorinated contaminants, marine biotoxins, uranium levels in water and on CS and PAVA sprays, for completion in 2006. Together with the Committee on Safety of Medicines, the Committee held an interesting workshop on diet and drug interactions. Other generic issues discussed included route-to-route extrapolation and toxicology of nanomaterials. The Committee currently has two working groups: on the long-term health effects of the Lowermoor incident and on variability and uncertainty in toxicology. Draft reports from both these working groups were discussed by the full Committee during the course of 2005.

All of this would not have been possible without the dedication and commitment of the extremely able body of experts on the Committee, to whom I am very grateful. Also, I would like to acknowledge the support of my Vice-Chair, Professor Ian Rowland. Finally I would like to add my sincere thanks and appreciation of the work of the administrative and scientific secretariats without whose excellent work the Committee would not be able to function.

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COT evaluations

Brominated organic contaminants: Preliminary discussion on toxicological evaluation

1.1 The Food Standards Agency (FSA) has commissioned a number of surveys on brominated organic contaminants. Farmed and wild fish and shellfish consumed in the UK, the 2003 total diet study samples and fish oil dietary supplement samples have been analysed to determine the concentrations of a number of brominated organic contaminants. These are: brominated flame-retardants (BFRs), i.e. polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs) hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA); as well as polybrominated dibenzo-p-dioxins (PBDDs) and polybrominated dibenzofurans (PBDFs).

1.2 The COT has previously evaluated PBDEs, HBCD and TBBPA, and was therefore invited to provide preliminary advice on PBDDs, PBDFs and PBBs. In particular, it was asked to consider whether data on various congeners should be combined and if so what method is most appropriate.

1.3 On the basis of the available data, the COT agreed that there was sufficient evidence to conclude a similar mechanism of action for the PBDDs, PBDFs and coplanar PBBs to their chlorinated analogues. Toxic equivalency factors (TEFs) have not been established for the brominated dioxin-like compounds. In particular, there is a lack of information on the rate of clearance in laboratory animals and in humans. However, assuming additivity and applying the TEFs derived for the chlorinated dioxins, furans and dioxin-like PCBs would be more protective than presuming independence of action. The Committee also confirmed that the total toxic equivalents (TEQs) for the brominated contaminants should be combined with the TEQs for the chlorinated dioxins to provide a measure of the total concentration of chemicals with dioxin-like properties for the purpose of evaluating the data on dietary exposure.

1.4 The COT will be presented with survey results combined in this manner for consideration at a future meeting.

Diet and drug interactions

1.5 As reported in the 2004 Annual Report, the COT and the Committee on the Safety of Medicines (CSM) held a joint open meeting in February 2005 to discuss the issue of diet-drug interaction.

1.6 Interaction between different drugs is a well-established phenomenon. There are also several well-characterised interactions between drugs and foods such as those between grapefruit juice and drugs metabolised by cytochrome P450 enzymes, but the overall extent of diet and drug interaction is uncertain.

1.7 The meeting provided a useful forum for discussion, there were brief presentations on the epidemiology and the mechanisms of interactions, practical aspects of managing interactions, the potential vulnerable groups and how interactions are assessed in a regulatory framework. The particular issues associated with herbal medicines and with obesity were also considered.

1.8 The COT and CSM noted that the clinical significance of diet-drug interactions was variable, and many interactions had only been demonstrated experimentally. Food itself was a complex mixture of macro and micronutrients making interactions hard to assess. Overall, the COT and CSM concluded that the issue of diet-drug interactions was a real one but of limited clinical significance. However, there were few data and further interactions might be identified in the future.

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

Food additives and developmental toxicology

1.10 The European Food Safety Authority (EFSA) has been asked to review the food additives currently permitted within the EU in order to determine whether full re-evaluation is required. The COT has been invited to contribute to the re-evaluation process, with a particular focus on neurotoxicity, because there have been recent developments in the toxicological approaches in this area.

1.11 The COT reviewed the animal toxicology data on twelve additives. These had previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Scientific Committee on Food (SCF). However, a number of the evaluations were conducted more than 20 years ago. The COT was asked to consider whether the potential for neurotoxicity and developmental effects was taken into account in setting the acceptable daily intake (ADI) for certain food additives and whether significant new data of relevance to neurodevelopmental effects had emerged since the ADI was set.

1.12 The additives considered were quinoline yellow (E104), sunset yellow (E110), carmoisine (E122), ponceau 4R (E124), indigo carmine (E132), brilliant blue (E133), sodium benzoate (E211), sulphur dioxide (E220), monosodium glutamate (E621), acesulfame K (E950), aspartame (E951) and saccharin (E954).

1.13 The COT concluded that there was no evidence of potential developmental neurotoxicity for Acesulfame K, brilliant blue, indigo carmine, saccharin, sodium benzoate or sulphur dioxide. There was some equivocal evidence suggesting the potential to produce developmental neurotoxicity at very high dose levels for carmoisine, sunset yellow and quinoline yellow. There was actual evidence suggesting the potential to produce developmental neurotoxicity at very high doses for aspartame, monosodium glutamate and Ponceau 4R. For no agents was there any evidence suggesting the potential for developmental neurotoxicity at current acceptable daily intakes. Therefore, although direct evaluations of developmental neurotoxicity were mostly absent and would, in principle, be desirable, the available data did not suggest that further investigations of any of these agents would be a priority given current dietary levels.

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

Furan in food

1.15 In February 2005, the COT was asked to consider a report from the EFSA expert panel on contaminants in the food chain (CONTAM) on furan in food. Furan is carcinogenic in animal models, although the mechanism of furan carcinogenicity has not yet been fully elucidated with both genotoxic and non-genotoxic mechanisms proposed.

1.16 The COT considered that a systematic mode of action assessment would be appropriate but requested further advice on the existing evidence for genotoxicity from COM and carcinogenicity from COC, and any needs for further research in these areas. The COM and COC discussions on furan are included in paragraphs 2.9 to 2.14 and 3.10 to 3.15 of this report, respectively.

1.17 The COT will consider the conclusions from COM and COC in 2006 and further research requirements in light of a European Union research call in December 2005.

Hydrogel filler for breast implants: Further studies

1.18 The COT had previously considered data to assess the safety of hydrogel implants in 1999, 2000 and 2002 and was asked by the Medicines and Healthcare products Regulatory Agency (MHRA) to comment on two new studies that had been sponsored by the company producing the Hydrogel implants.

1.19 COT had previously reviewed data from two 90-day rat implantation studies which were considered unsatisfactory in design, execution and reporting, and was not able to exclude the possibility that the reported lesions were indicative of a toxic or immunologically-mediated response. These lesions warranted further study, including investigation of the reversibility of any changes observed.

1.20 The manufacturer had now submitted reports of a one-year toxicity and a two-year carcinogenicity study in rats that had been exposed to the hydrogel subcutaneously. The COT concluded that the results of these two studies provided reassurance that the effects previously noted in the liver, kidney and lymph nodes were not indicative of a toxic effect. Marginal effects seen in the kidney in the new studies were considered to be treatment related but were not clearly adverse. Taking account of the susceptibility of older rats to kidney effects, especially in longer studies, the COT considered that similar effects would be unlikely to occur in humans.

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

Kava kava in food products

1.22 Kava-kava, also known as kava, is an herbal ingredient derived from the plant *Piper methysticum*, a member of the pepper family, which is native to many Pacific islands. The leaves and root of the plant are used in herbal preparations that are available as tablets, capsules, tinctures and drops. These products are largely considered medicinal, however some products are sold as foods such as teas.

1.23 The consumption of kava-kava has been associated with liver toxicity following a number of case reports that came mostly from Germany. In 1992, the Committee on Safety of Medicines (CSM) decided that steps should be taken to prohibit the use of kava-kava in unlicensed medicines other than for external use. A number of food products also contained kava-kava, and due to time constraints, urgent advice was requested of the COT. The risk assessment conducted at that time is in the COT annual report from 2002.

1.24 In 2002 the kava-kava in Food (England) Regulations were laid before parliament. At the time the FSA stressed its commitment to reviewing any new evidence that came to light in order to reassess the ban on kava-kava. In February 2005, the FSA consulted with stakeholders to assess whether any further information had emerged on kava-kava. The COT was invited to consider whether the new information altered its previous conclusions.

1.25 The COT considered that the new data were not sufficient to demonstrate the safety of food products containing kava-kava particularly considering the severe nature of the hepatotoxicity linked with kava-kava consumption. It was also agreed that, because the hepatotoxicity appeared to be an idiosyncratic reaction, it would be extremely difficult to predict or to propose a suitable animal model. The new studies on metabolism did not help to identify a mechanism of hepatotoxicity. Reports of hepatotoxicity were still occurring, including cases related to traditional kava-kava preparations. Concern was also expressed about the lack of controls over production of food such as the extraction method and the parts of the plant used in the extracts.

1.26 Overall, members concluded that the new data did not warrant a change in their previous advice. More information would be needed on the mechanism of hepatotoxicity before it would be possible to propose further research to establish a safe usage of kava-kava in foods.

Terephthalic acid: multigeneration reproduction study additional histopathological examinations

1.27 Terephthalic acid (TPA) is used as a starting material in the manufacture of polyethylene terephthalate (PET), which may be used to manufacture food cans and beverage bottles. In 2000 the Committee reviewed the health implications of the results of a survey of TPA migration from can coatings into food and recommended that appropriate studies should be carried out to determine whether TPA possesses endocrine disrupter activity.

1.28 In June 2003, BP Chemicals Ltd submitted the report of a full multigeneration reproduction toxicity study on TPA. The information provided in the report was sufficient to demonstrate that TPA did not have endocrine disrupting effects at the highest dose tested in this study. However, a number of histopathological changes in the urinary bladder and the kidney were reported at the high dose, but these organs had not been examined in the other groups. The company was asked to provide further information on the histopathology of these organs in the mid- and low-dose groups.

1.29 A new histopathology evaluation along with an expert report was submitted by BP Chemicals Ltd in 2005. The COT was 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) of 5000 ppm in the diet in the multigeneration study. It was noted that some effects on the thymus had also been observed in this study however these were considered artefacts associated with the necropsy technique used rather than being treatment related.

1.30 A statistically significant decrease in renal weights (adjusted for bodyweight) was present in all generations. Because this was also present in the parental generation, it was not viewed as a developmental effect. There was no associated histopathology or effect on renal function, and this effect was not observed in a chronic toxicity study using a different rat strain. The relevance of the

reduction in renal weights was considered by applying agreed criteria from the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) for distinguishing between adverse and adaptive effects. The COT concluded that the reduction in kidney weight in these rats was likely to represent a colony specific physiological adaptation to exposure to terephthalic acid TPA.

1.31 Additional mutagenicity data on TPA had previously been requested by the Committee on Mutagenicity (COM) and will need to be evaluated by the COM before a statement can be produced.

Tryptophan in food: responses to consultation on revision of the Regulations

1.32 In 1990 the COT endorsed a ban on the addition of the isolated amino acid tryptophan to foods (including dietary supplements) in the UK. This followed reports of a new epidemic illness in the USA, known as eosinophilia-myalgia syndrome (EMS) which was associated with the consumption of L-tryptophan-containing dietary supplements. EMS was a serious disorder which affected 1500 people in the USA and caused at least 37 deaths. Several cases of EMS also occurred in the UK.

1.33 In 2003-2004 the COT considered the available data on tryptophan and EMS and concluded that the balance of the data linked EMS to a contaminated batch of tryptophan made by one manufacturer. However it could not be completely ruled out that the apparent epidemic may have been due to the increased use of tryptophan as a supplement and awareness of EMS as a condition.

1.34 Data from a monitoring scheme, established in 1994 for a prescription tryptophan product provided reassurance that medicinal tryptophan produced to European Pharmacopoeia standards had not resulted in cases of EMS. Due to the remaining uncertainties, the COT applied an uncertainty factor of 10 to the mean therapeutic dose of 2228 mg tryptophan/day, a dose which was not associated with any adverse effects, and concluded that a dose of 220 mg tryptophan per day would not present a risk to health, provided that it met the purity criteria specified in the European Pharmacopoeia.

1.35 The COT advice was reflected in a proposed revision to the Tryptophan in Food (England) Regulations 1990, which were consulted upon in early 2005. The responses received were considered by the COT. In addition, the COT considered a number of additional studies of tryptophan in humans (mainly pre-dating the EMS epidemic) as well as updated data on adverse reactions to medicinal tryptophan.

1.36 The COT noted that the additional human studies involved small numbers of participants, took place over short duration's and were conducted to assess the effects of tryptophan on a variety of therapeutic endpoints. The side effects, where reported, were generally self reported rather than specifically assessed. The COT concluded that, overall, there were insufficient new data to reconsider their 2004 conclusion.

1.37 The COT agreed that an amendment should be made to the 2004 conclusions to state that the mean therapeutic dose was "without adverse effects" so that it was clear that it represented a NOAEL.

Urgent advice provided by COT

Mutagenicity of para red

1.38 The Food Standards Agency was informed on 20 April that some batches of spice used by one manufacturer contained an azo dye, para red, which is not a permitted colour under the Colours in Food Regulations 1995.

1.39 Para red is chemically very similar to Sudan 1. The Agency consulted the Chairs of the COC and COM who advised that, although there were very limited data available it would be prudent to assume that para red could be a genotoxic carcinogen.

1.40 A copy of the agreed opinion was provided to COT for information and is included at the end of this report.

Committee procedures and working groups

Horizon scanning

1.41 At the February 2005 meeting, members were provided with information on planned discussion items for the year, and invited to comment on emerging issues that might also need to be addressed.

1.42 It was considered appropriate for the COT statement on adverse trends in the development of the male reproductive system to be reviewed in the light of emerging data. A one-day meeting was subsequently planned for February 2006 to allow the COT to discuss new data with experts in the field.

1.43 The Department of Health was interested in seeking the Committee's view on specific aspects of odour perception and health risks due to airborne chemicals, in particular that resulting from bystander exposure to pesticide mixtures. A brief overview of incidents reviewed by the Pesticides Incidents Appraisal Panel (PIAP) between 1990 and 2002 showed that approximately one third of reported incidents considered "confirmed and likely" involved skin and/or eye irritancy. Possible mechanisms for reported effects were chemosensory stimulation or inflammation, or a psychogenic response. It was anticipated that the COT would be asked to provide advice based on a literature review, which would assist in further evaluation and identifying possible research in this area. It was suggested that data on organic solvents should be included. Certain organic solvents had been shown to have an additive effect in causing narcosis, and this might also occur for chemosensory irritation.

1.44 It was noted that advice on reducing intake of sodium chloride might lead to increased use of salt substitutes containing potassium chloride, both in the home and in processed foods. Excessive intakes of potassium lead to hyperkalaemia, which results in adverse effects including altered acid-base balance and altered respiratory and heart rates and may result in cardiac arrest. However, it was not clear what intakes of potassium were required to overwhelm homeostatic mechanisms and lead to hyperkalaemia. People with decreased renal function, including the elderly, would be expected to be more vulnerable to hyperkalaemia, as would people taking a number of medications which predispose to hyperkalaemia.

Consideration of high potassium intakes, leading to altered sodium/potassium balance might require additional types of expertise.

1.45 Processes such as chlorination and ozonation are used by the food industry to reduce the risk of microbial contamination in pre-packed foods. Current legislation specifies limits for chlorine residues but there may also be a concern about potential generation of by-products of chlorination or ozonation on or in the food. It was agreed that before any risk assessment could be undertaken there was a need for information on the nature and levels of the by-products that were formed. It was likely that by-products would include trihalomethanes, and it would be important to obtain information on the contribution of treated processed foods to total trihalomethane exposure.

1.46 RNA interference (RNAi) is a post-transcriptional mechanism of blocking or "silencing" of genes in order to test gene function. Since it was first demonstrated in 1998 in *Caenorhabditis elegans* (Fire *et al*., Nature 391: 806-811; 1998) it has been headlined as a technique that will speed up research in many fields and has significant potential as a tool in toxicological risk assessment. RNAi technology was rapidly being incorporated into experimental protocols and was being used in toxicology as an analytical tool. The COT agreed that an educational seminar would be useful, and this was subsequently arranged (see paragraphs 1.67-1.72).

1.47 Some pilots and cabin crews have claimed to be suffering from a disabling illness, from 'fume events' in the aircraft cabin. Organophosphates, which are used as additives in aircraft engine oil and auxiliary power unit (APU) oils, may transfer into some aircraft cabin air ventilation systems during 'fume events'. The Department of Health had asked the British Airline Pilots Association (BALPA) to provide the information they have available on ill health among pilots and cabin crew. This information would be considered by the Aviation Health Working Group (AHWG), and if the AHWG considers there is sufficient information, the Department of Health would commission a COT review.

1.48 Potential thyrotoxicants that do not produce frank hypothyroidism may have subtle effects on thyroid function, which could have irreversible effects on brain development. The Department of Health was to consider whether COT advice should be sought on this issue.

1.49 In addition a member suggested that consideration of exposure of the central nervous system (CNS) to chemicals via inhalation be considered. The nasal cavity was noted to increase bioavailability to the CNS. Members also suggested that the COT could re-examine some of the assumptions it makes, such as concepts of threshold and low dose effects. It was noted that a number of initiatives of the International Programme on Chemical Safety (IPCS) and EFSA were of relevance to this, and would be provided to members.

1.50 Members were reminded that they may draw additional issues to the attention of the Secretariat at any time.

Working group on Variability and Uncertainty in Toxicology

1.51 In 2003, the COT established a working group chaired by Professor Peter Aggett to review the approaches that are currently used, or that might in future be used, for dealing with variability and uncertainty in the biological data utilised in the risk assessment of chemicals in food.

1.52 The COT considered a preliminary draft report of the working group at its October 2005 meeting. The working group subsequently met in December 2005 to discuss revisions, and a draft report will be published for consultation early in 2006.

Lowermoor subgroup

1.53 The Lowermoor subgroup was established in 2001 under the chairmanship of Professor Frank Woods to consider the human health effects of the chemical exposure resulting from a water pollution incident which occurred in July 1988 in North Cornwall published a draft report on January 26, 2005. Its terms of reference were:

- to advise on whether the chemicals involved in the incident caused, or were likely to cause, delayed or persistent harm to health.

- to advise whether the existing programme of monitoring and research into the health effects of the incident should be augmented and, if so, to make recommendations.

1.54 The COT discussed the draft report during a public consultation which ended on 20 May 2005. The main conclusion was that the chemicals involved in the incident were unlikely to have led to delayed or persistent adverse health effects in the population. However, the report recommended further investigations to explore the neuropsychological status of those individuals who consumed the contaminated water and investigations into the cognitive, behavioural and intellectual development of individuals who were less than a year old at the time of the incident. Three of the polluting metals (lead, manganese and aluminium) are known neurotoxicants.

1.55 The Subgroup is currently finalising the revised report in the light of the public consultation.

Risk assessment strategies

Food Standards Agency Draft Science Strategy 2005-2010

1.56 The Food Standards Agency Draft Science Strategy 2005-10 was issued for public consultation in June 2005. The document considered the science needed to support the Agency's needs over the next five years and how the Strategy's aims would be achieved. The COT was asked to comment on the draft and in particular on the sections relating to obtaining and interpreting scientific evidence which include work of scientific advisor committees such as COT.

1.57 The COT noted that better integration of FSA science strategy with that of other government departments might be beneficial and reduce duplication. It was suggested that the Strategy was too focused on Agency structure, interests and committees rather than considering objective analysis of areas of uncertainty relating to food safety.

