

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

Committee on \_\_\_\_\_  
**TOXICITY**

Committee on \_\_\_\_\_  
**MUTAGENICITY**

Committee on \_\_\_\_\_  
**CARCINOGENICITY**

Annual Report 2005

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Toxicity  
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of Chemicals in Food,  
Consumer Products  
and the Environment

Annual Report

2005



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## About the Committees

This is the fifteenth joint annual report of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) and the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC).

The aim of these reports is to provide a brief toxicological background to the Committees' decisions. Those seeking further information on a particular subject can obtain relevant references from the Committee's administrative secretary or from the internet sites listed below.

In common with other independent advisory committees the members are required to follow a Code of Conduct which also gives guidance on how the commercial interests should be declared. Members are required to declare any commercial interests on appointment and, again, during meetings if a topic arises in which they have an interest. If a member declares a specific interest in a topic under discussion, he or she may, at the Chairman's discretion, be allowed to take part in the discussion, but they are excluded from decision making. The Code of Conduct is at Annex 2 and Annex 3 describes the Committees' policy on openness. Annex 4 contains a glossary of technical terms used in the text. Annex 5 is an alphabetical index to subjects and substances considered in previous reports. Previous publications of the Committees are listed in Annex 6.

These three Committees also provide expert advice to other advisory committees, such as the Advisory Committee on Novel Foods and Processes, and there are also links with the Veterinary Products Committee and the Advisory Committee on Pesticides.

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

COT: <http://www.food.gov.uk/science/ouradvisors/toxicity/>  
<http://www.advisorybodies.doh.gov.uk/cotnonfood/>

COC: <http://www.advisorybodies.doh.gov.uk/coc/>

COM: <http://www.advisorybodies.doh.gov.uk/com/>

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

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# 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.

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

# 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 durations 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.

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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](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 dermal 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 ( $\mu\text{m}$ ). 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)

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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 ( $\text{PO}_4^{3-}$ ).
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

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# 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<sup>a</sup> (CSM) held a joint meeting on the 2<sup>nd</sup> 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<sup>b</sup> and pharmacodynamic<sup>c</sup>. 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<sup>1</sup> 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<sup>2</sup> 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<sup>d</sup>. 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.

<sup>a</sup> The Committee on the Safety of Medicines is now known as the Commission on Human Medicines

<sup>b</sup> Pharmacokinetics describes the fate of a drug in the body including a mathematical account of their absorption, distribution, metabolism and excretion.

<sup>c</sup> Pharmacodynamics is the interaction of drugs with target sites such as receptors, leading to therapeutic or adverse effects.

<sup>d</sup> 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. Splanchnic blood flow (blood flow in the internal organs) can be increased by high protein meals but is unaffected by carbohydrate<sup>3</sup>; increased splanchnic 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<sup>4</sup>.
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<sup>5</sup>. 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<sup>6</sup>. The clinical significance of this latter interaction is uncertain.

Table 1. Summary of individual food-drug interactions.

Food	Drug	Interaction	Significance?	Reference
<i>Absorption</i>				
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
<i>Distribution</i>				
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
<i>Metabolism</i>				
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
<i>Excretion</i>				
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)<sup>e, 11</sup>. However curcumin is poorly absorbed *in vivo*<sup>12</sup> 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<sup>2</sup>. 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<sup>2</sup>.
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<sup>13</sup>.

<sup>e</sup> 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<sup>14</sup>. 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-enzyme 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<sup>15</sup>. 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<sup>16</sup>.
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<sup>17</sup>.

### *Alcohol*

16. Ethanol has both pharmacokinetic and pharmacodynamic effects, and can interact both with drugs and with food<sup>18</sup>. 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<sup>5</sup>. 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 CYP1A2 increasing warfarin metabolism and thus may reduce its effectiveness and increase the risk of clotting<sup>10</sup>. 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<sup>19</sup>. Other foods reported to reduce warfarin efficacy include large quantities of ice cream, soya beans and avocados<sup>7</sup>. 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<sup>20, 21</sup>. 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<sup>22</sup> 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<sup>23</sup>. St John's wort can also induce the MDRI transporter, reducing, for example, plasma concentrations of digoxin<sup>24</sup>.