1.58 The COT also emphasised the need for better and more pro-active communication of the scientific rationale for the FSA's actions and decisions and a more pro-active approach to the communication of the results of FSA funded research.

Interdepartmental Group on the Health Risk of Chemicals (IGHRC) report: Guidelines on route-to-route extrapolation of toxicity data when assessing health risks of chemicals

1.59 The Interdepartmental Group on Health Risks from Chemicals (IGHRC) is an informal group of representatives of UK government departments, agencies and research councils. Its aims include the production of guidance documents for non-specialist government officials or regulators. The COT had previously commented on IGHRC guidance documents on uncertainty factors (2001) and exposure assessment (2003)*.

1.60 The document aims to provide best practice for assessing the toxicity of a chemical when the only available data are for exposure by a different route to that being investigated. The most common scenario is the use of oral exposure data for risk assessment of dermal or inhaled exposure. It sets out essential criteria for deciding to use route-to-route extrapolation and stresses the need for a case by case assessment for individual chemicals and the need to apply expert judgement.

1.61 The COT noted that route-to-route extrapolation is being encouraged in several areas, especially for providing data for the EU REACH (Registration, Evaluation and Authorisation of Chemicals) proposal http://europa.eu.int/comm/environment/chemicals/pdf/0188_en.pdf. The major disadvantage of this approach is that the rule base for route-to-route extrapolation is very strict. The COT made a number of suggestions for changes and additions to the document, including an outline of when route-to-route extrapolation should be used, the dependence on level of exposure, information on physiologically-based pharmacokinetic modelling, and exposure via the lung and demal route. The difficulty of route-to-route extrapolation for sensitisation of the immune system was noted.

Nanomaterial toxicology

1.62 The risk assessment of nanomaterials was identified by COT/COC/COM as an area of interest during horizon scanning discussions in February 2004. In September 2004, the COT discussed the report by the Royal Society and the Royal Academy of Engineering 'Nanoscience and nanotechnologies: opportunities and uncertainties'. The UK Government's response to this report, published in February 2005, identified the COT, COC and COM as relevant scientific committees to provide advice on the development of nanotechnology.

* *Uncertainty factors: their use in human health risk assessment by UK government* (IGHRC, 2003) and *Guidelines for good exposure assessment practice for human health effects of chemicals* (IGHRC, 2004) are available on the IGHRC website at http://www.silsoe.cranfield.ac.uk/ieh/ighrc/igpublications.html

1.63 Nanomaterials were defined as having one dimension less than 100 nanometres (nm) or 0.1 micrometre (μm). Their small size means that a high proportion of total atoms are at the surface of the nanoparticle causing high surface reactivity. It is not known whether this results in nanomaterials having different toxicological characteristics to the corresponding bulk material.

1.64 The information presented to the Committees was based on a hazard assessment document published by the Health and Safety Executive (HSE) with additional papers and abstracts identified by the secretariat. There were considerable limitations in the number of materials tested, and in the toxicology data available. COT concluded that the current data available did not suggest the need for a new risk assessment paradigm for nanotechnology products. However, it was considered necessary to keep a watching brief of this developing area.

1.65 The Committees suggested a systematic tiered approach to initial toxicological studies, generating basic hazard identification data on nanomaterials, based on *in vitro* screening of selected materials followed by *in vivo* testing.

1.66 The joint COT/COC/COM statement is included at the end of this report.

RNA Interference

1.67 In response to the horizon scanning discussion noted above at paragraph 1.46, Dr Miguel Martins of the MRC Toxicology Unit at the University of Leicester gave a presentation to the COT on RNA interference (RNAi) technology.

1.68 The presentation included an explanation of the general principles of RNAi, and examples of its utility as a research tool. These included silencing specific genes in order to find out their role in a particular biological pathway or disease and the use of RNAi libraries to screen for candidate genes that may be involved in a biological process of interest.

1.69 The issue of specificity of gene silencing with RNAi was raised. In studies where a transfected gene is being targeted, the endogenous gene may also be affected if it contains a homologous target sequence. In addition, where silencing of non-target genes is seen, it is possible that this may have been mediated by endogenous microRNAs (miRNAs). miRNAs are single stranded RNAs that regulate gene expression. miRNAs bind mRNA with sequences that are often significantly, though not completely, complementary to the miRNA.

1.70 RNAi was reported as achieving up to 90% knockdown of protein expression. It was noted that it is not possible to determine whether 90% knockdown is achieved in 100% of cells, or if 100% knockdown is achieved in 9 out of 10 cells. In some situations, 100% knockdown may be required in order to see significant effects and in such cases, RNAi would not be as effective as gene knockout by homologous recombination techniques.

1.71 Questions were raised as to the duration of knockdown that can be achieved by RNAi. Lentiviral and retroviral vectors are integrated into the genome and should enable stable knockdown of genes, but these vectors are mutagenic. Transfection of short synthetic double stranded RNA (siRNA)

oligonucleotides is currently the preferred method, although they only produce a transient response in vitro as they are diluted by cell division.

1.72 The difficulty of targeting specific cell populations *in vivo* and the possibility of long-term adverse effects were also noted. Adverse effects are most likely to occur with viral vectors, due to their mutagenic properties. In contrast, siRNA oligonucleotides are not mutagenic as they target mRNA. While they have been shown to have epigenetic effects in fungi and plants, there is no evidence for such effects in mammalian cells.

Ongoing work

2-Chlorobenzylidene malonitrile (CS) and PAVA (Nonivamide) sprays: combined use

1.73 At the request of the Home Office Science Development Branch (HOSDB) the COT discussed potential effects of exposure to both 2-chlorobenzylidene malonitrile (CS) and pelargonic acid vanillylamide (PAVA). This item will be completed early in 2006.

Marine biotoxins

1.74 In December 2005, COT commenced discussion on the risk assessment and monitoring of marine biotoxins in support of public health. The discussions will continue in 2006.

Perfluorooctane sulfonate (PFOS)

1.75 The Food Standards Agency has commissioned research to determine the concentrations of a number of fluorochemicals in food following unexpected bioaccumulation and toxicological findings for these substances. The COT was invited to assess the toxicology of two perfluoroalkyl acids in order to advise on any health implications in advance of receiving the results of the analysis.

1.76 Perfluorooctane sulfonate (PFOS) has excellent surfactant properties and is widely used in the manufacture of plastics, electronics, textile and consumer material in the apparel, leather, and upholstery industries. A number of other compounds have the potential to degrade subsequently to PFOS either metabolically or through environmental processes.

1.77 A hazard assessment for PFOS has been produced under the Existing Chemicals Programme of the Organisation for Economic Co-operation and Development (OECD). Given the widespread occurrence of PFOS the OECD evaluation recommended that national or regional exposure information gathering and risk assessment may need to be considered. Consequently the Environment Agency for England and Wales concluded that PFOS meets the criteria for classification as a Persistent, Bioaccumulative and Toxic (PBT) substance.

1.78 COM and COC concluded that PFOS should be regarded as not mutagenic and that a threshold approach could be used for the risk assessment. The COT opinion will be finalised in 2006.

Perfluorooctanoic acid (PFOA)

1.79 Perfluorooctanoic acid (PFOA) is primarily used as an emulsifier in industrial applications, for example in the production of fluoropolymers such as polytetrafluoroethylene (PTFE). PFOA may also be found at low levels in some fluorotelomers, as an unintended by-product of the manufacturing process. Fluorotelomer derivatives are ingredients of fire-fighting foams and coatings, and are intermediates in the manufacture of stain-, oil-, and water-resistant additives for some textiles, coatings and food contact papers.

1.80 PFOA has not been evaluated by the Scientific Committee on Food (SCF) or the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The US Environmental Protection Agency (EPA) has recently submitted a draft risk assessment of the potential human health effects associated with exposure to PFOA and its salts to the EPA Science Advisory Board for peer-review.

1.81 The COM and COC concluded that, overall, PFOA was not mutagenic and that a threshold approach to establishing a tolerable intake was appropriate. The COT opinion will be finalised in 2006.

Uranium levels in water used to re-constitute infant formula

1.82 Uranium is a metallic element that is ubiquitous in the environment occurring in rocks, soil, air, food and water. Where present in water, this tends to be the major source of uranium intake. Due to dissolution from mineral deposits, notably granite, ground waters contain higher levels of uranium than surface waters, although the level will vary considerably depending on the local geology.

1.83 Uranium is known to be nephrotoxic in both animals and humans producing characteristic damage to the proximal tubules of the kidney, following high levels of exposure.

1.84 The WHO has established a Tolerable Daily Intake (TDI) and a guideline value for uranium in drinking water of 15 μg/L. The Committee were asked to comment on the potential health implications for infants consuming formula milk made up with water containing uranium at this guideline level.

1.85 The discussion will be completed in 2006.

Statements of the COT

Phosphate and the calcium-parathyroid hormone axis

Introduction

1. Phosphorus was one of the nutrients considered in the recent report of the Expert Group on Vitamins and Minerals (EVM, 2003). The review relates to phosphorus compounds, primarily phosphates, expressed as elemental phosphorus to allow comparison of exposures to different compounds. There were insufficient data to establish a Safe Upper Level but a guidance level of 250 mg/day supplemental phosphorus was established, in addition to the estimated maximum intake of phosphorus from food of 2110 mg/day. The guidance level took into account both the occurrence of osmotic diarrhoea in human volunteer studies and the possible adverse effects of phosphorus on the parathyroid hormone-calcium axis in subjects with hypovitaminosis D who would be vulnerable to the effects of hyperparathyroidism. The guidance level represents a supplemental intake which would not be expected to result in any adverse effects. The EVM noted that physiological changes in calcium and parathyroid hormone (PTH) levels have been associated with intakes of 1500 mg/day and above of supplemental phosphorus. An uncertainty factor of 3 was then applied to a NOAEL of 750 mg/day (Brixen *et al*., 1992) to allow for inter-individual variability, resulting in a guidance level of 250 mg/day phosphorus.

2. In the interests of maintaining choice and promoting consumer understanding, the Food Standards Agency is working with manufacturers to develop a labelling initiative, which would allow higher amounts of phosphorus (as phosphate) to be present in food supplements than those recommended by the EVM provided that appropriate advisory statements were also included.

3. The COT was asked to consider data on phosphate, PTH, calcium balance and bone health in detail, in order to allow the FSA to formulate the appropriate consumer advice. This review included new data which were not available to the EVM.

Background

4. Phosphorus is a non-metallic, group V element. It is most commonly found in its pentavalent form in combination with oxygen as phosphate (PO_4^{3-}).

5. Phosphorus is present in a wide variety of foods largely as phosphate. Particularly rich sources are red meats, dairy products, fish, poultry, bread and other cereal products. Food additives are also a significant source of phosphate (Calvo and Park, 1996). Phosphorus intakes in the UK in 2000 were estimated to be 1494 (mean) and 2381 (high level) mg/day in men and 1112 (mean) and 1763 (high level) mg/day in women (Henderson *et al*., 2003). These intakes had increased since the previous survey in 1986/6 (Gregory *et al*., 1990).

6. Food supplements provide doses of up to 1100 mg/day phosphorus (EVM, 2003), though 150 mg or less is more common (OTC, 2003). Tablet forms of supplement products may also contain calcium phosphates as fillers or diluents. It has been suggested that these could represent a dose of up to 250 mg phosphorus per day (personal communication, Proprietary Association of Great Britain). The phosphate may not be declared on the label as an active ingredient.

7. The solubility of the inorganic phosphate salts used in supplements is variable. Calcium phosphate is soluble at acid pH but much less soluble in neutral conditions (Carr and Shangraw, 1987), suggesting that it would be soluble in the stomach but less so in the small intestine. Salts such as sodium and potassium phosphate are much more soluble at neutral pH.

Phosphate: Absorption, distribution, metabolism and excretion

8. Phosphate is readily absorbed throughout the small intestine (Koo and Tsang, 1997). The majority of phosphate absorption occurs via passive diffusion but active transport, dependent on potassium and calcium ions, sodium-potassium ATPase and vitamin D, also occurs. Active transport of phosphate is regulated by PTH which promotes calcium absorption via increased synthesis of calcitriol. However, even at low levels of calcitriol, substantial phosphate absorption occurs, such that dietary phosphate content is the most important determinant of phosphate absorption. To some extent, the degree of phosphate absorption varies with need, being 90% in infants fed human milk with low phosphate levels and 60-70% in adults consuming a normal diet, but it is essentially linear over the normal physiological range (Lemann, 1996).

9. Soluble phosphates from meat or milk are almost completely absorbed, whereas the phosphate present as phytate in certain vegetable fibres may not be (Koo and Tsang, 1997). Phosphate absorption is affected by calcium, with an insoluble calcium phosphate precipitate being formed in the intestine (Calvo and Heath, 1988; Calvo and Park, 1996). However it is likely that excess calcium affects phosphate absorption rather than phosphate affecting calcium absorption (IOM, 1996).

10. Phosphorus compounds are important constituents of body tissues, with over 85% being contained in the skeleton (Ilich and Kerstetter, 2000). The quantities present in intracellular pools are small, but they are critical to many aspects of cell structure and function, being present in phospholipids, nucleic acids and the phosphoproteins required for mitochondrial function (Stoff, 1982). Phosphate is involved in the regulation of the intermediary metabolism of proteins, fats and particularly carbohydrates and may regulate a number of enzymatic reactions. Phosphate is also the source of the high energy bonds of ATP.

11. Phosphate is largely excreted in the urine. Plasma phosphate levels are maintained via phosphate reabsorption in the kidney by a sodium dependent active transporter. This is achieved both by a PTH dependent mechanism and by a second mechanism mediated by soluble factors or "phosphatonins". The latter pathway has not yet been fully characterised and the significance of it with respect to normal phosphate levels is unclear (Jan de Beur and Levine, 2002). There are few data on the effect of high phosphate levels on phosphatonins so the effect of dietary increase on this aspect of phosphate regulation is unclear.

Bone metabolism

12. Bone is an active tissue which undergoes a continual cycle of resorption and renewal via osteoclasts and osteoblasts respectively (COMA, 1998). When resorption exceeds renewal, loss of bone mass occurs e.g. osteoporosis. Calcium is extracted from bones by osteoclasts and laid down by osteoblasts in response to changes in serum calcium levels. This process is mediated by PTH and vitamin D.

Effects of high phosphate intakes on the calcium-PTH axis and markers of bone health

13. As noted previously, calcium and phosphate are thought to form an insoluble precipitate in the intestine reducing absorption. However, the data suggest that calcium is more likely to reduce phosphate absorption than vice versa, and that the reduction of calcium absorption by phosphate would be significant only where calcium levels were very low (IOM, 1997).

14. At high dietary intake, phosphate is also thought to react with calcium to form an insoluble complex in the plasma, thus reducing free (ionised) calcium levels. It has been argued that the resulting decrease in serum calcium causes an increase in PTH secretion and thus in the subsequent resorption in calcium from bone to maintain plasma calcium homeostasis. The increased PTH stimulates the synthesis of calcitriol (1,25-dihydroxy vitamin D) via the renal enzyme 1-alpha-hydroxylase, which then promotes calcium absorption from the gut. PTH secretion is then subject to feedback regulation by calcitriol and calcium. Phosphate may also have a direct effect on PTH at a post-transcriptional level, possibly by stabilising the PTH mRNA. The effects of high phosphate are difficult to distinguish from the effects of low calcium *per se* since this would also trigger increased levels of PTH.

15. Although increased PTH would be expected to increase bone resorption to release calcium, intermittent PTH treatment is used therapeutically to prevent osteoporotic bone loss and to stimulate bone formation. The mechanism for this paradoxical effect is unclear, but may involve the uncoupling of bone resorption and bone formation. PTH infusion has been shown to increase the number of collagen cross-links and it has been suggested that it may stimulate the activity and differentiation of osteoblast progenitors. Similarly, phosphate is used therapeutically to lower serum calcium and to "activate" bone remodelling; bone resorption is then stopped pharmacologically, so mineralisation without resorption occurs. The relationship between PTH and phosphate is complex and may be different in acute compared to chronic exposure.

16. It has however been argued that high phosphate levels may not have adverse effects on bone if calcium intakes are adequate, or until the Ca:P ratio becomes very low (IOM, 1996).

17. The data from human volunteer studies are conflicting. In acute human studies, single doses of 1-1.5 g phosphorus (as phosphates) result in changes in PTH, serum and urinary calcium and phosphate levels, but more specific markers of bone resorption such as deoxypyridilone and carboxy terminal telopeptide of type 1 collagen are generally unaffected. Markers indicating bone formation have been reported to decrease.

18. Repeat dose human studies, generally using phosphate supplements at doses of 1-3g/day or manipulating dietary phosphate content to a similar extent, have resulted in a variety of effects. These have included increased levels of PTH and related markers and decreased urinary calcium levels. However, specific bone resorption markers were generally unchanged; this may indicate that the studies concerned were not of a sufficient duration for adverse effects to be apparent (lasting generally 1 week to 1 month in contrast to the calcium resorption/deposition cycle in bone which takes 4-8 months) or that the changes were small changes reflecting calcium homeostasis. In 7 post-menopausal women taking 1g phosphorus/day as phosphate for up to 15 months, calcium balance was "improved" (Goldsmith *et al*., 1976). Similarly in a 4 month study by Heaney and Recker (1987) urinary calcium was decreased and there was no evidence of bone remodelling in 8 female volunteers given 1.1 g/day supplemental phosphorus. However neither of these studies looked at specific markers of bone formation or resorption.

19. It has also been noted that some human studies may not detect changes in PTH because of the sampling procedures used (frequently in the morning after an overnight fast) (Calvo and Park, 1996). PTH has a strong diurnal rhythm, peaking in the late afternoon and early evening. This rhythm can be altered by dietary manipulation.

Effects of high phosphorus intakes on bone health *in vivo*.

20. Epidemiological studies of subjects exposed to high dietary phosphate levels are conflicting. In some studies, high phosphate levels have been associated with increased fracture risk, while this is not apparent in other studies. Calcium intakes are low in the majority of the studies and it has been argued that the association of fracture risk may be with low calcium consumption or low calcium:phosphate ratio rather than high phosphorus *per se*.

21. Where studies have specifically investigated the effects of beverages containing the acidulant phosphoric acid on indicators of bone health such as bone mineral density or fracture risk, the results are also conflicting. Rather than phosphate having a direct effect on bone, it has been suggested that intake of such beverages is a surrogate for physical activity which increases beverage consumption for rehydration purposes (Wyshak *et al*., 1989), and increases risk of fracture due to enhanced activity (Petridou *et al*., 1997). Phosphate containing beverages may also displace milk in the diet, reducing calcium intake (McGartland *et al*., 2003). Other possibilities suggested have been that it is the caffeine, which increases calcium excretion (Heaney and Rafferty, 2001), rather than the phosphate content that may be having an effect on bone health (Garcia-Contreras *et al*., 2000).

22. In animal studies, increased phosphate or decreased Ca:P ratios have resulted in marked bone loss in a number of species; soft tissue calcification, particularly in the kidney, has also been observed. These studies have used doses of up to 3 g/day supplemental phosphate in dogs or up to 1.2% dietary phosphorus in mice. However, studies in primates have much less marked effects at comparable phosphate intakes and it has been argued that they may be a better model for humans than other laboratory species (Anderson *et al*., 1977). Minor osteoporotic changes have been observed microscopically in baboons given a diet with Ca:P ratios of 1:4 and 1:2.1, similar to that of the human diet (Pettifor *et al*., 1984). Where calcium concentrations have been increased, the effects of phosphate are partially offset and the bone loss induced by high phosphate and/or low calcium can be reversed by reducing phosphate and/or increasing calcium. It has been argued that the Ca: P ratio of animal diets is lower than that of human diets, making animals more susceptible to metastatic calcium and other adverse effects (IOM, 1996).