#### *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<sup>25</sup>. Drug-food effects may be less direct; for example antibiotics may alter enteric micro-organisms resulting in reduced absorption of fat-soluble vitamins<sup>26</sup>. 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<sup>27</sup> 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 MDRI 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<sup>28</sup>, 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<sup>29</sup>. Renal excretion is also reduced in older people. Many older people may be taking multiple medicines increasing the potential for interactions<sup>26</sup>.
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<sup>28</sup>.

#### *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<sup>30</sup>. 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<sup>31</sup>. 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,  $\alpha$ -1-acid glycoprotein may increase the degree of protein binding of triazolam and propranolol<sup>31</sup>. However, albumin binding is generally unaffected<sup>32</sup>.
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<sup>33</sup>.
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<sup>34</sup>.
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<sup>35</sup>. 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<sup>31</sup>. 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<sup>31</sup> changes in oestradiol metabolism, and increased resistance to insulin<sup>30</sup>. 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<sup>31</sup>.
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  $\beta$ -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*<sup>36</sup>. 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, 17 $\beta$ -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<sup>37</sup>. 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.

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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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## 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<sup>1</sup>. In the absence of these, a standard Functional Observational Battery in the F<sub>1</sub> 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)<sup>2</sup>.

## Summary of data available

### Quinoline Yellow (E104)

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

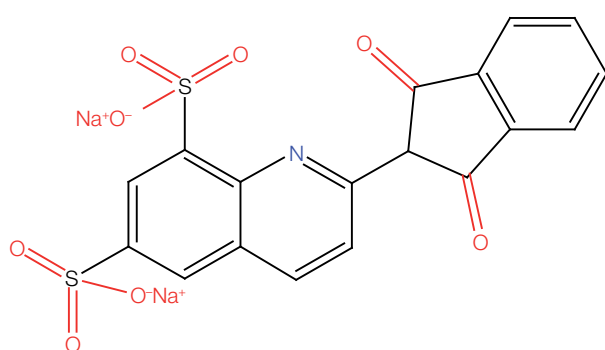


Figure 1. Quinoline yellow (disodium 2-(1,3-dioxo-2-indanyl)-6,8-quinolinesulphate)

7. Quinoline yellow was evaluated by the SCF<sup>3</sup> and by JECFA<sup>4</sup>. 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 OF1 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.<sup>5</sup> 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).

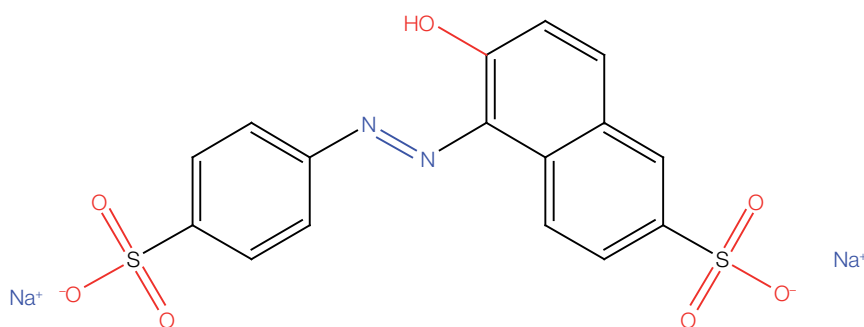


Figure 2. Sunset yellow (disodium 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalene-sulfonate)