Discussion

23. The effects of high phosphate levels are difficult to distinguish from those of low calcium. However, the appropriate level of calcium in the diet is also unclear, with conflicting results obtained from both metabolic balance studies in human volunteers and epidemiological investigations (Kanis, 1994).

24. Many of the changes resulting from phosphate treatment appear to represent homeostatic mechanisms to maintain plasma calcium levels. However, as indicated in the animal studies, very high levels of phosphate or low Ca:P ratios can result in significant adverse effects on bone.

Conclusions

25. The Expert Group on Vitamins and Minerals was asked to advise on a level of phosphate supplementation that would not be expected to result in any type of adverse effect. Since the data were inadequate to establish a robust Safe Upper Level, a guidance level was established by applying appropriate uncertainty factors to the limited data available.

26. Numerous studies have shown that phosphate loads result in an increase in PTH levels and changes in plasma calcium levels. The EVM concluded that phosphorus could result in adverse effects on the parathyroid hormone-calcium axis and that subjects with hypovitaminosis D could be particularly vulnerable to hyperparathyroidism. The EVM noted that physiological changes in calcium and PTH levels were associated with intakes of 1500 mg/day and above supplemental phosphorus. An uncertainty factor of 3 was applied to a NAOEL of 750 mg/day to allow for inter-individual variability resulting in a guidance level of 250 mg/day supplemental phosphorus. In addition, this level would not be expected to result in adverse gastrointestinal effects.

27. We were asked to consider the relationship of phosphate intake and bone health in detail, and to advise on the levels of phosphate intake that might be associated with adverse effects on bone. Our consideration included new data on epidemiology and on the regulation of serum phosphate that were not available to the EVM. In the light of this *we conclude* that the elevated PTH levels associated with supplemental phosphorus intakes reflect a short term adjustment to maintain plasma calcium levels and do not necessarily represent an adverse effect of phosphate on bone health. The long term effects on bone health of elevated PTH resulting from high phosphate intakes are unknown.

28. Low calcium and vitamin D intakes are known to have adverse effects on bone health since calcium in bone is resorbed to maintain serum calcium levels. It is possible that high phosphate intakes may exacerbate such effects but this is uncertain.

29. Inorganic phosphate salts have different physiological properties. It is likely that salts such as di and tri calcium phosphates, which are less soluble and also provides a source of calcium, would be of less concern with regard to adverse effects on bone.

COT statement 2004/07
October 2004

References

1. Anderson, M.P., Hunt, R.D., Griffiths, H.J., McIntyre, K.W., Zimmerman, R.E. (1977).

2. Long Term Effect of Low Dietary Calcium Phosphate Ratio on the Skeleton of *Cebus albifrons* monkeys. Journal of Nutrition, 107, 834–839.

3. Calvo, M.S. Heath, H. (1988) Acute Effects of Oral Phosphate-Salt Ingestion on Serum Phosphorus, Serum Ionized calcium, and Parathyroid Hormone. American journal of Clinical Nutrition, 47, 1025–1029.

4. Calvo, M.S. and Park, Y.K. (1996). Changing Phosphorus Content of the US Diet: Potential for Adverse Effects on Bone. Journal of Nutrition, 126, 1168S–1180S

5. Carr, C.J. and Shangraw, R.F. (1987). Nutritional and Pharmaceutical Aspects of Calcium Supplementation. American Pharmacy, NS27, 49–50, 54–57.

6. EVM (2003). Safe Upper Levels for Vitamins and Minerals. Expert Group on Vitamins and Minerals, 2003.

7. Garciá-Contreras, F., Paniagua, R., Avila–Díaz, M., Cabrera-Muniz, I., Foyo–Niembro, E., Amato, D. (2000). Cola Beverage Consumption Induces Bone Mineralization Reduction in Ovariectomized Rats. Archives of Medical Research 31, 360–365.

8. Goldsmith, R.S., Jowsey, J., Dubé, W.J., Riggs, B.L., Arnaud, C.D., Kelly, P.J. (1976). Effects of phosphorus supplementation on serum parathyroid hormone and bone morphology in osteoporosis. J Clin Endocrin. Metab. 43, 523–532.

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

10. Heaney, R.P. and Recker, R.R. (1987). Calcium Supplements: Anion Effects.Bone and Mineral Research, 2, 433–439.

11. Henderson, L., Irving, K., Gregory, J. (2003). The National Diet and Nutrition Survey: Adults aged 19–64.

12. Ilich, J.Z. and Kerstetter, J.E. (2000). Nutrition in one Health Revisited: A Story Beyond Calcium. Journal of the American College of Nutrition, 19 (6) 715–737.

13. IOM (1997). Institute of Medicine. Food and Nutrition Board (1997). Dietary Reference values for calcium, phosphorus, magnesium, vitamin D and Fluoride. Report of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. National Academy Press, Washington.

14. Jan de Beur, S.M., Finnegan, R.B., Vassiliadis, J., Cook, B., Barberio, D., Estes, S., Manavalan, P., Petroziello, J., Madden, S.L., Cho, J.Y., Kumar, R., Levine, M.A., Schiave, S.C. (2002). Tumours Associated with Oncogenic Osteomalacia Express Genes Important in Bone and Mineral Metabolism. Journal of Bone and Mineral Research, 17, 1102–1110.

15. Kanis, J.A. (1994). Calcium Nutrition and its Implications for Osteoporosis. Part 1. Children and Healthy Adults. European Journal of Clinical Nutrition, 48, 757–767.

16. Koo, W.W.W. and Tsang, R.C. (1997). Calcium, Magnesium and Phosphorus. In Nutrition during Infancy, 2nd ed, pp 175–189. Cincinnati.

17. Lemann, J. (1996). Calcium and Phosphate Metabolism: An Overview in Health and Calcium Stone Formers. In Coe, F.L., Favus, M.J., Pak, C.Y.C.,

18. Parks, J.H and Preminger G.M. (Eds) Kidney Stones: Medical and Surgical Management. Lipincott-Raven, Philadelphia.

19. McGartland, C., Robson, P.J., Murray, L., Cran, G., Savage, M.J., Watkins, D., Rooney, M., Boreham, C. (2003). Carbonated Soft Drink Consumption and Bone Mineral Density in Adolescence: The Northern Ireland Young Hearts Project. Journal of Bone and Mineral Research, 18, 1563–1569. OTC (2003). OTC Directory 2003/2004, Proprietary Association of Great Britain, London.

20. Pettifor, J.M., Marie, P.J., Sly, M.R., Du Bruyn, D., Ross, F., Isdale, J.M., deKlerk, W.A., van der Walt, W.H. (1984). The Effect of Differing Dietary Calcium and Phosphorus Contents on Mineral Metabolism and Bone Histomorphometry in Young Vitamin D-Replete Baboons. Calcified Tissue International, 36, 668–676.

21. Stoff, J.S. (1982). Phosphate Homeostasis and Hypophosphatemia. The American Journal of Medicine, 72, 489-495. Wyshak, G., Frisch, R.E., Albright, T.E., Albright, N.L., Schiff, I., Witschi, J. (1989). Non-alcoholic Carbonated Beverage Consumption and Bone Fractures Among Women Former College Athletes. Journal of Orthopaedic Research, 7 91–99.

Joint COT/CSM one day meeting on diet and drug interactions

Introduction

1 Interaction between different drugs is a well-understood phenomenon. There are also a small number of well-characterised examples of interactions between food and drugs, but the extent and significance of diet and drug interaction is unclear.

2 The COT and the Committee on the Safety of Medicines[a] (CSM) held a joint meeting on the 2nd February 2005 to consider the issue of interactions between drugs and the diet. A range of topics covering many aspects of the subjects was considered. Information from the talks and subsequent discussions is summarised in the following statement.

Background

3. Interactions between different drugs may occur as a result of previous exposure or concomitant use. Interactions can be divided into two types, pharmacokinetic[b] and pharmacodynamic[c]. Pharmacokinetic interactions involve alterations to the absorption, metabolism and excretion of a drug in the body, while pharmacodynamic interactions involve more direct alterations in the effect of the drug (e.g. via common receptors), and are less common. Elderly patients in long term care may be taking an average of 7 medications[1] so that the potential for interactions in some individuals is great.

4. Some foods or food components may also have effects on drugs (referred to in this statement as food-drug interaction). It has been suggested[2] that there are over 200 drugs whose action or toxicity is affected by food. Many of these interactions occur via a limited number of pathways such as stimulating or inhibiting a particular enzyme system, thus effects occurring in one system may be applicable to multiple drugs. The effect that the presence or absence of food may have on a particular drug and the activities of metabolic enzymes are routinely considered in the investigations of new drugs (see paragraph 24).

5. The risk of food-drug interactions is likely to be highest in individuals taking multiple medications, or drugs with a narrow therapeutic index such as warfarin, lithium, theophylline, phenytoin and levodopa, or, where individuals may be taking high doses of vitamin, mineral or herbal supplements or functional foods[d]. Foods which have a high potential for interactions include grapefruit juice, milk and dairy products, fatty foods and potassium rich foods. Not only can some food components affect drug action or toxicity, drugs may affect the way an individual handles food and thus their nutritional status (referred to here as drug-food interactions). As with drug-drug interactions, food-drug interactions can be divided into pharmacokinetic and pharmacodynamic interactions. Fewer drug-food interactions have been identified and so they are normally considered individually.

[a] The Committee on the Safety of Medicines is now known as the Commission on Human Medicines

[b] Pharmacokinetics describes the fate of a drug in the body including a mathematical account of their absorption, distribution, metabolism and excretion.

[c] Pharmacodynamics is the interaction of drugs with target sites such as receptors, leading to therapeutic or adverse effects.

[d] Functional foods are foods which are claimed to have health promoting or disease preventing properties, such as margarines containing plant sterols.

Types of food-drug interaction

Pharmacokinetic interactions

6. Some food components may lead to reduced, increased or delayed absorption of drugs depending on the intestinal contents. Drugs may form inactive complexes with food components, while the fat content of food will affect the absorption of lipid-soluble and non lipid-soluble drugs in opposing ways. Splachnic blood flow (blood flow in the internal organs) can be increased by high protein meals but is unaffected by carbohydrate[3]; increased splachnic blood flow will increase drug absorption. Ketoconazole absorption is increased by the acidity of the intestinal environment which is food dependent. The first peak in the plasma levels of a drug occurs after initial gastric emptying, a second peak in plasma levels may occur when drugs are taken with food if the presence of the food delays further gastric emptying. Non specific food-drug effects may also occur due to changes in the normal motility of the intestinal tract.

7. Examples of more specific effects of foods on the absorption, distribution, metabolism and excretion of drugs are summarised in Table 1. It should be noted that while some foods can result in a clinically significant effect on drug pharmacokinetics, many of the interactions demonstrated experimentally may not affect the overall clinical effect of a drug in most people. For example, consumption of 250 g of cabbage and Brussels sprouts was necessary to cause a decrease in plasma area under the curve (AUC) and increased clearance of oxazepam, but this did not result in a change to the mean plasma half life of the drug[4].

8. The effect of food components on the metabolism of particular drugs may be different depending on whether short term inhibition or chronic induction of an enzyme system has occurred. Reported effects on drug metabolising enzymes have mostly involved the cytochrome P450 and conjugating enzyme systems, but other enzymes may also be involved. For example, vitamin B6 (pyridoxine) stimulates peripheral dopa-decarboxylase activity which increases the metabolism and decreases the efficacy of the drug[5]. Consequently, decarboxylase inhibitors are used with L-dopa to compensate for this effect. Vitamin B6 has also been reported to reduce plasma phenytoin and phenobarbital levels because of their metabolism by pyridoxine-dependent hydroxylase[6]. The clinical significance of this latter interaction is uncertain.

Table 1. Summary of individual food-drug interactions.

Food	Drug	Interaction	Significance?	Reference
Absorption				
Milk and dairy products	Antibiotics (tetracycline and 4-quinolones)	Formation of non-absorbed chelates with calcium and magnesium ions.	Clinically significant, patients advised to leave 2 hr gap between drug and dairy consumption.	7
Milk and dairy products	Bismuth chelate	Ulcer-healing properties decreased	Clinically significant if milk volume is large.	7
High fat or carbohydrate diets	Theophylline	Bioavailability decreased by carbohydrate, increased by fat.	Could be clinically significant due to narrow therapeutic index	2
Distribution				
High fat diets	Various (includes warfarin)	Displacement of drug from albumin binding sites by free fatty acids	Significance unclear, may depend on drug concerned	8
Metabolism				
High protein, low-carbohydrate diets	Theophylline	Cytochrome P 450 (CYP) metabolism stimulated. Precise mechanism uncertain.	Could be clinically significant due to narrow therapeutic index	9
Grapefruit juice, St John's wort	Various (includes cyclosporine, terfenadine, statins, protease inhibitors)	Inhibition of CYP 3A4 enzyme-mediated metabolism	Yes, dosage adjustment may be required.	10
Grapefruit juice, St John's wort	Various	Inhibition of plasma glycoprotein drug efflux transporter MDR1	Unclear	10
Cruciferous vegetables	Antipyrine, phenacetin	Indoles induce CYP1A2	Unlikely to be of significance. Modest effect on CYP1A2 induction.	5 10
Cooked meats	Phenacetin	CYP1A induced by heterocyclic amines decreases bioavailability.	Modest effects only	10
Broccoli	Chlorzax azone	CYP2E1 inhibited by sulphorane	Unclear	10
Brussels sprouts, cabbage	Paracetamol	Glucuronidation and clearance increased	Unclear – likely to be modest at normal dietary intakes.	4
	Oxazepam	Clearance also increase but not by increased glucuronidation	Unlikely to be significant.	4
Brussels sprouts, cabbage.	Paracetamol metabolites (and other compounds detoxified by glutathione conjugation.	Glutathione S transferase induced by sulphoranes, increases metabolism.	Unlikely to result in significant effects	10
Vitamin B6	L-dopa	Stimulation of peripheral dopa-decarboxylase	Yes, supplementation with pyridoxine not recommended	5

Table 1. Summary of individual food-drug interactions *(continued)*.

Food	Drug	Interaction	Significance?	Reference
Excretion				
Fruit, vegetables	Phenobarbital	Increasing urinary alkalinity increases excretion	Unclear	8
Meat, eggs	Amphetamine	Increasing urinary acidity increases excretion	Unclear	8

Pharmacodynamic interactions

9. Pharmacodynamic interactions, which include pharmacological and receptor interactions are less common. Interactions of food constituents with drug targets are known to occur *in vitro* but often the concentrations occurring *in vivo* may be too low to be significant clinically. For example, curcumin a component of turmeric is known to be an inhibitor of COX-2 (cyclo-oxygenase 2)[e, 11]. However curcumin is poorly absorbed *in vivo*[12] and so is unlikely to have any effect at realistic dietary intakes.

10. One of the most well documented pharmacodynamic interactions involves monoamine oxidase inhibitors (MAOIs) and the amino acid tyramine which is found in a variety of aged, overripe and pickled foods and to a lesser extent in chocolate and yeast-containing foods[2]. Tyramine is indirectly sympathomimetic, that is, it mimics the actions of hormones of the sympathetic nervous system such as noradrenaline. By suppressing its metabolism, MAOIs elevate noradrenaline levels in the circulation. However, tyramine metabolism is also suppressed, thus intact tyramine can then enter the circulation and release noradrenaline from local stores in the nerve endings. This prolongs the action of noradrenaline on the adrenergic receptors, potentially resulting in a marked increase in blood pressure, cardiac arrhythmia, hyperthermia and cerebral haemorrhage. Similarly Ma huang, an ephedra containing herbal medicine, can react with MAOIs resulting in a hypertensive crisis[2].

11. The medicinal herb St John's wort which is used to treat depression, has mild inhibitory effects on monoamine oxidase and serotonin reuptake, which may result in pharmacodynamic interactions with SSRI (selective serotonin reuptake inhibitor) drugs such as fluoxetine and paroxetine[13].

[e] An enzyme which mediates prostaglandin synthesis during inflammation.

Notable diet-drug interactions

Grapefruit juice

12. Among the best known food–drug interactions are those of grapefruit juice and drugs metabolised by CYP3A4. Grapefruit juice inhibits CYP3A4 which is the major form of cytochrome P450 in the human liver and gut. Inhibition of CYP3A in the gut by the furanocoumarins present in grapefruit juice may result in increased drug bioavailability, this in turn may result in enhanced or reduced drug activity and/or increased side effects. Up to half of clinically used drugs are metabolised by CYP3A4, resulting in a number of potentially significant adverse effects. For example, the effects of calcium channel blockers are enhanced resulting in a risk of hypertension and myocardial ischaemia[14]. Similarly, the central nervous system (CNS) depressant effects of benzodiazepines are increased as a result of increased absorption. Other drugs affected by grapefruit juice include hydroxymethylglutaryl Co-enxyme A (HMG-Co-A) reductase anti-cholesterol drugs, immunosuppressants such as cyclosporin, protease inhibitors, and anti-histamines.

13. The multidrug resistance 1 (MDR1) glycoprotein is a drug transporter that is inhibited by grapefruit juice. MDR1 transports drug molecules from the cells of the gut wall back into the intestinal lumen, thus inhibition of MDR1 can also result in increased bioavailability of the drug[15]. Thus the effect of grapefruit juice on the bioavailability of certain drugs can be further enhanced by its effects on the MDR1 glycoprotein since many substrates for CYP3A4 are also substrates for MDR 1.

14. The plasma concentration of the anti-histamine fexofenadine is decreased by grapefruit juice as result of inhibition of intestinal organic anion transporting polypeptide A[16].

15. Alteration to drug metabolism may occur with consumption of both processed and freshly squeezed grapefruit juice and grapefruit segments. The maximum interaction occurs between 0 and 4 hours of consuming grapefruit but some interaction may persist for up to 24 hours[17].

Alcohol

16. Ethanol has both pharmacokinetic and pharmacodynamic effects, and can interact both with drugs and with food[18]. Pharmacokinetic interactions generally occur in the liver and involve many classes of drugs including antibiotics and antihistamines. For example, ethanol induces CYP2E1, which may lead to either increased or decreased efficacy or toxicity, depending on the drug. The ethanol induced increase in CYP2E1 enhances the breakdown of paracetamol increasing the formation of the toxic quinonimide products and the depletion of glutathione, which in turn may result in an increased risk of paracetamol hepatotoxicity[5]. Ethanol may potentiate the sedative effect of opiates and benzodiazepines by pharmacodynamic interactions with the GABA (gamma amino butyric acid) complex of receptors.

17. Ethanol can also contribute towards vitamin deficiency, hinder the absorption of drugs and result in abnormal vitamin D metabolism, via interference with steroid metabolism. Inhibition of alcohol dehydrogenase by cephalosporins and ketoconazole reduces ethanol breakdown.

Warfarin

18. The indoles present in green vegetables are able to induce CYPIA2 increasing warfarin metabolism and thus may reduce its effectiveness and increase the risk of clotting[10]. Warfarin efficacy is also reduced by competition with high levels of dietary vitamin K which favour synthesis of clotting factors. Cranberry juice has been reported to reduce warfarin metabolism, possibly via inhibition of CYP2C9 by the flavanoids present in the juice[19]. Other foods reported to reduce warfarin efficacy include large quantities of ice cream, soya beans and avocados[7]. The clinical significance and prevalence of these latter reports is unclear.

Lithium

19. Dietary sodium restriction may increase tubular reabsorption of therapeutic agents in the kidney as normal homeostatic mechanisms operate to maintain sodium levels. For example, serum lithium is reabsorbed along with sodium and thus can be increased to potentially toxic levels[20, 21]. Increased sodium intake may have the converse effect. Once stabilised on lithium, patients should not alter their sodium intake.

Herbals

20. Herbal products may have the legal status of either medicines or of food. A recent literature review[22] of theoretically possible interactions involving complementary and alternative medicine reported that in the last 15 years, there were 56 articles reporting interactions between drugs and herbs, 44 of which concerned St John's Wort (see paragraph 22 below) compared to 44 articles reporting interactions between food and drugs, 24 of which were related to grapefruit juice.

21. There are several aspects of herbal products which increase their potential for interaction with food or drugs. Synthetic drugs tend to have one active ingredient, while herbal medications usually have several active components, thereby increasing the potential for interactions with other drugs or components of the diet. The composition of synthetic drugs is constant, the composition of herbal medicines can vary. In addition, the therapeutic window of herbal drugs is usually wide, while that of synthetic drugs tends to be narrow. Identification of interactions may be difficult because many patients do not inform their doctors that they are taking herbal or other complementary products.

22. The medicinal herb St John's wort can interact with a variety of drugs. It can induce CYP3A4 activity resulting in a reduction in plasma levels of drugs such as cyclosporin or the anti-HIV drug indinavir. It is thought that the effect on CYP3A4 may occur via interaction with the pregnane X receptor (PXR) which induces CYP3A4 activity[23]. St John's wort can also induce the MDR1 transporter, reducing, for example, plasma concentrations of digoxin[24].

Drug-food interactions

23. As noted above, there are a few examples of drugs which affect an individual's handling of food and consequently their nutritional status. This may be due to the mode of action of the drug, for example, diuretics may promote urinary mineral loss and anti-cholesterol drugs may deplete fat soluble vitamins. It is therefore important to seek a balance between optimising a patient's nutritional needs and the drug levels necessary for efficacy. More specifically, anti-convulsant drugs such as phenytoin may act as folate antagonists and precipitate folate deficiency[25]. Drug-food effects may be less direct; for example antibiotics may alter enteric micro-organisms resulting in reduced absorption of fat-soluble vitamins[26]. Drug-induced enzyme activity may increase the metabolism of vitamins.

Prediction of potential interactions

24. Pharmaceutical companies are required to consider potential drug-drug interactions as part of the pre-clinical data supporting applications for marketing authorisations for new chemical entities. An interaction is considered to be clinically relevant when the therapeutic activity or toxicity of the drug is affected to such an extent that adjustment of the medication or medical intervention is required, or when concomitant use of drugs could occur when both are used as therapeutically recommended.

25. Guidelines are available[27] which cover pharmacokinetic and pharmacodynamic issues, though these do not take into account factors such as age, gender, ethnic origin, physical activity and time of administration (which may affect drug disposition). Relevant factors that should be studied include: the effect of food on the drug; the physicochemical properties of the drug and formulation; pharmacokinetics, pharmacodynamic properties such as effects on the gut (motility, pH, bile secretion, blood flow); the potential for toxic damage to the gastrointestinal tract. Potential inhibition of MDR1 should be considered, as should factors affecting drug distribution which may influence interactions. For example, the displacement of drugs from plasma proteins would be assessed by protein binding, tissue distribution and other studies.

26. Metabolic interaction studies are mainly required where metabolic pathways account for 30% or more of drug elimination or where active or toxic metabolites are formed by minor pathways. The primary CYP isozymes responsible for drug metabolism are determined, and it also is established whether the drug is an inducer or inhibitor of these systems. Phase II metabolism (conjugation of the initial metabolites produced by Phase I metabolism) should also be assessed. A variety of these enzyme systems could be affected by food or food components, but this is not specifically assessed.

27. Pharmacodynamic effects may be predictable from the pharmacokinetic profile of the drug, if it is likely to be co-administered with drugs which have a mechanism or end organ response that could result in additivity, synergy, or opposing effects. Examples include MAOIs and other antidepressants. Information is necessary on whether the mechanism is primary or secondary. The product would undergo *in vitro* receptor profiling with a range of ligand binding studies (on receptors, ion channels and transporters); positive binding results might require *in vitro* or *in vivo* functional tests to look at agonist or antagonist effects. It should then be considered whether any effects would occur at the therapeutic dose.

28. In the EU, specific interactions between drugs and food do not have to be considered and clinical trials are not conducted using a standard diet.

Vulnerable groups

29. It is possible that certain population sub-groups may be more vulnerable to the effects of food-drug and drug-food interactions, although information on this area is limited.

30. The elderly may have differences in body composition which affect drug distribution[28], while reduced absorption occurs in the frail elderly who are also prone to malabsorption syndrome, eat inappropriate food and who may suffer from inflammatory bowel disease or other chronic diseases. While the activity of some metabolic enzymes is unchanged in older people, liver blood flow and size are reduced so that overall metabolic clearance of xenobiotics is reduced[29]. Renal excretion is also reduced in older people. Many older people may be taking multiple medicines increasing the potential for interactions[26].

31. Children may be vulnerable to potential interactions as they have high inter-individual variability and different, frequently higher, nutrient requirements. Whilst dosages for children can be estimated by allowing for bodyweight or body surface area this does not account for other age-related pharmacokinetic differences such as organ development[28].

Obesity

32. Diet may have an indirect effect on drug action mediated by body weight. Obesity is characterised by a number of changes in body composition[30]. These include increased adipose and lean tissue mass, increased organ mass with fatty infiltration, increased cardiac outflow and blood volume and changes in plasma protein binding.

33. The absorption of drugs in the clinically obese may occur in an unpredictable fashion[31]. The volume of distribution for a drug might be difficult to predict, for example, whether lipophilic drugs will be absorbed into fat tissues and, if so, what the consequences of such absorption might be. Obese patients may have an increased volume of distribution into adipose tissue for barbiturates and diazepam, whereas digoxin and cyclosporin may have a lower volume of distribution than expected.

34. Characteristic alterations in lipoproteins may inhibit protein binding of drugs, for example, α-1-acid glycoprotein may increase the degree of protein binding of triazolam and propranolol[31]. However, albumin binding is generally unaffected[32].

35. Obese patients are at an increased risk of venous thrombotic embolism (VTE). An even greater risk of VTE is associated with being overweight (BMI ›25) and using combined oral contraception, where the risk of VTE is 10-fold higher than in women of lower body weight[33].

36. In females, obesity is linked with an increased conversion of oestradiol to oestrone and an increased production of testosterone. There is also an association between increased body weight (›70 kg) and an increased risk of contraceptive failure[34].

37. Obesity may also alter hepatic oxidative metabolism of some drugs, for example, CYP 2E1 levels are increased resulting in a predisposition to drug toxicity mediated by this enzyme[35]. Changes have also been reported for the phase II conjugation pathways in the liver, such as glucuronidation and sulphation. Drugs such as lorazepam and oxazepam are more rapidly cleared from the blood in obese patients due to increased excretion as a glucuronide conjugate[31]. However, the pharmacokinetics of other pharmaceuticals, such as salicylates and procainamide, are not significantly altered.

38. Increased adipose tissue results in increased clearance of prednisolone, prednisone and carbamazepine[31] changes in oestradiol metabolism, and increased resistance to insulin[30]. Some of the reported changes are reversed on weight loss. Renal function can also be affected in obesity. Glomerular filtration rates and tubular secretion are increased and as a result, drugs such as ciprofloxacin, cimetidin, procainamide and lithium are more rapidly cleared[31].

39. Treatment for obesity may also influence drug action. For example, obese patients may undergo gastric surgical procedures to limit the ability to digest consumed food, or be prescribed pancreatic lipase inhibitors such as orlistat, which blocks the hydrolysis of dietary fat and thereby inhibits its absorption. This may have an impact upon, for example, the absorption of fat-soluble vitamins.

Future issues

40. The range of foods available is developing rapidly, resulting in a potential for more interactions in the future. Some of these possibilities are considered below.

41. Some functional foods are designed to promote well being by altering the microbiological content of the intestine. The enterohepatic circulation of many drugs depends on microfloral ß-glucuronidase which itself depends on the microfloral population. Thus functional foods of this type might result in food-drug interactions.

42. The phytoestrogen genistein is an isoflavanoid found in soya. Genestein has multiple effects on receptors, enzymes and a variety of their activities *in vivo* and *in vitro*[36]. These include induction via the steroid and xenobiotic pregnane X receptor, inhibition of thyroid peroxidase, topoisomerase II, protein kinases, *in vivo*, cell proliferation. Genestein is also a potent sulphotransferase inhibitor, and inhibits the enzymes glucuronidase and aromatase, 17ß-hydroxysteroid oxidoreductases. It can modulate sex hormone binding protein and has a high affinity for the estrogen receptor. These multiple activities could have the potential for both pharmacokinetic and pharmacodynamic interactions affecting hormone control and therapy.

43. Current health advice is to reduce the sodium content of the diet. However, it should not be assumed that replacement of sodium by potassium salts, either domestically or by food manufacturers, would necessarily have health benefits. Increased potassium levels can lead to hyperkalaemia and in turn to cardiac abnormalities. Drugs such as potassium-sparing diuretics, ACE inhibitors and COX-2 anti-inflammatory agents are known to result in elevated potassium as a result of impaired potassium clearance; in combination with a potassium rich diet, serious interactions could occur[37]. Elevated potassium is of particular concern in subjects with impaired renal function who are unable to regulate potassium levels.

Conclusions

44. Food and food constituents have the potential to affect all aspects of the absorption, distribution, metabolism and excretion of drugs. The effects may vary depending on whether there is single, concomitant or prolonged exposure. Certain food constituents alter metabolic parameters such as enzymes which can have opposing or unpredictable effects on different drugs. Often the most appropriate practical advice is that patients stabilised on a particular drug should not change their diet.

45. Food itself is a complex mixture of both macro and micronutrients, making interactions and potential interactions difficult to assess. The use of food supplements such as micronutrients and herbal products also increase the scope for interactions.

46. Interactions have most frequently been demonstrated under experimental conditions. To assess the clinical significance of individual reactions, it is necessary to consider the likely consumption of the food and drug and the severity of the potential outcome. For example the well-documented interactions between grapefruit juice and a range of drugs is widely known and clinically important since it could result in severe outcomes in a few individuals. In contrast, milk and dairy products can reduce the uptake of certain antibiotics, and both milk and dairy products and antibiotics are widely consumed. In practice, however, interactions between them are likely to be overlooked as they are unlikely to be life threatening.

47. There are few data on vulnerable groups but it seems that the most likely population subgroups vulnerable to food-drug interactions are the elderly, particularly the frail elderly, and children.

48. In some cases practitioners have built up knowledge about interactions in specific groups of patients taking particular drugs such as the interaction between St John's wort and anti-HIV drugs such as indinavir. It is important that this type of information is appropriately disseminated.

49. On the basis of current evidence the issue of food-drug interaction is a real one but not one of great clinical significance for the majority of the population. However, data are limited and further interactions may be identified in the future. Rather than widespread public information, it is most appropriate that practitioners and prescribers should be aware of the potential for food-drug interactions and should consider them both when prescribing drugs and where lack of efficacy or unexpected adverse reactions have occurred.

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References

1. KUMC (2005) Kansas University Medical Center. http://classes.kumc.edu/som/amed900/polypharmcay/polypharmacy-Know.htm#

2. Bland, S.E. (1998). Drug-food interaction, *Journal of the Pharmacy Society of Wisconsin*, Nov/Dec 1998, 28-35.

3. D'Arcy,P.F. (1995). Nutrient-drug interactions. *Adverse Drug React Toxicol Rev*, 14, 233-354.

4. Pantuck, E.J., Pantuck, C.B., Anderson, K.E., Wattenberg, L.W., Conney, A.H., Kappas, A. (1984). Effects of Brussels sprouts on drug conjugation. *Clinical Pharmacology and Therapeutics*, 35, 161-169.

5. Williams, L., Hill, D.P., Davis. J.A., Lowenthal, D.T. (1996). The influence of food on the absorption and metabolism of drugs: an update. European *Journal of Drug Metabolism and Pharmacokinetics*, 21, 201-211.

6. Hansson, O., Sillanpaa, M. (1976). Pyridoxine and serum concentration of phenytoin and phenobarbitone. *The Lancet*, 7953, 256.

7. Mason, P. (2002). Drug-food interactions (1). Food and medicines. *The Pharmaceutical Journal*, 269, 571-573.

8. Hathcock, J.N. (1985). Metabolic mechanisms of drug-nutrient interactions. *Federation Proceedings*, 44, 124-129.

9. Kappas, A., Anderson, K.E., Conney, A.H., Alvares, A.P. (1976). Influence of dietary protein and carbohydrate on antipyrine and theophylline metabolism. *Diet and Drug Metabolism*, 20, 643-653.

10. Harris, R.Z., Jang, R.G., Tsunoda, S. (2003). Dietary effects on drug metabolism and transport. *Clinical Pharmacokinetics*, 42, 1071-1088.

11. Plummer, S.M., Holloway, K.M., Manson, M.M., Munks,R.J., Kaptein, A., Farrow, S., Howells,L. (1999). Inhibition of cyclo-oxygenase 2 expression in Colin cells by the chemopreventative agent curcumin Involves inhibition of NF-kappa-B activation via the NIK/NKK signalling complex. *Oncogene*, 18, 6013-6020.

12. Garcea, G., Jones, D.J., Singh,R., Dennison, A.R., Farmer, P.B., Sharma, R.A., Steward, W.P., Gescher, A.J., Berry, D.P. (2004). Detection of curcumin and its metabolites in hepatic tissue and portal blood of patients following oral administration. *British Journal of Cancer*, 90, 1011-1015.

13. MCA (2000). Medicines Control Agency. Fact sheet for healthcare professionals. Interactions of St John's Wort (*Hypericum perforatum*) preparation. http://medicines.mhra.gov.uk/ourwork/licensingmeds/herbalmeds/furtherissues.htm#stjohns

14. Dahan, A., Altman, H. (2004). Food-drug interaction: Grapefruit juice augments drug bioavailability – mechanism, extent and relevance. *European Journal of Clinical Nutrition*, 58, 1-9.

15. Dresser, G.K., Bailey. D.G. (2003). The effects of fruit juices on drug disposition: a new model for drug interactions. *European Journal of Clinical Investigation*, 33, 10-16.

16. Dresser, G.K., Kim, R.B., Bailey. D.G. (2005). Effects of grapefruit juice volume on the reduction of fexofenadine bioavailability: Possible role of organic anion transporting polypeptide. *Clinical Pharmacol Ther*, 77, 170-177.

17. Bailey, D.G. Malcolm, J., Arnold, O., Spence, JD. (1998). Grapefruit juice- drug interactions. *British Journal of Clinical Pharmacology*, 46, 101-110.

18. Weathermon, R., Crabb, D.W. (1999). Alcohol and medication interactions. *Alcohol Research and Health*, 23, 40-54.

19. Suvarna, R., Pirmohamed, M., Henderson, L. (2003). Possible interaction between warfarin and cranberry juice. *British Medical Journal*, 327, 1454.

20. Bennett, W.M. (1997). Drug interactions and consequences of sodium restriction. *American Journal of Clinical Nutrition*, 65 (suppl) 678S- 681S.

21. Gray , G.E., Gray, L.K. (1989). Nutritional aspects of psychiatric disorders. *Journal of the American Dietetic Association*, 89, 1492-1498.

22. Schulz V. (2004). Arzneimittelinteraktion- Relevanz für Phytopharmaka? (Pharmacokinetic- Drug Interaction. Significance for Herbal Medicnes?) *Zeitschr Phytother*, 25, 283.

23. Moore, L.B., Goodwin, B., Jones,S.A., Wisley, G.G., Serabjit-Singh, C.J., Wilson, T.M., Collins, J.L., Kliewer, S.A. (2000). St John's Wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proceeding of the National Academy of Sciences*, 97, 7500-7502.

24. Ohnishi, N., Yokoyama, T. (2004). Interactions between medicines and functional foods or dietary supplements. *Keio Journal of Medicine*, 53, 137-150.

25. Matsui, M.S,, Rozovski, S.J. (1982). Drug-nutrient interaction. *Clinical Therapeutics*, 4, 423-441.

26. Thomas, J.A. (1995) Drug-nutrient interactions. *Nutrition Reviews*, 53, 271-282.

27. EMEA (1997) The European Agency for the Evaluation of Medicinal Products, Committee for Proprietary Medicinal Products, Note for Guidance on the Investigation of Drug Interactions. CPMP/EWP/560/95.

28. Singh, B.N. (1999). Effects of food on clinical pharmacokinetics. *Clin Pharmacokinetics*, 37, 213-255.

29. Thomas, J.A., Burns, R.A. (1998) Important drug-nutrient interactions in the elderly. *Drugs & Ageing*, 13, 199-209.

30. Kopelman, P.G. (2000). Obesity as a medical problem. *Nature*, 404, 635-643.

31. Blouin, R.A., Warren, G.W. (1999). Pharmacokinetic considerations in obesity. *Journal of Pharmaceutical Sciences*, 88, 1-7.

32. Abernethy, D.R., Greenblatt, D.J. (1986). Drug disposition in obese humans. an update. *Clinical Pharmacokinetics*, 11 199-213.

33. Abdollahi. M., Cushman, M., Rosendaal, F.R. (2003). Obesity: risk of venous thrombosis and the interaction with coagulation factor levels and oral contraceptive use. *Thromb Haemostat*, 89, 493-498.

34. Mansour, D. (2004). Implications of the growing obesity epidemic on contraception and reproductive health. *Journal of Family Planning and Reproductive Health*, 30, 209-21.

35. O'Shea, D,, Davis, S.N., Kim, R.B., Wilkinson, G.R. (1994). Effect of fasting and obesity in humans on the 6-hydroxylation of chlorzoxazone: A putative Probe of CYP2E1 Activity. *Clin Pharmacol Ther*, 56, 359-367.

36. COT (2003) Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment. Report on Phytoestrogens and Health. Food Standards Agency, London. http://www.food.gov.uk/science/ouradvisors/toxicity/reports/phytoestrogensandhealthcot

37. Hay, E., Dereaon, H., Bukish, N., Katz, L., Kruglyakov, I., Armoni, M. (2002). Fatal hyperkalaemia related to combined therapy with a COX-2 inhibitor, ACE inhibitor and potassium rich diet. *The Journal of Emergency Medicine*, 22, 349-352.

Food additives and development neurotoxicity

Background

1. The European Food Safety Authority (EFSA) has been asked to review the food additives currently permitted within the EU in order to determine whether full re-evaluation is required. The COT has been invited to contribute to the re-evaluation process, with a particular focus on neurotoxicity, because there have been recent developments in the toxicological approaches in this area.

2. The additives reviewed by the COT have previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Scientific Committee on Food (SCF). However, a number of the evaluations, particularly those for the colours, were conducted more than 20 years ago. It is appropriate therefore to consider whether the potential for neurotoxicity and developmental effects was taken into account in setting the acceptable daily intake (ADI) for certain food additives and whether significant new data of relevance to neurodevelopmental effects have emerged since the ADI was set. It should be noted that the original data for many of these assessments were submitted directly to the committees concerned and due to the passage of time are unlikely to be available except in a summary form.