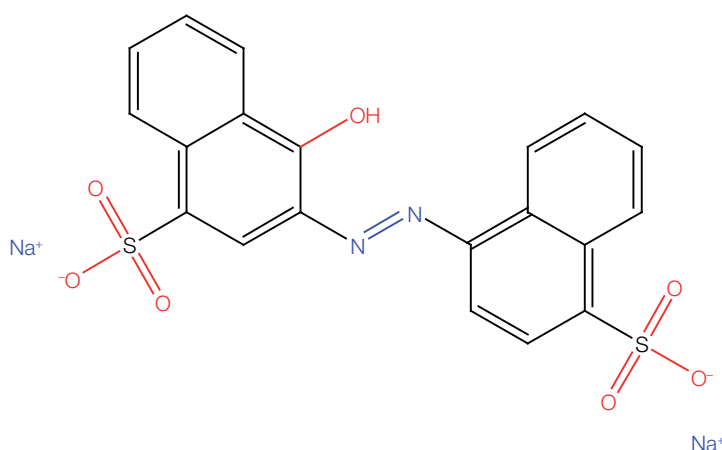
12. Sunset yellow was evaluated by JECFA<sup>6</sup> and the SCF<sup>3</sup>. The toxicity data assessed by the committees included acute and short-term studies, multi-generation, reproduction and teratology studies and long-term studies in the rat, mouse and dog.
13. JECFA established an ADI of 0-2.5 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<sup>7</sup> 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<sub>2b</sub> 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<sub>50</sub>) 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<sup>8</sup> 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)<sup>9</sup> 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<sub>1</sub> 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)<sup>5</sup> 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)<sup>10</sup> 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<sup>11</sup> and the SCF<sup>3</sup>. 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<sup>12</sup>.
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<sup>13</sup>. Rats from the 3<sub>0</sub> 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<sub>50</sub> study<sup>14</sup> 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<sup>15</sup> rats were fed up to 1200 mg/kg bw/day carmoisine, the F<sub>1</sub> generation being exposed for 110-115 weeks. No adverse clinical effects or effects on behaviour or reproductive performance were reported in the F<sub>0</sub> generation. Mortality in the F<sub>1</sub> 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)<sup>10</sup> 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)<sup>10</sup> also reported that in rats fed 4 mg/kg bw carmoisine or naphthionic 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 naphthionic 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).

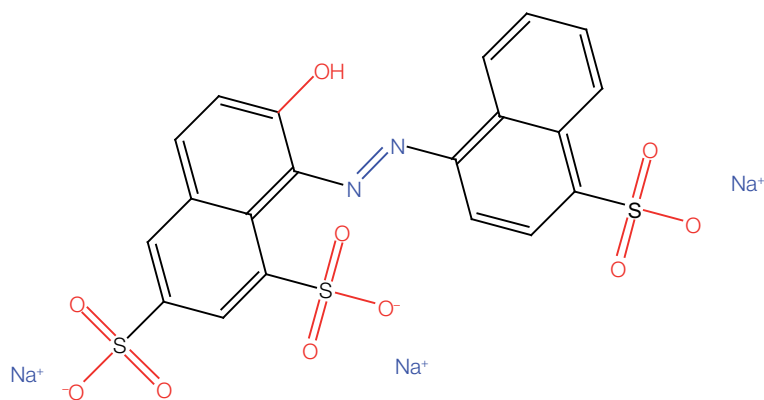


Figure 4. Ponceau 4R (trisodium 2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate)

33. Ponceau 4R was evaluated in 1983 by JECFA<sup>11</sup> and the SCF<sup>3</sup>. 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<sub>3</sub> animals.
36. The RTECS report stated that Ponceau 4R had behavioural effects in rodent studies. The report is based on an LD<sub>50</sub> study<sup>16</sup> 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)<sup>17</sup> 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<sub>2</sub> but not the F<sub>3</sub> generation. For the F<sub>3</sub> 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<sub>3</sub> animals but there were no treatment related histopathological findings.
38. In a subsequent two-generation study<sup>18</sup> 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<sub>0</sub> generation. The F<sub>1</sub> 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<sup>19</sup> and by the SCF<sup>3</sup>. 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.