3. The additives considered were quinoline yellow (E104), sunset yellow (E110), carmoisine (E122), Ponceau 4R (E124), indigo carmine (E132), brilliant blue (E133), sodium benzoate (E211), sulphur dioxide (E220), monosodium glutamate (E621), acesulfame K (E950), aspartame (E951) and saccharin (E954).

4. The data for most of the colouring agents are limited and the original assessments were conducted more than 20 years ago, with few details of the studies reviewed being included in the reports. There are some new data on some of the colours that may be of relevance when considering whether these substances have neurotoxic potential.

5. Developmental neurotoxicity studies are most useful where there is potential for significant pre- or post-natal exposure of the developing brain. They are particularly likely to be needed if the test agent is known to have neurotoxic potential in adults. The best tests are specially devised developmental neurotoxicity screens for learning and behaviour, and quantitative or semi-quantitative morphological examinations of a range of brain structures[1]. In the absence of these, a standard Functional Observational Battery in the F_1 generation as young adults, and general morphological observations on the brain based on at least 3 cross-sectional levels would be a substitute that should detect gross effects, though this would be of low sensitivity. If these measures are absent, the simple ability to feed and mate, and a normal appearance of the brain at 1 or 2 sectional levels in a standard two generation study do provide some reassurance, but may not be very sensitive indices of developmental neurotoxicity. However, it has been noted that for some pesticides, the NOAELs and LOAELs were not significantly lower in specific developmental tests than in standard toxicology tests (developmental toxicity, multi-generation toxicity, acute and short-term toxicity)[2].

Summary of data available

Quinoline Yellow (E104)

6. Quinoline yellow is a synthetic, yellow food colouring (see Figure 1).

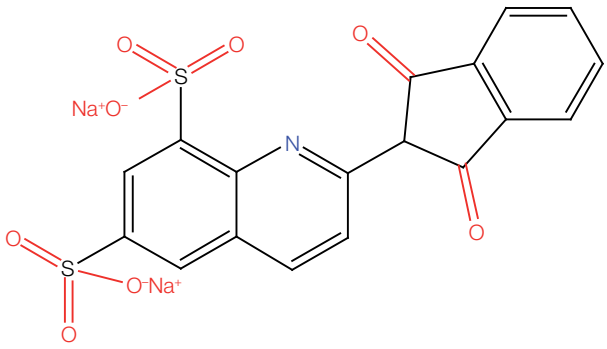


Figure 1. Quinoline yellow (disodium 2-(1,3-dioxo-2-indanyl)-6,8-quinolinesulphate)

7. Quinoline yellow was evaluated by the SCF[3] and by JECFA[4] . The toxicity data assessed by the committees included acute and short-term studies, long-term studies in the rat and mouse and multigeneration, reproduction and teratology studies

8. Both the SCF and JECFA derived an ADI from a two-generation study in which OFI mice were fed diet containing up to 1% quinoline yellow. A No Observed Adverse Effect Level (NOAEL) of 1% of the diet (equivalent to 1000 mg/kg bw/day) was observed and an ADI of 0-10 mg/kg bw/day established. Neurotoxicity was not specifically investigated in the study. However, histological examination of a number of tissues including the brain, optic nerve, and spinal column did not reveal any abnormalities. Brain weights were also unaffected by treatment.

9. A subsequent study by Osman et al.[5] reported that quinoline yellow could inhibit both true (acetyl) and pseudo (butyryl) cholinesterase (ChE) enzymes in human erythrocytes and plasma in vitro. A dose dependent decrease in the activity of pseudo and true ChE activity of up to 53% and 87% respectively was reported. Blood acetylcholinesterase inhibition greater than 20% is generally considered to be adverse, whereas inhibition of pseudo-ChE by itself is not usually considered adverse. The authors did not comment on the in vivo relevance of the concentrations tested.

10. COT view: A biologically relevant reduction in ChE activity would be clearly visible in animal studies. A 70% decrease in activity would result in cholinergic activity such as increased salivation, urination and defecation. These symptoms have not been reported in any of the available animal studies. Structurally, quinoline yellow would be unlikely to cross the mature blood-brain barrier but the developing brain could be exposed via lactation. The two-generation study provided limited reassurance that no gross effects occurred since the parental animals were able to feed and rear a second generation. Adverse histological effects were not observed in the brain or in other nervous tissues, but details of the histopathology were not available. It is unclear what observations were made of the animals' behaviour

Sunset Yellow (E110)

11. Sunset yellow is a synthetic, yellow azo dye that is used as a food colouring (see Figure 2).

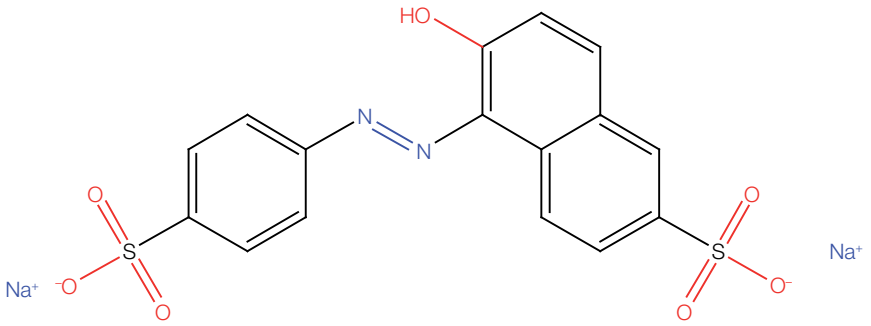


Figure 2. Sunset yellow (disodium 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalene-sulfonate)

12. Sunset yellow was evaluated by JECFA[6] and the SCF[3]. The toxicity data assessed by the committees included acute and short-term studies, multi-generation, reproduction and teratology studies and long-term studies in the rat, mouse and dog.

13. JECFA established an ADI of 0-2.5 mg/kg bw/day on the basis of a NOAEL of 2% in the diet (equivalent to 500 mg/kg bw) in a 7-year feeding study in dogs and a NOAEL of 1% in the diet (equivalent to 500 mg/kg bw) in long term rat studies.

14. The SCF also established an ADI of 0-2.5 mg/kg bw/day on the basis of a NOAEL of 250 mg/kg bw in a long term feeding study (the precise duration is not stated) in dogs though this was not the same one as that used by JECFA).

15. A summary of a three-generation reproductive study in rodents[7] was available to both JECFA and the SCF. The actual doses used (1, 10, 30 and 100 times the ADI) were not reported although it was noted that no doses in excess of 1000 mg/kg bw/day were used. No effects on reproductive performance were reported through to the F_{2b} generation. Neurotoxicity does not appear to have been specifically investigated in this study.

16. The Registry of Toxic Effects of Chemical Substances (RTECS) report prepared by the National Institute for Occupational Safety and Health (NIOSH) listed sunset yellow as having behavioural effects in two rodent studies including coma and effect on seizure threshold. However, both were acute (LD_{50}) studies in which very high doses of sunset yellow were administered by intraperitoneal (i.p.) injection, convulsions and coma occurring prior to death in some animals. The report is based on studies[8] which additionally reported that doses of up to 6g/kg bw in mice and 10/kg bw in rats were without adverse effects other than slight diarrhoea.

17. Tanaka (1996)[9] investigated the effect of sunset yellow on selected reproductive and neurobehavioural parameters in rats. Sunset yellow was administered in the diet at dose levels of 0, 243, 493 and 999 mg/kg bw/day during gestation, the middle dose being approximately equivalent to the NOAEL identified by JECFA and used to derive the ADI.

18. There were no adverse effects on litter size, weight or sex ratio. Some changes in the body weights and survival of offspring in some groups were reported but these were not dose related. A range of neurobehavioural parameters was then measured in the F_1 generation. Of these, swimming direction on post-natal day (PND) 4 was significantly affected in the mid and top dose group males and in females in all dose groups. Swimming head angle was also affected on PND 4 in the mid and top dose group females. Treatment effects on water T-maze performance were also reported, but with no clear pattern. The authors concluded that sunset yellow had some adverse effects on reproductive and neurobehavioural parameters. It was not possible to determine a NOAEL from this study.

19. Osman *et al.* (2002)[5] reported that sunset yellow could inhibit both true and pseudo-ChE enzymes in human erythrocytes and plasma *in vitro* in a concentration dependent manner with inhibition of pseudo-ChE and true ChE of 43% and 70% respectively at the highest concentration used.

20. Osman *et al.* (2004)[10] reported that in rats fed 4 mg/kg bw sunset yellow or its metabolite sulphanilic acid, pseudo ChE enzymes in plasma were inhibited by 23 and 14.5% respectively and true-ChE in red blood cells by 14.5 and 30.6%. The authors did not report whether any clinical symptoms were apparent. In studies with human blood *in vitro*, sulphanilic acid was reported to result in dose related inhibition of pseudo and true ChE activity of up to 50%.

21. COT view: A biologically relevant reduction in brain ChE activity would be clearly visible in animal studies. A 70% decrease in activity would result in cholinergic activity such as increased salivation, urination and defecation. Of these symptoms, only diarrhoea has been reported in the available animal studies (see paragraph 16). Structurally, sunset yellow would be unlikely to cross the mature blood-brain barrier but the developing brain could be exposed via lactation. Potential adult neurotoxicity was only seen at lethal doses of sunset yellow, given by i.p. injection. The three-generation study provides limited reassurance that no gross effects occurred since parental animals were able to feed and rear two further generations.

22. Sunset yellow is the only colour of those evaluated here that has undergone specific neurodevelopmental assessment. The Tanaka study had multiple endpoints increasing the possibility of confounding, and gave mixed results. Sunset yellow appears to have had an effect on swimming behaviour at 4 days of age, though this did not persist at later ages. In contrast treatment appeared to enhance maze learning at 7 weeks of age but this may have been due to poor performance in the controls.

Carmoisine (E122)

23. Carmoisine (azorubine, E122) is a synthetic, red azo dye that is used as a food colouring agent (see Figure 3).

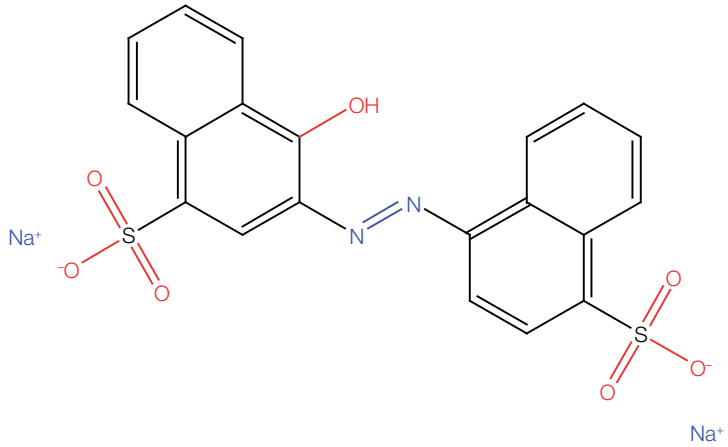


Figure 3. Carmoisine (disodium 4-hydroxy-3-(4-sulfonato-1-naphthylazo)-1-naphthalene-sulfonate)

24. Carmoisine was evaluated in 1983 by JECFA[11] and the SCF[3]. The animal studies assessed included acute and short-term studies, multigeneration, reproduction and teratology studies and long-term studies in the rat and mouse. Both JECFA and the SCF derived an ADI of 0-4 mg/kg bw/day on the basis of a NOAEL equivalent to 400 mg/kg bw/day in a 1-year study in rats[12].

25. In a four-generation rat study using doses up to 2% in the diet, there were no adverse effects on fertility, viability or lactation indices[13]. Rats from the 3_b generation were subsequently exposed to dietary concentrations of carmoisine up to 2% for 1-year. There were no adverse effects on bodyweight gains, urinalysis, haematology, gross pathology or histology (the latter did not include brain tissue).

26. Two additional multi-generation reproduction studies of carmoisine were also available to the committees, in which no treatment related adverse effects were apparent. However, neurological effects were not specifically investigated in any of the multigeneration reproduction studies.

27. The RTECS report listed carmoisine as having behavioural effects in rodent studies including somnolence, coma and convulsions or effect on seizure threshold. The report is based on an LD_{50} study[14] where coma and convulsions often preceded death following i.p. administration of high doses of carmoisine. However it was also reported that oral doses of up to 8g/kg bw in mice and 10/kg bw in rats were without adverse effect other than slight lethargy.

28. Since the evaluation of carmoisine by JECFA and SCF, two additional studies have been published. In a two-generation reproduction study[15] rats were fed up to 1200 mg/kg bw/day carmoisine, the F_1 generation being exposed for 110-115 weeks. No adverse clinical effects or effects on behaviour or reproductive performance were reported in the F_0 generation. Mortality in the F_1 generation was not affected by treatment. The tissues sampled for histopathology included brain, sciatic nerve and spinal cord and no adverse effects or differences in tumour incidence were reported. Neurotoxicity was not specifically investigated in this study.

29. Osman *et al.* (2004)[10] reported that carmoisine inhibited both true and pseudo-ChE enzymes in human erythrocytes and plasma in a dose-dependent manner with up to 50% inhibition of both pseudo and true ChE produced at the highest concentrations of carmoisine tested. Inhibition of these enzymes was reversible. Naphthionic acid, a metabolite of carmoisine, was also able to inhibit both pseudo and true ChE *in vitro*.

30. Osman *et al.* (2004)[10] also reported that in rats fed 4 mg/kg bw carmoisine or napthionic acid for up to 7 days pseudo-Che was inhibited by 15.1 and 18.5% respectively. True ChE was inhibited by 27.8% in the animals fed carmoisine but was unaffected in those given napthionic acid. The authors did not mention whether any adverse clinical effects were apparent.

31. COT view. A biologically relevant reduction in brain ChE activity would be clearly visible in animal studies. A 70% decrease in activity would result in cholinergic activity such as increased salivation, urination and defecation. Symptoms of this type were not reported in any of the available animal studies. Structurally, carmoisine would be unlikely to cross the mature blood-brain barrier but the developing brain could be exposed via lactation. Potential adult neurotoxicity was only seen at lethal doses, given by i.p. injection. The three-generation study provides some reassurance that no gross effects occurred since parental animals were able to feed and rear through two further generations.

Ponceau 4R (E124)

32. Ponceau 4R is a synthetically produced azo dye that is used as a red colouring for foodstuffs (see Figure 4).

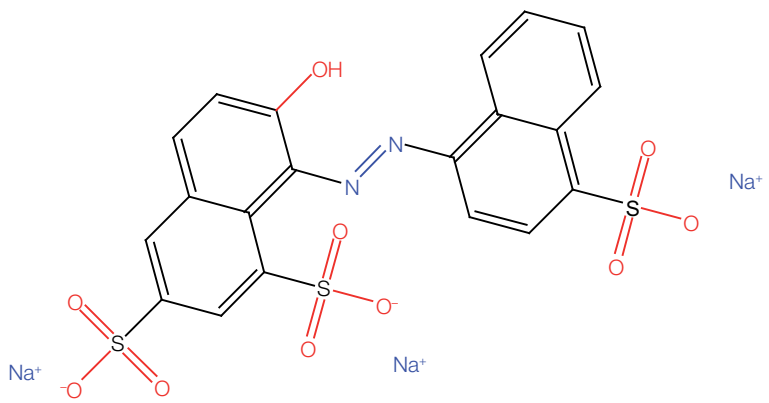


Figure 4. Ponceau 4R (trisodium 2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate)

33. Ponceau 4R was evaluated in 1983 by JECFA[11] and the SCF[3]. The animal studies assessed included acute and short-term studies, multigeneration, reproduction and teratology studies and long-term studies in the rat and mouse.

34. Both Committees identified a NOAEL equivalent to 375 mg/kg bw/day from a long term mouse study, adverse renal effects being reported at higher doses, this was used to derive an ADI of 0-4 mg/kg bw/day.

35. Ponceau 4R had no adverse effects in a 3-generation reproductive study in rats at dietary concentrations of up to 1250 mg/kg bw/day. There was no effect on the incidence of pre- and post-implantation losses or the weight or appearance of foetuses. The postnatal development of offspring as judged by survival, bodyweight and "developmental milestones" was not affected by treatment. There were no treatment related differences in histopathology (tissues not specified) between the control and F_3 animals.

36. The RTECS report stated that Ponceau 4R had behavioural effects in rodent studies. The report is based on an LD_{50} study[16] where coma and sometimes convulsions preceded death following i.p. administration of high doses of Ponceau 4R. However, it was further reported that oral doses of up to 8 g/kg bw in mice and rats were without adverse effect other than slight lethargy.

37. Following evaluation of Ponceau 4R by JECFA and the SCF, two additional studies have been identified. Brantom *et al.* (1987)[17] conducted a three-generation reproduction study in rats fed up to 1250 mg/kg bw/day Ponceau 4R in the diet. There were no treatment related clinical effects or deaths during the study. No differences in bodyweight, food or water intakes were observed. Fertility of treated dams and viability of litters was unaffected by treatment. Some differences in litter development were apparent in the F_2 but not the F_3 generation. For the F_3 generation, two additional responses, the righting response and the startle response, were assessed but were not affected by treatment. Some changes in organ weights were reported in the F_3 animals but there were no treatment related histopathological findings.

38. In a subsequent two-generation study[18] rats were fed Ponceau 4R at doses up to 1250 mg/kg bw/day. There were no treatment-related effects of Ponceau 4R in the F_0 generation. The F_1 generation received the same dietary concentrations for up to 114 or 188 weeks. Higher brain weights were reported in the top dose male rats, these changes became significant when adjusted for bodyweight. Degeneration of the granular layer neurones in the cerebellum was also reported in females at the highest dose (3/53 examined compared to 0/96 in the controls). The authors did not comment on this finding, but noted a number of statistically significant lesions were observed which were age related and confined to one sex. A NOAEL of 500 mg/kg bw/day was identified, comparable to that used to establish the ADI. Neurotoxicity was not specifically reported in either of these studies.

39. COT view. Structurally Ponceau 4R would be unlikely to cross the mature blood-brain barrier or placenta but the developing brain could be exposed via lactation. The three-generation study provides limited reassurance that no gross effects occurred since parental animals were able to feed and rear two further generations. Neurotoxic symptoms have been reported in adults but only at lethal doses, given i.p. However, of more concern are the effects on cerebellar granule cells in the two generation study since, although the effect was only seen at the highest dose, the qualitative measure used may have been insufficiently sensitive to detect neuronal loss at a lower dose.

Indigo carmine (E132)

40. Indigo carmine (indigotine) is a synthetically produced blue food colouring (see Figure 5).

41. Indigo carmine has been evaluated by JECFA[19] and by the SCF[3]. The animal studies assessed included acute and short-term studies and teratology studies. Data on long-term studies in the rat and mouse were available in summary form.

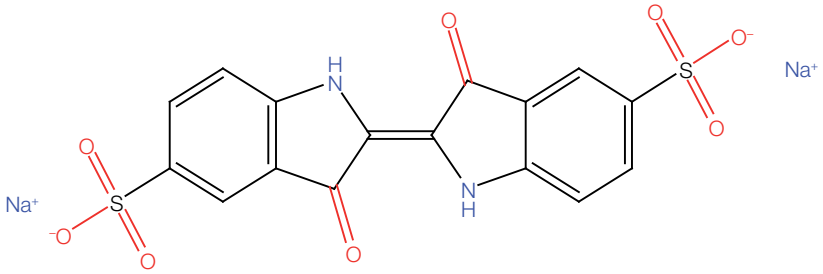


Figure 5. Indigo carmine (disodium 3-3' dioxo-2,2'bi-indolylidene-5-5' disulfonate)

42. Both Committees established an ADI of 0-5 mg/kg bw/day on the basis of a NOAEL equivalent to 500 mg/kg bw in a 2-year study in rats. JECFA reported that no adverse effects were noted on reproduction, gross or microscopic pathology. A similar study in mice did not indicate any adverse effects on histopathology. No neurotoxicity or multi-generation studies were available to JECFA or the SCF.

43. Since the evaluations were conducted an additional study has been published[20], which investigated the chronic toxicity and carcinogenicity of indigo carmine in rats treated with indigo carmine *in utero*. The F_0 generation received up to 2% indigo carmine in the diet prior to mating with no effects on bodyweight or mortality. Following mating, there were no treatment-related effects on fertility or pup viability. Animals from the F_1 generation received up to 2% indigo carmine in the diet for a maximum of 29-30 months. There were no treatment-related effects on mortality or on physical observations. Some changes in organ weights were measured but brain weights were unaffected. No treatment-related effects were apparent after histopathological examination of tissues including three sections of the brain (frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons) optic nerve, sciatic nerve and spinal cord. Overall, the authors reported that there was no evidence of toxicity or carcinogenicity. Neurotoxicity was not specifically investigated.

44. COT view. The two-generation study provides limited reassurance that no gross effects occurred since parental animals were able to feed and rear a further generations. Histopathological examination of the brain and other nervous tissue did not reveal any adverse effects. There was no suggestion of potential to produce neurotoxicity in the adult at any dose. Structurally, indigo carmine would be unlikely to cross the mature blood-brain barrier or placenta but the developing brain could be exposed via lactation.

Brilliant blue (E133)

45. Brilliant blue is a synthetic, blue food colouring (see Figure 6)

46. Brilliant blue has been evaluated by JECFA[21] and the SCF[3]. The animal studies assessed included acute and short-term studies, reproduction and teratology studies and long-term studies in the rat and mouse.

47. In 1969, JECFA established an ADI of 0-12.5 mg/kg bw/day on the basis of a NOAEL equivalent to 2500 mg/kg bw/day in a two-year rat study. In 1983, the SCF established an ADI of 0-10 mg/kg bw/day on the basis of a NOAEL equivalent to 1000 mg/kg bw in rodent studies. In the SCF review, no compound related effects were reported in reproductive and teratology studies in the rat. Neurological effects were not specifically investigated in these studies. There are no additional details of the studies reviewed by the SCF.

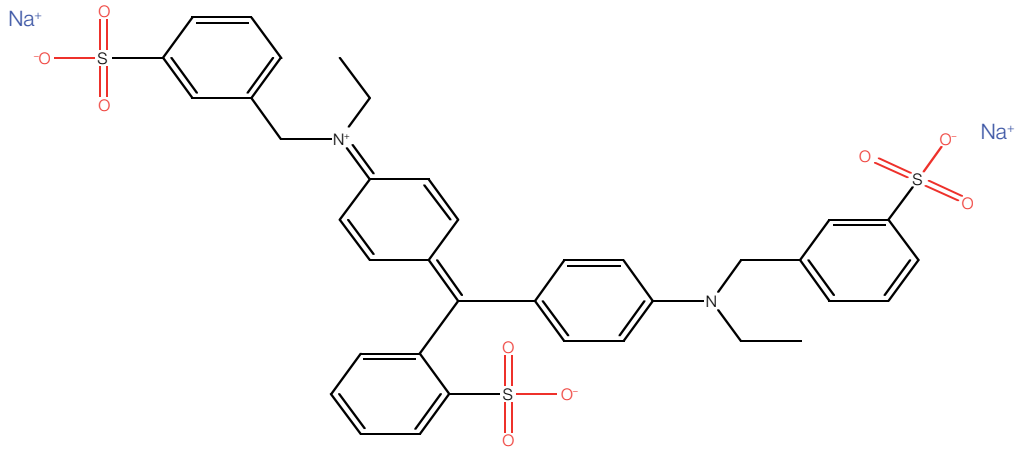


Figure 6. Brilliant blue (disodium α-[4-(N-ethyl-3-sulfonatobenzylamino)phenyl]-α-[4-(N-ethyl-3-sulfonatobenzylimino)cyclohexa-2,5-dienylidene] toluene-2- sulfonate

48. The RTECS report lists brilliant blue as having behavioural effects including convulsions and effects on seizure threshold in mice. This is taken from an LD_{50} study in which the effects were reported following i.p. administration of 4600 mg/kg, a lethal dose.

49. Following the JECFA and SCF evaluations, one additional paper has been published, Borzelleca *et al.* (1990)[22] investigated the lifetime toxicity and carcinogenicity of brilliant blue in rats and mice exposed to brilliant blue *in utero*. In the rat study, the F_0 generation received up to 2% in the diet for 2 months prior to mating. There were no consistent treatment-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or on the number of live or stillborn pups. The F_1 generation was also exposed to up to 2% brilliant blue for up to 111-116 weeks. Some effects on food consumption and mean bodyweights were reported in some dose groups and survival was significantly reduced in females at 2%. No treatment related clinical, haematological or urinalysis findings were reported. There were no effects on any of the organ weights measured including brain. In addition, there no treatment related gross or histological changes following examination of tissues including three sections of the brain

(frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons) optic nerve, sciatic nerve and spinal cord. NOAELs of 2% (1072 mg/kg bw/day) and 1% (631 mg/kg bw/day) for males and females respectively were identified.

50. In the mouse study, brilliant blue was administered in the diet at concentrations of up to 5% for 104 weeks. There were no compound related adverse effects on general physical observations, food consumption, survival or on haematological parameters. Group mean bodyweights were slightly lower in treated mice at various intervals, but this was not consistent throughout the study. The NOAEL in the mouse study was 5% (7354 and 8699 mg/kg bw/day for males and females respectively). The same tissues were examined histologically as in the rat study. No adverse effects were apparent.

51. COT view. Structurally brilliant blue would be unlikely to cross the mature blood-brain barrier or placenta but the developing brain could be exposed via lactation. Potential adult neurotoxicity was only seen at lethal dose levels given by i.p. injection. The two-generation study provides some reassurance that no gross effects occurred since parental animals were able to feed and rear a further generation. Histopathological examination of the brain did not reveal any effects.

Sodium benzoate (E211)

52. Sodium benzoate is used as a food-preserving agent (see Figure 7).

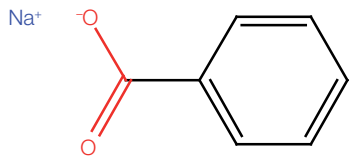


Figure 7. Sodium benzoate

53. Sodium benzoate has been evaluated by the SCF[23] and JECFA[24]. Both Committees established an ADI of 0-5 mg/kg bw/day on the basis of the NOAEL of 500 mg/kg bw/day from a four-generation study in rats. There were no adverse effects on fertility or lactation, the only parameters investigated. Developmental studies were available in the mouse, rat, rabbit and hamster. Neurotoxicity was not specifically investigated in any studies considered in the JECFA or SCF reviews.

54. The SCF evaluation noted that high, acute doses of sodium benzoate were associated with effects on the central nervous system in humans due to the disruption of acid-base balance, but were rapidly reversible. Such effects were not expected to occur at the level of the ADI. Sodium benzoate is detoxified via glycine conjugation and urinary excretion thus benzoate toxicity is reduced by glycine supplementation.

55. No additional relevant studies have been identified since sodium benzoate was evaluated.

56. COT view. The structure of benzoate suggests that it could penetrate the blood brain barrier or cross the placenta, possibly via the organic anion transporter, though metabolic studies with radiolabelled benzoate reviewed by the SCF and JECFA indicate that the vast majority is excreted with little accumulation in the organs. No evidence of neurotoxicity is apparent in the available data which includes a four-generation reproduction study. Effects on the central nervous system have been noted but this is due to the disruption to acid-base balance rather than specific neurotoxicity.

Sulphur dioxide (E220)

57. Sulphur dioxide is used as a food-preserving agent. It has been evaluated by JECFA[25] and the SCF[23]. Both Committees established an ADI of 0-0.7 mg/kg bw/day based on a NOAEL of 70 mg/kg b.w. in long term studies in rats and pigs.

58. In the rat study, the animals were treated for up to two years and over three generations. The brain was weighed and brain, spinal cord and femoral nerve examined histopathologically. No treatment related changes relevant to neurotoxicity were observed. The animals were bred for 2 further generations. There were no treatment-related decreases in mortality; body weight gain was reduced in some of the treated animals in the F_1 and F_2 generations, but a dose-response relationship was not apparent. The number of F_{2a} young were reduced in the treated animals but this was not dose-related and did not occur in the F_{2b} litter.

59. No additional relevant studies have been identified since sulphur dioxide was evaluated.

60. COT view. Structurally sulphur dioxide would be unlikely to cross the mature blood-brain barrier or placenta but the developing brain could be exposed via lactation. The two-generation study provides limited reassurance that no gross effects occurred since parental animals were able to feed and rear a further generation. No neurotoxic effects were apparent in other animal studies. Limited histopathology in brain and other nervous tissue did not suggest any adverse effects.

Monosodium glutamate (E621)

61. Monosodium glutamate is a flavour-enhancing agent. It is a salt of L-glutamic acid, an amino acid representing approximately 20% of ingested protein.

62. Monosodium glutamate has been evaluated by JECFA[26] and by the SCF[27]. Both Committees established a group ADI 'not specified' on the basis of the data provided and since large intakes of glutamates are consumed in the normal diet.

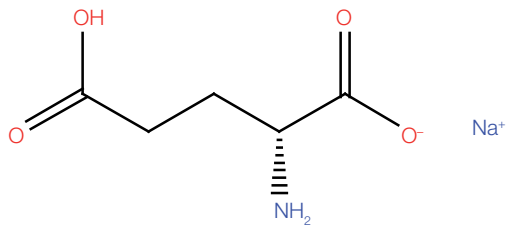


Figure 9. Monosodium glutamate

63. There is a large body of information regarding the neurological effects of MSG. The SCF stated that some studies demonstrated a strain dependant, variable vulnerability of the developing rat or mouse CNS to high levels of glutamate alone or in combination with other amino acids in massive doses but that no brain lesions occurred in mouse, rat and hamster studies in which the animals had ingested large doses of MSG in their diet. In addition, some of the acute effects in humans observed after ingestion of more than 3g glutamate per person were also observed with other foods not containing glutamates. The symptoms are not specified by the SCF but high plasma glutamate levels are associated with nausea and vomiting[26]. Acute symptoms of burning, facial pressure and chest pains have also been reported[28].

64. Since the evaluations were conducted, four additional neurotoxicity studies have been identified. Bawari *et al.* (1995)[29] reported that subcutaneous administration of MSG to rat pups resulted in significantly increased lipid peroxidation and catalase levels in the mid-brain region. A significant reduction in total as well as non-protein SH groups was also reported. There were no effects in the frontal cortex. Similar findings were reported by Babu *et al.* (1994)[30]. Hsieh *et al.* (1997)[31] reported that subcutaneous administration of MSG resulted in sex-specific and area-specific changes in neuronal density (both number and volume) in rats.

65. Jing *et al.* (1994)[32] injected pregnant mice s.c. with monosodium glutamate on alternate days until parturition. Memory and Y-maze spatial discrimination learning were damaged in the offspring of the high dose (2.5g/kg bw) MSG group compared to the low dose group (1g/kg bw). There was significant destruction of neurones in the arcuate nucleus and ventromedial nucleus of the hypothalamus exhibiting cytoplasmic swelling, nuclear pyknosis and a reduction in the number of neurones. MSG treatment affected the amount of ^{3}H-glutamate binding in the hypothalamus and hippocampus of the mice. It was also reported that MSG can increase the calcium ion concentration in individual neurones by inducing an influx of extracellular calcium and releasing intracellular calcium stores which could be responsible for the observed cell damage and behavioural changes.

66. COT view. Assessment of MSG assumes that the sodium salt behaves in the same way as the glutamate derived from dietary protein breakdown. Glutamate is a neuroactive compound and can cross the blood-brain barrier and placenta. Hence there is potential for brain exposure at all developmental stages. However, glutamate is present in normal blood and brain, and brain levels are well controlled by active physiological regulation, so there is unlikely to be a hazard at realistic dose levels. Neurotoxic effects have been seen in animal studies but only at very high doses, often administered by s.c. injection. Such high doses would be expected to overcome physiological regulation of brain glutamate concentration. There is a substantial body of work investigating MSG at lower doses with no indication of any adverse effects, as well as extensive human exposure data.

Acesulfame K

67. Acesulfame K (E950) is a synthetic sweetening agent (see Figure 10).

68. Acesulfame K has been evaluated by JECFA[33] and the SCF[34]. JECFA established an ADI of 0-15 mg/kg bw/day on the basis of a NOAEL equivalent to 1500 mg/kg bw/day in a chronic study in rats. In the SCF review, the dog was considered to be the most relevant species, due to greater kinetic similarities. The NOAEL in the dog study was 900 mg/kg bw/day from which an ADI of 0-9 mg/kg bw/day was established.

69. Neurotoxicity was not specifically investigated in a four-generation rat study at dietary concentrations of up to 3%. No adverse effects on fertility, pups per litter, birth weight and mortality during lactation were reported. Growth rate was slightly affected in the top dose animals of the F_0 and F_1 generations. No dose-related effects were found in a second reproduction study in which rats were treated with up to 3% acesulfame K in the diet. Tissues such as brain, spinal cord and sciatic nerve were examined histologically in a variety of studies, with no adverse effects being apparent.

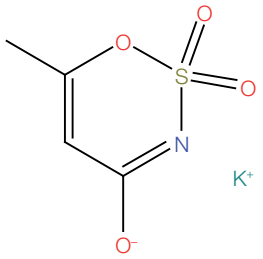


Figure 10. Acesulfame K (potassium salt of 6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide.

70. No additional relevant studies have been identified since acesulfame K was evaluated.

71. COT view. Structurally acesulfame K would be unlikely to cross the mature blood-brain barrier or placenta to a significant extent but the developing brain could be exposed via lactation. However, the four-generation study provides reassurance that no gross effects occurred since parental animals were able to feed and rear subsequent generations. There was no suggestion of potential to produce neurotoxicity in the adult at any dose. The limited histopathology available from other animal studies did not indicate any adverse neurological effects.

Aspartame (E951)

72. Aspartame (is a synthetic sweetener (see Figure 11 overleaf).

73. Aspartame has been evaluated by JECFA[35] and the SCF[36]. Both Committees derived an ADI of 0-40 mg/kg bw/day on the basis of a 104-week study in rats.

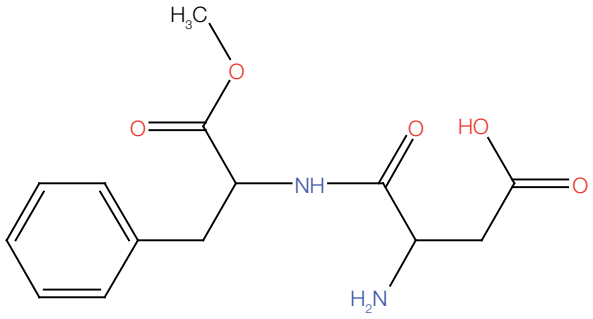


Figure 11. Aspartame (6-methyl-1,2,3-oxathiazine-4(3H)—one-2,2-dioxide salt of L-phenylalanyl-2-methyl-L-α-aspartic acid.

74. The 2002 SCF review considered neurological symptoms in particular detail. They concluded that despite targeted animal studies, no consistent effect of aspartame on neurotransmitters or their precursors had been observed. Human studies indicate that there were no changes in behaviour, cognition, mood or learning associated with aspartame nor was it more likely to be associated with headaches than placebo. No additional relevant studies have been identified since the most recent SCF evaluation.

75. COT view. Aspartame has been extensively studied in both humans and animals, with no indication of any adverse effects being apparent from either aspartame or its breakdown products (which include the natural amino acid phenylalanine). Only doses high enough to increase plasma phenylalanine well beyond the normal level would have the potential to be neurotoxic. As well as conventional animal studies, the data available include behavioural studies in animals and assessment of reported human side effects, including in potentially sensitive individuals none of which indicate neurotoxic potential.

Saccharin (E954)

76. Saccharin is a synthetic sweetening agent (see Figure 12).

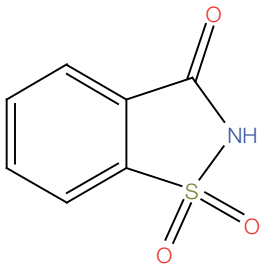


Figure 12. Saccharin (3-oxo-2,3-dihydrobenzo[d]isothiazol-1,1-dioxide)

77. Saccharin has been evaluated by JECFA[37] and the SCF[38]. Both Committees derived an ADI of 0-5 mg/kg bw on the basis of a NOAEL equivalent to 500 mg/kg bw/day for bladder tumours in a 2-generation feeding study in the rat.

78. Neurological effects were not specifically investigated in young rats exposed to saccharin from parturition (up to 5% in the diet) or in two multi-generation reproductive studies (up to 7.5% in the diet). However, no adverse effects on reproductive parameters were apparent.

79. No additional relevant studies have been identified.

80. COT view. Structurally saccharin would be unlikely to cross the mature blood-brain barrier or placenta to a significant extent but the developing brain could be exposed via lactation. However, the available two-generation reproduction studies do not indicate any gross effects. No evidence of neurotoxicity is apparent from other animal studies. The limited histopathology available did not indicate any adverse neurological effects.

Summary and Discussion

81. Neurotoxic symptoms have been reported in acute studies for some of the additives but these have been LD50 studies using very high doses administered ip and may not be relevant to assessing the use of these colours as food additives.

82. For most of the substances there were insufficient data to fully assess developmental neurotoxicity. Gross neurotoxic effects would be apparent in conventional animal studies but developmental and behavioural effects can be much more sensitive than other end points, for example, as with studies of lead and mercury toxicity. However, certain factors would indicate whether there was need for concern and thus assist in prioritising the additives for review. These could include chemical structure, indicating whether the compound could be likely to cross the blood brain barrier or the placenta, and the neurotoxic potential in adults since almost all neurodevelopmental toxins in children are also neurotoxins in adults. It was agreed that a tiered approach should be taken in assessing these additives, considering both the potential for adult toxicity and potential routes of exposure to establish whether significant exposure could occur *in utero* or via lactation.

83. Quinoline yellow, sunset yellow (and its metabolite sulphanilic acid) and carmoisine (and its metabolite naphthionic acid) have been reported to inhibit pseudo and true cholinesterase activity *in vitro*. Sunset yellow and carmoisine have also been reported to reversibly inhibit cholinesterase following administration to rats. The significance of these data is currently unclear and cholinergic symptoms have not been observed in conventional animal studies even after high dose and/or chronic exposure.

84. Sunset yellow has been assessed for effects on a variety of neurobehavioural endpoints at doses in the region of the NOAEL used to derive the ADI. Some non-persisting effects on behaviour were observed.