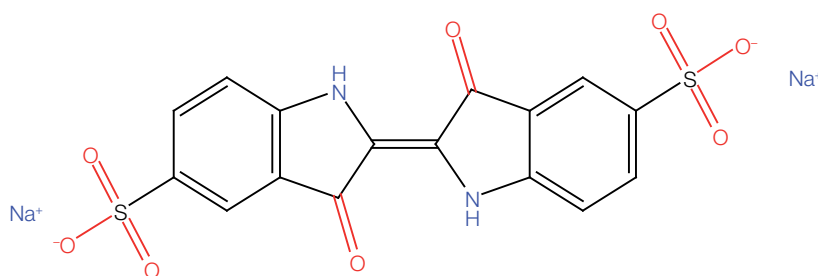
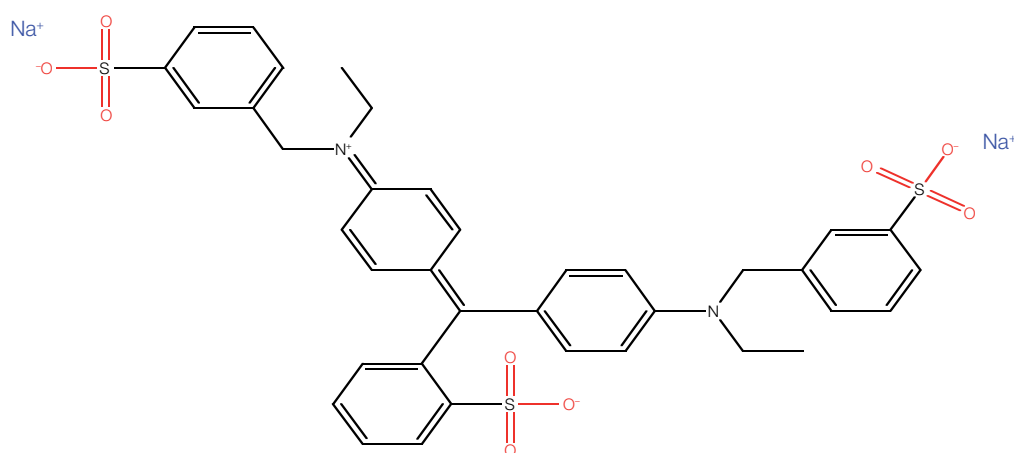


Figure 5. Indigo carmine (disodium 3,3'-dioxo-2,2'-bi-indolylidene-5,5'-disulfonate)

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<sup>20</sup>, which investigated the chronic toxicity and carcinogenicity of indigo carmine in rats treated with indigo carmine *in utero*. The F<sub>0</sub> 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<sub>1</sub> 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<sup>21</sup> and the SCF<sup>3</sup>. 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  $\alpha$ -[4-(N-ethyl-3-sulfonatobenzylamino)phenyl]- $\alpha$ -[4-(N-ethyl-3-sulfonatobenzylimino)cyclohexa-2,5-dienylidene] toluene-2- sulfonate**

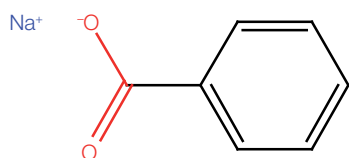
48. The RTECS report lists brilliant blue as having behavioural effects including convulsions and effects on seizure threshold in mice. This is taken from an LD<sub>50</sub> study in which the effects were reported following i.p. administration of 4600 mg/kg, a lethal dose.
49. Following the JECFA and SCF evaluations, one additional paper has been published, Borzelleca *et al.* (1990)<sup>22</sup> 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<sub>0</sub> 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<sub>1</sub> 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<sup>23</sup> and JECFA<sup>24</sup>. 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.

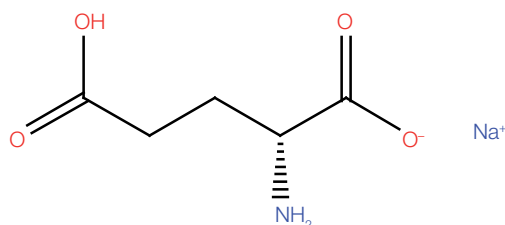
56. COT view. The structure of benzoate suggests that it could penetrate the blood brain barrier or cross the placenta, possibly via the organic anion transporter, though metabolic studies with radiolabelled benzoate reviewed by the SCF and JECFA indicate that the vast majority is excreted with little 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<sup>25</sup> and the SCF<sup>23</sup>. 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<sub>1</sub> and F<sub>2</sub> generations, but a dose-response relationship was not apparent. The number of F<sub>2a</sub> young were reduced in the treated animals but this was not dose-related and did not occur in the F<sub>2b</sub> 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<sup>26</sup> and by the SCF<sup>27</sup>. 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<sup>26</sup>. Acute symptoms of burning, facial pressure and chest pains have also been reported<sup>28</sup>.
64. Since the evaluations were conducted, four additional neurotoxicity studies have been identified. Bawari *et al.* (1995)<sup>29</sup> 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)<sup>30</sup>. Hsieh *et al.* (1997)<sup>31</sup> 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)<sup>32</sup> 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 <sup>3</sup>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<sup>33</sup> and the SCF<sup>34</sup>. 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<sub>0</sub> and F<sub>1</sub> 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.