85. Indigo carmine and brilliant blue have not been specifically investigated for neurotoxicity. Chronic toxicity and reproductive studies were available for the original evaluations, and some additional studies have been conducted since. No overt effects on behaviour and no effects on fertility or reproductive parameters have been reported. This suggests that gross neurotoxic effects would have been detected. Although more subtle behavioural or neurodevelopmental effects have not been investigated, the available studies do not provide any indications of neurotoxic potential, or that the ADI is inadequate.

86. Ponceau 4R has not been specifically investigated for neurotoxicity. Chronic toxicity and reproductive studies were available for the original evaluations, and some additional studies have been conducted since. No overt effects on behaviour and no effects on fertility or reproductive parameters have been reported. A conventional two-generation study however, gave clear evidence of neuronal loss, and hence of developmental neurotoxicity, at a high dose. The possibility that a quantitative evaluation might have shown more subtle effects at lower doses cannot be excluded.

87. Sodium benzoate, sulphur dioxide, acesulfame K and saccharin were evaluated more recently and no new relevant studies have emerged since. Overall the data do not provide indications of concern for neurotoxicity.

88. For MSG and aspartame, where concerns have been expressed, neurotoxicity has been considered in more detail by the SCF and JECFA. There are several additional studies on MSG which indicate damage to nervous tissue but these have required administration of sufficiently high MSG doses to overcome physiological control of brain glutamine and the relevance of this route to its use as a food additive is unclear.

Conclusions

89. In summary, the Committee concluded that there was no evidence of properties that would suggest a potential to produce developmental neurotoxicity for acesulfame K, brilliant blue, indigo carmine, saccharin, sodium benzoate or sulphur dioxide. There is some equivocal evidence suggesting the potential to produce developmental neurotoxicity at very high dose levels for carmoisine, sunset yellow and quinoline yellow. There was actual evidence suggesting the potential to produce developmental neurotoxicity at very high doses for aspartame, monosodium glutamate and Ponceau 4R. For no agents was there any evidence suggesting the potential for developmental neurotoxicity at current acceptable daily intakes. Therefore, although direct evaluations of developmental neurotoxicity were mostly absent and would, in principle, be desirable, the available data did not suggest that further investigations of any of these agents would be a priority given current dietary intakes.

COT statement 2006/02
January 2006

References

1. Makris, S, Raffaele, K, Sette, W, Seed, J. (1998). A Retrospective Analysis of Twelve Developmental Neurotoxicity Submitted to the USEPA Office of Prevention, Pesticides, and Toxic Substances.

2. JMPR (2002). Pesticides Residues in Food-2002 Joint FAO/WHO meeting on Pesticide Residues. Studies of Developmental Neurotoxicity and their use in establishing acute references doses and acceptable daily intakes. http://www.inchem.org/documents/jmpr/jmpmono/2002pr15.htm

3. SCF (1983). Reports of the Scientific Committee for Food. 13th Series.

4. WHO (1984). World Health Organization. WHO Food Additives Series, 19.

5. Osman MY, Sharaf IA, el-Rehim WM, el-Shharkawi AM (2002). Synthetic organic hard capsule colouring agents: in vitro effect on human true and pseudo-cholinesterases. *British Journal of Biomedical Sciences*. 59:212-217.

6. WHO (1982). World Health Organization. WHO Food Additives Series, 17.

7. Pierce E.C., Agersborg,H.P.K., Borzelleca, J.F., Burnett, C.M., Eagle, E., Ebert, A.G., Kirschman, J.C., Scala, R.A. (1974). Multi-generation studies with certified colours in rats. Abstract XIII. *Toxicology and Applied Pharmacology*, 136: 259.

8. Gaunt I.F., Farmer, M., Grasso, P., Gangolli, S.D. (1967a). Acute (rat and mouse) and short-term (rat) toxicity studies on sunset yellow FCF. *Food and Cosmetic Toxicology*, 5:747-754.

9. Tanaka T (1996). Reproductive and neurobehavioural effects of sunset yellow FCF administered to mice in the diet. *Toxicology and Industrial Health*. 12:69-79.

10. Osman MY, Sharaf IA, Osman HMY, El-Khouly ZA, Ahmed EI (2004). Synthetic organic food colouring agents and their degraded products: effects on human and rat cholinesterases. *British Journal of Biomedical Sciences*. 61:128-132.

11. WHO (1983). World Health Organization. WHO Food Additives Series, 18.

12. Holmes, P.A., Pritchard, A.B., Kirschman, J.C. (1978a). A one year feeding study with carmoisine in rats. *Toxicology*, 10:185-193.

13. Holmes, P.A., Pritchard, A.B., Kirschman, J.C. (1978b). Multigeneration reproduction studies with carmoisine in rats. *Toxicology*, 10:169-183.

14. Gaunt I.F., Farmer, M., Grasso, P., Gangolli, S.D. (1967b). Acute (rat and mouse) and short-term (rat) toxicity studies on carmoisine. *Food and Cosmetic Toxicology*, 5:179-185.

15. Ford GP, Stevenson BI, Evans JG (1987). Long term toxicity study of carmoisine in rats using animals exposed in utero. *Food Chemical Toxicology*. 25:919-925.

16. Gaunt I.F., Farmer, M., Grasso, P., Gangolli, S.D. (1967c). Acute (rat and mouse) and short-term (rat) toxicity studies on Ponceau 4R. *Food and Cosmetic Toxicology*, 5:187-194.

17. Brantom PG, Stevenson BI, Ingram AJ (1987). A three-generation reproduction study of ponceau 4R in the rats. *Food Chemical Toxicology*. 25:963-968.

18. Brantom PG, Stevenson BI, Wright MG (1988). Long-term toxicity study of ponceau 4R in rats using animals exposed in utero. *Food Chemical Toxicology*. 25:955-962.

19. WHO (1974). World Health Organization. WHO Food Additives Series, 6.

20. Borzelleca JF, Hogan GK, Koestner A (1985). Chronic toxicity/carcinogenicity study of FD&C Blue No.2 in rats. *Food Chemical Toxicology*. 23:551-558.

21. WHO (1969). World Health Organization. WHO FAO Nutrition Meetings Report Series 46a.

22. Borzelleca JF, Depukat K, Hallagan JB (1990). Lifetime toxicity/carcinogenicity studies of FD&C Blue No.1 (Brilliant Blue FCF) in rats and mice. *Food Chemical Toxicology*. 28: 221-234.

23. SCF (1994). Reports of the Scientific Committee for Food. 35th Series.

24. WHO (1996). World Health Organization. WHO Food Additives Series, 37.

25. WHO (1998). World Health Organization. WHO Food Additives Series, 42.

26. WHO (1987). World Health Organization. WHO Food Additives Series, 22.

27. SCF (1991). Reports of the Scientific Committee for Food. 25th Series.

28. Gosselin, R.E., R.P. Smith, H.C. Hodge. (1984). Clinical Toxicology of Commercial Products. 5th ed. Baltimore: Williams and Wilkins, II-407.

29. Bawari M, Babu NG, Ali MM, Misra UK (1995). Effect of neonatal monosodium glutamate on lipid peroxidation in adult rat brain. *NeuroReport*. 6: 650-652.

30. Babu GN, Bawari M, Ali MM (1994). Lipid peroxidation and antioxidant status of circumventricular organs of rat brain following neonatal monosodium glutamate. *Neurotoxicology*. 15: 773-778.

31. Hsieh, Y., Hsu, C., Lue, S., Hsu, H., Peng, M. (1997). The neonatal neurotoxicity of monosodium L-glutamate on the sexually dimorphic nucleus of the preoptic area in rats. *Developmental Neuroscience*, 19: 342-347.

32. Jing G, Juan, W, Xiaoning Z Weining Z, Yitu Z, Zuxuan Z. Effects of monosodium glutamate passing through the placental barrier on the structures and functions of specific areas of the brain in infant mice. *Acta Physiologica Sinica*. 48:44-51, 1994. [English translation of Chinese original]

33. WHO (1990). World Health Organization. WHO Food Additives Series, 28.

34. SCF (2000). Opinion of the SCF expressed on 9th March 2000.

35. WHO (1981). World Health Organization. WHO Food Additives Series, 15.

36. SCF (2002). Opinion of the SCF expressed on 4th December 2002.

37. WHO (1993). World Health Organization. WHO Food Additives Series, 32.

38. SCF (1995). Opinion of the SCF expressed on 2nd June 1995.

Further toxicity studies in the rat of a hydrogel filler for breast implants

Introduction

1 During 2000, because of concerns raised by clinicians about the safety of the fillers used in breast implants, the Medical Devices Agency (MDA) had decided to review the safety data on all breast implant fillers available in the UK. These included a hydrogel pre-filled breast implant manufactured by Poly Implant Prostheses. In September 2000, at the request of the MDA, the Committee considered a submission questioning the significance of the findings in a 90-day toxicity study in rats implanted with this hydrogel. The product was voluntarily withdrawn from the UK market in December 2000 and an MDA Device Alert was issued to advise plastic surgeons and implanted women. MDA indicated that further advice on the safety of these implants would be provided as soon as it became available.

2 COT considered additional data in February 2002 and concluded (COT Statement COT/02/1 – March 2002) :

i. The Committee considered that the conclusion of the new study, namely that there were no pathological findings in the organs examined, was not supported by the limited experimental results provided. There were limitations in the design of the study, the interpretation of its findings and the report was considered to be imprecise and inadequate.

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

iii. The Committee considered that the new studies provided no further information to permit clarification of the extent or significance of toxicological risks.

iv. The Committee repeated its previous conclusion that further testing should be undertaken including the administration of single doses of the filler gel with longer-term follow-up. The Committee stressed the need for the design and reporting of further studies to be compatible with current guidelines for chronic toxicity tests.

The implant

3 The hydrogel filler originally comprised 92% of physiological saline gelled with 8% of a polysaccharide. This filling material has subsequently been modified and the saline replaced by a buffer. It is understood that the polysaccharide is based on a cellulose derivative that forms long, linear chains linked by bridges. This gel is contained within a silicone elastomer shell.

4 Degradation of the filling material

5 Limited data were provided previously on the potential for *in vivo* degradation of the filling materials

(both buffered and unbuffered). A substantial proportion of the material dosed to animals was not recovered and the fate of this material had not been ascertained. No additional information has been provided. Therefore we still consider that the potential degradation of the hydrogel had not been adequately addressed.

The new rat toxicity studies

6 The manufacturer had provided results from a 2-year subcutaneous carcinogenicity study and a 1-year implantation study, intended to address the concerns raised by COT in relation to the earlier studies.

7 In the 2-year study, groups of fifty female Wistar Han rats were injected in each flank subcutaneously with 1/60 of their body weight of the modified gel filler material divided between two sites or with saline as a control. Groups of dosed and control rats were killed after 24 months. Limited observations were made during life with detailed examinations at necropsy. In addition haematology and biochemistry were evaluated in sub-groups of 20 implanted and control animals at 12, 18 and 24 months, biochemistry was assessed on all animals at 24 months. Urinalysis was performed on sub-groups of 10 implanted and control animals at 18 and 24 months. A more extensive range of organs was examined histopathologically than in the earlier studies.

8 In the 1-year study, groups of fifteen male and fifteen female Sprague-Dawley rats were injected in each flank subcutaneously with 1/60 of their body weight of the modified gel filler material divided between two sites or with saline as a control. Limited observations were made during life with detailed examinations at necropsy. In addition haematology and biochemistry were evaluated in sub-groups of 10 implanted and control animals per sex at 3, 6 and 12 months, biochemistry was assessed on all animals at 12 months. Urinalysis was performed on sub-groups of 10 implanted and control animals per sex at 3 and 6 months and all animals at 12 months. An extensive range of organs was examined macroscopically with a more limited range examined histopathologically.

9 The glomeruli of the kidneys of the animals in the test group of the 2-year study were significantly dilated compared to those of the controls and the test group showed significantly higher incidences of tubular nephropathy, sometimes associated with interstitial nephritis. Changes in creatinine levels in serum were observed in the first twelve months of the 2-year study but these these were no longer evident after 24 months. Diuresis, excretion of urinary proteins and creatine clearance were similar to control values at the end of the study. The dilatation of the glomeruli of the kidney was associated with the presence of what was thought to be test material contained in macrophages.

Evaluation of the findings

10 The previously observed effects in the liver, kidney and lymph nodes in the earlier inadequately designed and reported studies of shorter duration were not confirmed in these new studies and hence should no longer be considered as indicative of a toxic effect. The changes in creatinine level indicated a functional effect in the first twelve months of the study, but by 24 months no difference was apparent although there was substantial variability between animals at this time. As a result, any

difference was not statistically significant, but there may still have been a biologically significant effect. However due to the limited urinalysis for kidney function in the studies, it was difficult to determine if significant damage to the kidneys was occurring.

11 Any effect in the kidney could have been the result of a combination of factors in individual animals rather than representing a functional toxic effect of the hydrogel. We concluded that the marginal effects seen in the kidney were most likely a treatment related effect but not clearly adverse. We noted that, compared to most other species, older rats are very susceptible to interstitial nephritis, especially in studies of longer duration.

12 We noted that exposure to the hydrogel in the new studies was intended to represent a worst case situation. In women generally the amount is likely to be lower and duration of exposure is likely to be limited, however similar exposure on a body weight basis could occur in women following simultaneous rupture of both implants.

13 We were informed that the ongoing rate of rupture for all breast implants is 1-5% per annum. When rupture is detected (usually by change of volume), the implants are usually removed and replaced. Therefore, we considered that exposure to hydrogel would, in most cases, be for a relatively short period of time.

14 We recognised that so-called 'silent' ruptures, where leakage may take time to become apparent, could result in longer exposure times. Although we recognise that there is additional uncertainty, we considered hydrogel unlikely to have acute toxic effects.

15 We were concerned that there seemed to be minimal follow-up to monitor kidney function in women with these implants, and that continued surgeon-patient contact was limited. We were informed that the Independent Review Group on Silicone Gel Breast Implants had previously expressed similar concerns over inadequate follow-up. Women with implants are often reluctant to attend for follow-up consultations and therefore there had been increased provision of information to patients on possible adverse events after surgery.

Conclusions

16 We conclude that the results of the two studies provide reassurance that the effects previously noted in the liver, kidney and lymph nodes were not indicative of a toxic effect.

17 We conclude that the marginal effects seen in the kidney in the new studies were most likely a treatment related effect but not clearly adverse. Taking account of the susceptibility of older rats to kidney effects, especially in longer studies, we considered that similar effects would be unlikely to occur in humans.

18 The exposure of rats to the hydrogel in the new studies was generally greater in amount and duration than that occurring in women following rupture of their implants. We conclude that the results of these studies when considered together with the existing data, suggest that subcutaneous exposure to the hydrogel would not lead to toxic effects in women with these implants.

19 We recognise that removal of the implants is a clinical decision which would need to be based on all relevant medical factors, including the risks associated with removal of the implants.

COT statement 2006/03
January 2006

Joint statements of the COT with the COM and COC

Nanomaterial toxicology

Background

1. In June 2003 the UK Government commissioned the Royal Society, the UK national academy of science, and the Royal Academy of Engineering, the UK national academy of engineering, to carry out an independent study of likely developments in nanotechnology and of whether nanotechnology raises or is likely to raise new ethical, health and safety or social issues which are not covered by current regulation.[1] Their report "Nanoscience and nanotechnologies: opportunities and uncertainties" was published on 29 July 2004.[2] The UK Government's response to the joint Royal Society and Royal Academy of Engineering report was published on 25 February 2005.[3] The Committees on the Toxicity, Carcinogenicity and Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COT, COC and COM) were identified in an annex to the Government report along with six other independent expert scientific committees as relevant scientific committees to provide advice on the development of nanotechnology. The Government stated in its reply to the Royal Society that it would ask for advice from COT/COC/COM on issues as they arise and seek to ensure that nanotechnologies will be explicitly mentioned in their terms of reference.

2. The COT, COC and COM carry out regular horizon scanning exercises as part of their annual remit (see appended internet links at the end of this statement). The COT identified nanomaterials as an emerging issue at its February 2004 meeting. Following the Royal Society's review of nanotechnology in 2004 (which was discussed at the COT's September 2004 meeting), all three committees identified the risk assessment of nanomaterials as an area of interest and asked for appropriate information to be provided for consideration.

Introduction to current review

3. Overview papers on the available toxicological data were prepared for the committees to assist in preparing an initial joint statement.[4-6] The information presented to the committees was based on a hazard assessment document published by the Health and Safety Executive (HSE)[7], a literature review prepared by the secretariat which identified a number of additional published scientific papers (which are cited in the overview papers) and information published in abstracts from the US Society of Toxicology (SOT) meeting held March 6–10, 2005, in New Orleans, Louisiana, USA.[8] The HSE captured published information up to July 2004 and the additional review prepared for the committees captured information up to March 2005.

4. The Royal Society defined nanomaterials as having one dimension less than 100 nanometres (nm) or 0.1 micrometre (μm).[2] However, the Committees (COT,COC,COM) agreed that this should not be viewed as a rigid definition and that a pragmatic case-by-case approach should be adopted with regard to nanomaterials. There are two basic approaches to generating novel nanomaterials. 'Top down' technologies use machining and etching methods to create particulates which are usually found in

micrometre sizes, but can also be produced in nanometre dimensions. Examples include engineered surfaces and surface coatings (e.g. fuel cells and catalysts) and microcrystalline materials (potential uses are in textiles, cosmetics, and paints). 'Bottom-up' nanotechnologies involve the production of nanomaterials from individual molecules. The nanomaterials thus generated are novel, e.g. carbon nanotubes and nanofoam, nanodots and fullerenes. Some examples of 'bottom up' nanomaterials are shown below. The committees noted that nanoparticles were also produced during combustion, food cooking and from vehicle exhausts.

	Carbon nanotubes	Fullerenes	Nanodots	Carbon nanofoam
Structure	Rolled up sheets of graphite, with one end capped	Molecules of carbon formed into hollow cage like structures	Crystalline structures of compounds e.g. cadmium, selenium, tellurium, sulphur	Clusters of carbon atoms in a web like structure
Properties	Extreme strength and electrical conductivity. Insoluble in water. Biologically non-degradable			Lightweight, spongy solid, can act as semiconductor. Magnetic property

5. Nanomaterials have a high surface to volume ratio. This means that a high proportion of the atoms will be at the particle surface, and consequently surface reactivity will be high. These particles may adopt structures that are different to the bulk form, with different physical and chemical properties. The kinetic behaviour of nanoparticles follows basic laws of gaseous diffusion, with extensive interactions between particles. It is likely these collisions lead to agglomeration, and reactions between nanoparticles and other airborne molecules (water or pollutants).[7]

COT/COC/COM Review of toxicological information on nanomaterials

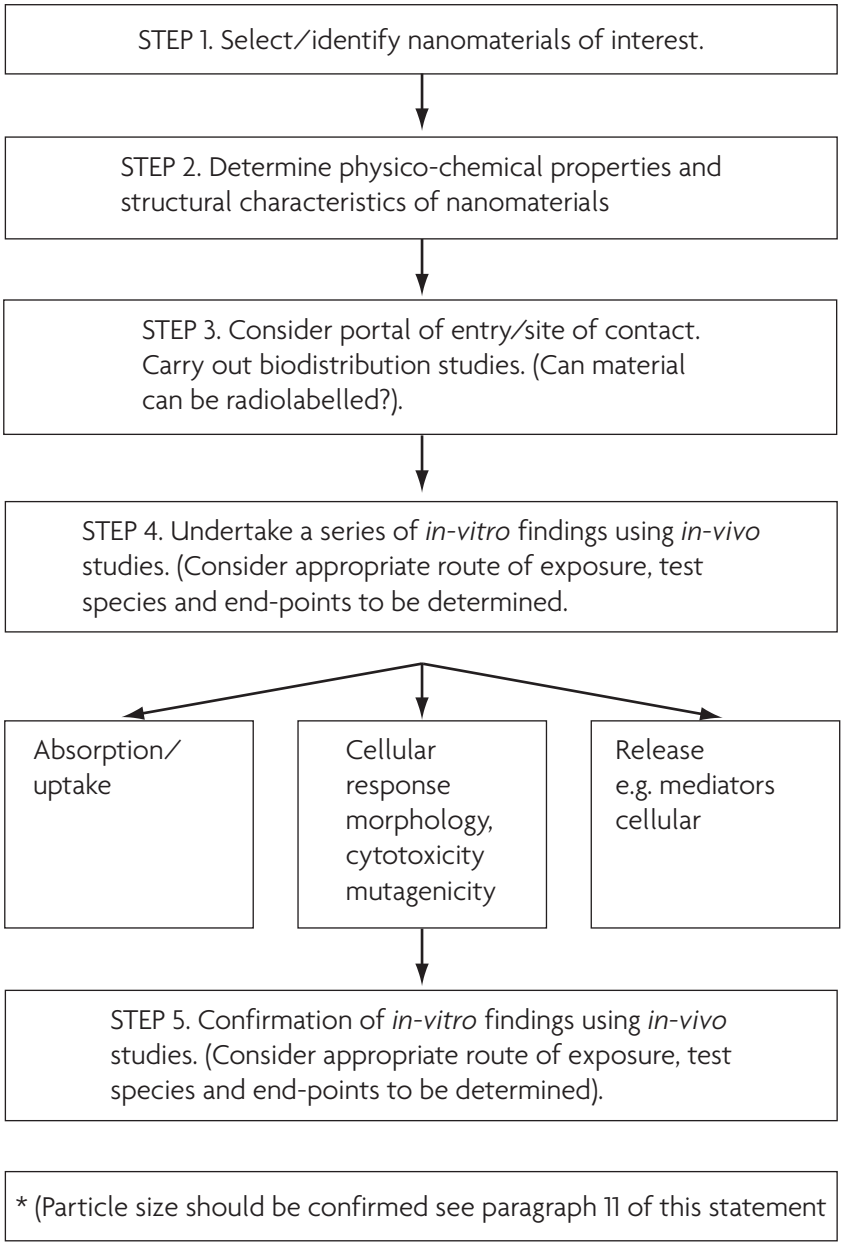
Proposed approach to initial toxicological studies with nanomaterials

6. The Committees agreed that the objective of the review was to provide a baseline statement on the available information on nanomaterials toxicology. At the present time, there are considerable limitations in the number of materials tested, and in the toxicology data available. However, it is expected there will be considerable growth in the number of nanomaterials produced industrially and their potential commercial applications. There is also virtually no information on potential human exposure resulting from environmental exposure. To some extent this reflects the limited commercial applications to date (excluding medicinal/cosmetic uses which are considered under regulatory assessment schemes). In addition the review provided to COT/COC/COM did not cover the exposure to nanoparticulate material present in air pollution (e.g. resulting from industrial processes, diesel emissions etc). The Committees noted the importance of particle size, surface area and surface chemistry as determinants of nanomaterial toxicity. The main methods of hazard identification used included comparison of hazard data for micrometre sized and nanometre sized equivalent materials.

7. Possible biological effects were discussed, including a contribution of nanoparticles in the genesis of oxidative stress processes. It was suggested that the mechanisms leading to these processes probably depend on particle size and chemical composition. Some of the SOT abstracts reported studies suggesting that surface area might not be the most appropriate metric for describing the dose of nanoparticles, which contrasted with the information available in the HSE review document.[7,8] The Committees noted the "Seaton" hypothesis regarding potential cardiovascular effects of inhaled particles.[9] The Committee considered that there was scope for further research into the potential systemic effects associated with inhalation of nanomaterials. This would include information on uptake and systemic distribution and potential for systemic effects (such as procoagulation).

8. The Committees suggested a systematic tiered approach to initial toxicological studies with nanomaterials. Given the paucity of toxicological data indicating which are the vulnerable cell types, and the likelihood that this will be variable depending on nanoparticle surface properties, *in-vitro* assessment should initially be directed towards those cell types shown to receive the highest nanoparticle dose in biodistribution studies (where this information is available). Because of the likely routes of exposure, such an approach would normally involve epithelial cells (e.g. respiratory and gastrointestinal tract) and macrophages (i.e. professional phagocytic cells) for assessment of cytotoxicity, adsorption/uptake, changes in oxidative status, release of mediators. Such studies would provide basic data that could be used for comparison between nanomaterials. This would be followed by a second tier of *in-vivo* studies using appropriate routes of exposure. It was noted that evidence of oral uptake of one type of single-walled carbon nanotube (SWCNT) had been identified.[10] The Committees recognised the need for identifying ranges of standardised nanomaterials for these initial investigations to produce baseline information on structural influences on toxicological responses (e.g. the impact of surface chemistry). It was acknowledged that the range of nanomaterials and uses would be very diverse. This approach can be summarised in the following figure.

Proposed approach to initial toxicological studies with nanomaterials



9. The Committees confirmed that there was no need to develop a new approach to risk assessment of nanomaterials but there was a clear need to provide hazard identification data on the widest possible range of nanomaterials. It was noted that in the absence of such data it was not possible to derive conclusions about the spectrum of toxicological effects which might be associated with nanomaterials. Thus it was noted that nanoparticles resistant to degradation could accumulate in secondary lysosomes, which in cells with a long survival such as neurones or hepatocytes might lead to chronic toxicity.

Additional comments from COM on mutagenicity evaluation.

10. The COM reviewed a number of publications where mutagenic effects *in vitro* had been specifically attributed to nanoparticulate titanium dioxide[11] and zinc oxide[12]. However the COM noted inconsistency in the available mutagenicity data and in the information on the specification of the test materials used. It was therefore not able to conclude that any specific mutagenic activity had been documented which would not also be reported for studies using micrometre sized equivalents.

11. The COM considered that specific information on particle size was required to assess mutagenicity studies undertaken with nanomaterials. Thus, there was insufficient information on titanium dioxide to allow an assessment of the agglomeration/disagglomeration of particles in the vehicles used and it was not possible to conclude which particles had been tested. The COM agreed that it might be appropriate to support *in-vitro* mutagenicity tests with imaging data on particle sizes.

12. The Committees agreed that particle size was a generic factor which should be considered with all *in-vitro* testing of nanomaterials.

Additional comment from COC on carcinogenicity evaluation

13. The Committees discussed whether SWCNTs and other carbon nanotubes might have carcinogenic potential analogous to fibres such as asbestos. Some recent information from the SOT abstracts using gold labelled SWCNT had demonstrated that some of these fibres may evade macrophage engulfment, although granuloma formation was still reported. It was considered they would not reach the mesothelium. The COC considered that more information (including detailed structural data, and absorption and cellular response in macrophages) was required on a range of single- and double-walled carbon nanotubes before any definite conclusions could be reached.

Epidemiological aspects of exposure to nanomaterials

14. The Committees noted that there were no published epidemiological studies of nanomaterials available. They also noted that the Royal Society report had highlighted problems in the detection of nanoparticles. It was agreed that estimating human exposure to nanoparticles would be exceptionally difficult particularly where there was exposure to a range of both nanometre-sized and micrometre-sized particles. Similarly, assessment of the toxicity would need to distinguish effects arising from the nanoparticle form and those due to chemical composition. HSE have confirmed that the Health and Safety Laboratory (HSL) in Buxton is working with the US National Institute for Occupational Safety and Health (NIOSH) to develop techniques to carry out such monitoring in the future.

Concluding remarks

15. The Committees noted that the current review did not include information on mixtures of nanoparticles such as in environmental air pollution. Members considered that information from environmental epidemiology and volunteer studies of nanomaterials, predominantly from the field of air pollution research, might be informative in identifying end points for initial screening and possible hazards. It was suggested that liaison with other relevant expert groups such as the Committee on the

Medical Effects of Air Pollutants (COMEAP) would be valuable. In addition information on medical applications of nanoparticles might be important to the COT discussions. Such information might be potentially relevant with regard to information on structure activity. The secretariat was asked to liaise with the Medicines and Healthcare products Regulatory Agency (MHRA).

16. The Committees reached the following overall conclusions:

i) We note that there is the potential for a wide range of nanomaterials to be produced by many different methods and that there is also the potential that they may be used for many different purposes. Two safety concerns arise: firstly, the intrinsic toxicity of the nanomaterial itself and secondly, the fact that products with potential for widespread human exposure (e.g. paints) may be delivered in future using nanotechnology.

ii) We have proposed a systematic tiered approach for initial toxicological studies on novel nanomaterials based on *in-vitro* screening of selected materials supported by biodistribution studies to aid in the identification of cell types for study, followed by appropriate *in-vivo* testing.

iii) We believe from the available toxicological data that current approaches to risk assessment should be appropriate for nanomaterials. However there are limited toxicological data on nanomaterials at present and we consider it is necessary to keep a watching brief of the developing area of nanomaterial toxicology.

iv) We note the difficulties in determining exposures to nanomaterials but consider this to be a high priority for further research so that appropriate risk assessments can be undertaken.

v) We suggest close collaboration and exchange of information between COT/COC/COM and COMEAP and the MHRA so that information on environmental air pollution and human medicines can be included in further reviews of nanomaterials. Such information may help to identify potential areas of hazard and risk assessment for nanomaterials used in manufactured products.

vi) We consider this subject should be subject to regular reviews by COT/COC/COM.

December 2005

References

1. UK Government (2003). The Government's Response to Better Regulation Task Force's report on Scientific Research: Innovation with Controls. http://www.brtf.gov.uk/docs/pdf/scienceresponse.pdf

2. The Royal Society and Royal Academy of Engineering (2004). Nanoscience and nanotechnologies: Opportunities and Uncertainties, published 29 July 2004, http://www.nanotec.org.uk/finalReport.htm

3. HM Government in consultation with devolved administrations (2005). Response to The Royal Society and Royal Academy of Engineering report "Nanoscience and nanotechnologies: Opportunities and Uncertainties", http://www.ost.gov.uk/policy/issues/nanotech_final.pdf

4. COT (2005). Overview of nanomaterial toxicology. http://www.food.gov.uk/multimedia/pdfs/TOX-2005-19.pdf

5. COC (2005). Overview of nanomaterial toxicology. http://www.advisorybodies.doh.gov.uk/pdfs/cc055.pdf

6. COM (2005). Overview of nanomaterial toxicology. Consideration of mutagenicity data. http://www.advisorybodies.doh.gov.uk/pdfs/mut0515.pdf

7. HSE (2004) Health effects of particles produced for nanotechnologies, EH75/6. http://www.hse.gov.uk/horizons/nanotech/index.htm

8. Society of Toxicology (2005) 44th Annual Meeting to be held March 6–10, 2005, in New Orleans, Louisiana. Abstracts. Published in *The Toxicologist*, volume 84, March 2005.

9. Seaton, A., MacNee, W., Donaldson, K. and Godden, D. (1995) Particulate air pollution and acute health effects. *The Lancet* 345, 176-178.

10. Wang H, Wang J, Deng X, Sun H, Shi Z, Gu Z, Liu Y and Zhao Y (2004). Biodistribution of carbon single-wall carbon nanotubes in mice. *J Nanoscience nanotechnology*, 4, 1019-1024.

11. Rahman Q, Lohani M, Dopp E, Pemsel H, Jonas KL, Weiss DG and Schiffmann D.(2002). Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts. *Environmental Health Perspectives*, 110, 797-800.

12. SCCNFP (2003). Final evaluation and opinion on zinc oxide. SCCNFP/0649/03 final.

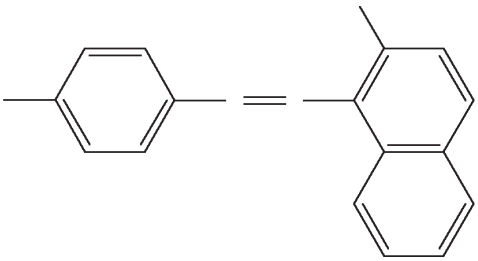
13. Minutes of 2004 COT horizon scanning: http://www.food.gov.uk/science/ouradvisors/toxicity/cotmeets/cot_2004/169681/cotmin2february2004

COM/COC horizon scanning papers for 2004:
http://www.advisorybodies.doh.gov.uk/pdfs/MUT0422.pdf
http://www.advisorybodies.doh.gov.uk/pdfs/cc0432.pdf

Para Red risk assessment

1. There is only one study on the genotoxicity of para red available from the literature, an *in vitro* genotoxicity study in *S. typhimurium* (Milvy and Kay 1978). Para red was positive with metabolic activation in two strains (TA 1538 and TA 98), but was negative in these strains without metabolic activation. It has not been tested for carcinogenicity.

2. The structure of para red is given below.



1-((4-nitrophenyl)azo)-2-naphthalenol.

3. Although a different structure was illustrated in the Milvy and Kay paper the CI number they quoted corresponded with the above structure. The metabolites of para red described by Milvy and Kay (*p*-nitroaniline and 1-amino-2-naphthol) would be derived from the structure illustrated above. The structure illustrated in the Milvy and Kay paper would not have the dye colour properties of para red and this would have been obvious to the authors. It was concluded based on the CI number, dye properties and description of metabolites that although incorrectly illustrated the material tested by Milvy and Kay was para red.

4. A report in Dutch by the RIVM, attached to the RASFF, notes these mutagenicity data and points out that azo dyes can be metabolised to aromatic amines, a number of which are known genotoxic carcinogens. The RIVM report states that based on the structure of para red it could plausibly be metabolised to the aromatic amine p-nitroaniline.

5. The data on p-nitroaniline are also limited. It has produced positive results in a number of *in vitro* genotoxicity studies (*S. typhimurium* strain TA98, with and without metabolic activation, chromosomal aberrations in CHO cells with S9, trifluorothymidine resistance induction in L5178Y mouse lymphoma cells without S9) (NTP, 1993). In an NTP carcinogenicity study there were equivocal results in male mice, based on marginal increases in haemangiosarcoma of the liver and haemangioma or haemangiosarcoma (combined) at all sites, and negative results in female mice (NTP, 1993).

Conclusions

6. Despite the very limited data available, the Chairs of COC and COM considered that it is prudent to assume that para red could be a genotoxic carcinogen based on the azo structure and the positive *in vitro* genotoxicity finding. Dietary exposure should therefore be as low as reasonably practicable (ALARP).

References

1. Milvy P & Kay K (1978); Mutagenicity of 19 major graphic arts and printing dyes. J Toxicol Environ Health 4:31-36.

2. NTP (2003). NTP Technical Report on the Toxicology and Carcinogenesis studies of p-nitroaniline (CAS 100-01-6) in B6C3F_1 Mice (Gavage Studies). National Toxicology Program. TR 418. NIH publication no. 93-3149.

May 2005

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Declaration of members' interests during the period of this report

	Personal Interest		Non Personal Interest	
MEMBER	COMPANY	INTEREST	COMPANY	INTEREST
Professor I Hughes (chairman)	Pfizer	Education Adviser	Archives of Disease in Childhood	Commentary Editor
	BP Amoco	Shares	Academy of Medical Sciences	Fellow
	BP Amoco	Daughter is an employee of this Company	Society for Endocrinology	Member
	Topical Endocrinology	Editorial Board 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 Fund	Funds received from all these sources for Departmental research and education in medicine and health related topics
Professor J Ashby	Syngenta	Employee, Salary; Share Option	NONE	NONE
Dr D Bell	Alliance & Leicester	Shares	FSA	Research Contract
	BAA		Astrazeneca	BSRC CASE studentship
	BG Centrica HBOS Plc International Power RT Group Rolls Royce Scottish Power Thus United Facilities National Grid Transco	Employee Shareholder		

	Personal Interest		Non Personal Interest	
MEMBER	COMPANY	INTEREST	COMPANY	INTEREST
Professor A Boobis OBE	Abbey National Scottish Power Centrica BG Group Halifax Barclays National Grid Transco BT Group	Shares	GlaxoSmithKline FSA ILSI HESI Elsevier	Support by Industry Research Contract Member of Board of Trustees Editor-in-Chief; Food and Chemical Toxicology
Dr P Carthew	Unilever Provalis	Employee Shareholder	NONE	NONE
Dr R Dearman	Syngenta CTL AstraZeneca	Salary Shareholder	NONE	NONE
Dr P Greaves	Marix Drug Development Ltd Shire Pharmaceutical Development Ltd Experimental Pathology Laboratory USA Sun Coast Tox USA Sanofi-Aventis AstraZeneca	Consultancy	NONE	NONE
Dr J Hinson	GlaxoSmithKline	Shareholder	NONE	NONE
Dr P Jackson	British Heart Foundation Mitchell & Butler Intercontinental Hotels Marks & Spencer	Lecture Fees Share Ownership	Medtronic AVE	Research Grant
Professor J Lunec	NONE	NONE	Scilucent LLC USA	Funding research group to investigate toxicology of soya-bean oil implants
Professor D Ray	Medical Research Council	Employer	Consortium of: Bayer, DuPont, FMC Sytenta & Valent	Research Grant

	Personal Interest		Non Personal Interest	
MEMBER	COMPANY	INTEREST	COMPANY	INTEREST
Professor I Rowland	Alpro Foundation Woolwich Halifax	Consultancy Shares	Valio (Finland) Alpro (Belgium) Alpro VK Muehlen (Germany) Biohit (Finland) Danone (France) Orafti (Belgium) Unilever UK ILSI Europe Cerestar (Belgium) Geest Viatcress Yakult UK Nicobrand	Partner in EC funded project CAST PhD studentship Partner in EC funded project CAST PhD studentship Partner in EC funded project Funded Research CAST PhD studentship
Dr L Rushton	Transport & General Workers Union Friends Provident Northern Rock Unilever	Consultancy – completed Shares Consultancy – advice on design of an epidemiological survey relating to dermatitis	European Silica International Manganese Institute American Chemistry Council	Ongoing Cohort Study Contract to IEH – completed Contract to IEH to prepare criteria document – completed Contract to IEH for systematic review and meta analysis – completed
Dr G Rylance	NONE	NONE	NONE	NONE

	Personal Interest		Non Personal Interest	
MEMBER	COMPANY	INTEREST	COMPANY	INTEREST
Dr L Stanley	CXR Biosciences	Salary	Alizyme	Company Contract
	Agan	Company Contract	Arrow	
	Proctor & Gamble		Bayer	
	Toxel		Cyclacel	
	Association of Plastics Manufacturers	Consortium Client	Entremed	
	Europe Eurochlor		Etiologics	
	European Council for Plasticisers and Intermediates		Ferring	
	Halogenated Solvents Industry Association		Grupovita	
	AstraZeneca	Research Collaboration	Guerbet	
	GlaxoSmithKline		Ionix	
	NovoNordisk		Nestle	
	Pfizer		Neuroseach	
	Wyeth		Oncosense	
			Serono	
			Stiefel	
			Strakan	
			Yamanouchi	
Professor S Strobel	NONE	NONE	NONE	NONE
Miss A Ward	NONE	NONE	Barking, Havering and Redbridge NHS Trusts	Non-Executive Director
Mrs A Williams	NONE	NONE	NONE	NONE
Dr C deVries	NONE	NONE	Schering AG	Research Grant
			Yamanouchi	