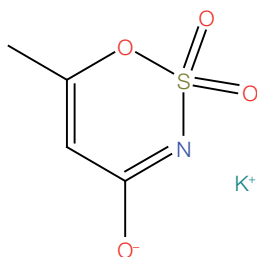


Figure 10. Acesulfame K (potassium salt of 6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide).

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<sup>35</sup> and the SCF<sup>36</sup>. Both Committees derived an ADI of 0-40 mg/kg bw/day on the basis of a 104-week study in rats.

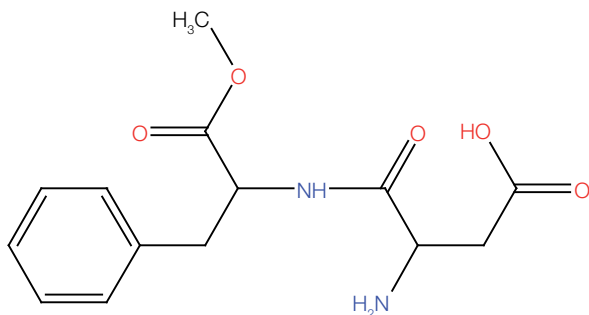


Figure 11. Aspartame (6-methyl-1,2,3-oxathiazine-4(3H)—one-2,2-dioxide salt of L-phenylalanyl-2-methyl-L- $\alpha$ -aspartic acid).

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).

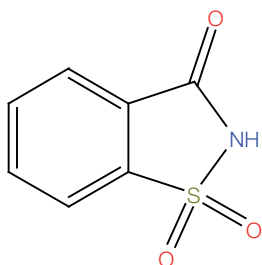


Figure 12. Saccharin (3-oxo-2,3-dihydrobenzo[d]isothiazol-1,1-dioxide)

77. Saccharin has been evaluated by JECFA<sup>37</sup> and the SCF<sup>38</sup>. 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 sulphanic 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.

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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

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# 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 Protheses. 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 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.<sup>1</sup> Their report “Nanoscience and nanotechnologies: opportunities and uncertainties” was published on 29 July 2004.<sup>2</sup> The UK Government’s response to the joint Royal Society and Royal Academy of Engineering report was published on 25 February 2005.<sup>3</sup> 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.<sup>4-6</sup> The information presented to the committees was based on a hazard assessment document published by the Health and Safety Executive (HSE)<sup>7</sup>, 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.<sup>8</sup> 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 ( $\mu\text{m}$ ).<sup>2</sup> 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
<b>Structure</b>	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
<b>Properties</b>	Extreme strength and electrical conductivity. Insoluble in water. Biologically non-degradable			Lightweight, spongy solid, can act as semiconductor. Magnetic property

- 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).<sup>7</sup>

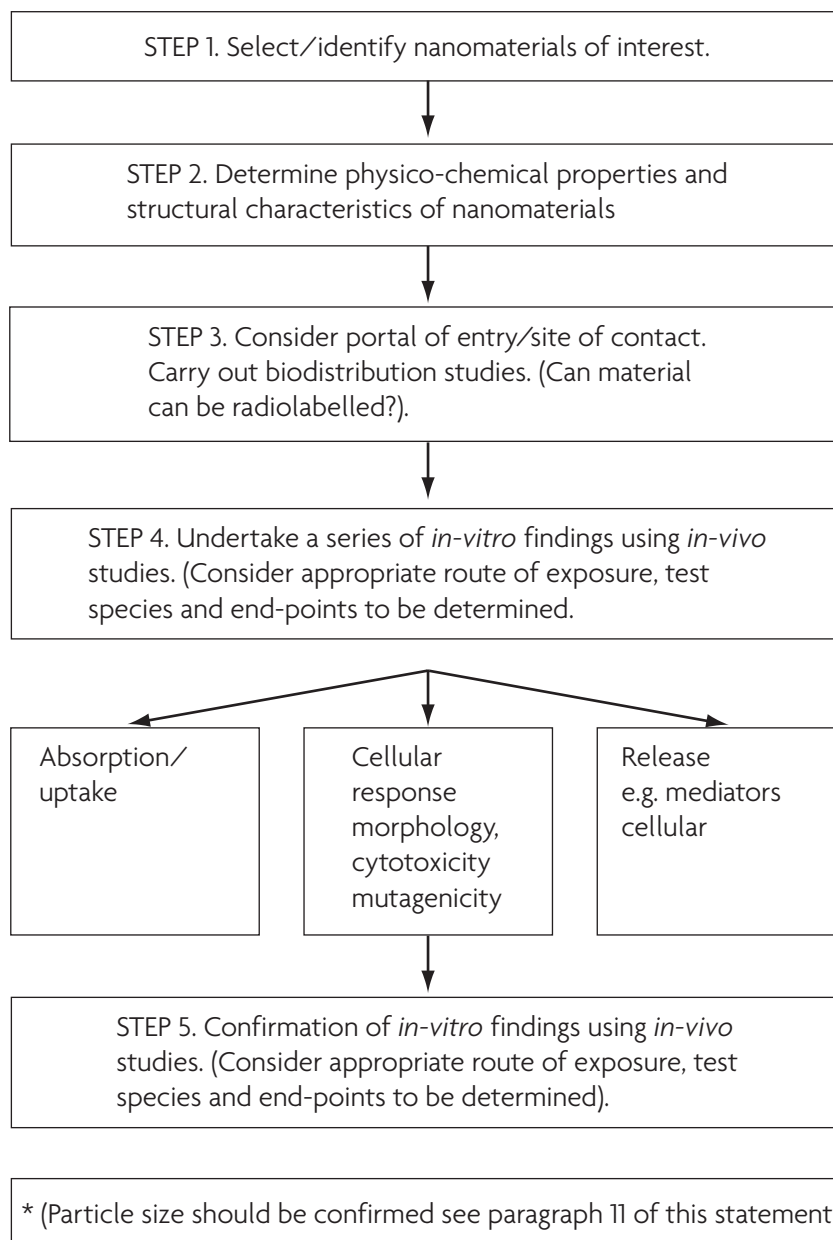
### COT/COC/COM Review of toxicological information on nanomaterials

#### *Proposed approach to initial toxicological studies with nanomaterials*

- 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.

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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.<sup>7,8</sup> The Committees noted the “Seaton” hypothesis regarding potential cardiovascular effects of inhaled particles.<sup>9</sup> 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.<sup>10</sup> 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<sup>11</sup> and zinc oxide<sup>12</sup>. 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

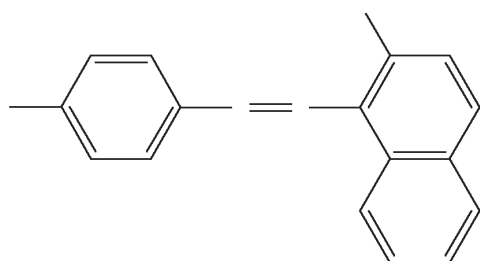
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COM/COC horizon scanning papers for 2004:  
<http://www.advisorybodies.doh.gov.uk/pdfs/MUT0422.pdf>  
<http://www.advisorybodies.doh.gov.uk/pdfs/cc0432.pdf>

### 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).

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May 2005

# 2005 Membership of the Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment

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Scientific Secretary

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Administrative Secretary

**Mr J Battershill** BSc MSc

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**Ms G Aherne** BSc

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(until August 2005)

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**Dr N Rajapakse** BSc PhD

**Dr C Tahourdin** BSc PhD

**Dr N Thatcher** BSc PhD

**Miss T Gray**

**Miss J Murphy**

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

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor I Hughes (chairman)	Pfizer	Education Adviser	Archives of Disease in Childhood	Commentary Editor
	BP Amoco	Shares	Academy of 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 Medical Research Council Pfizer Aventis NovoNordisk Diabetes UK Wellcome Trust Juvenile Diabetes Fund	Fellow; Senior Examiner; Regional Academic Advisor Member of Advisory Board 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	Employee Shareholder		
	Centrica			
	HBOS Plc			
	International Power			
	RT Group			
	Rolls Royce			
	Scottish Power			
	Thus			
United Facilities				
National Grid Transco				

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
<b>Professor A Boobis OBE</b>	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
<b>Dr P Carthew</b>	Unilever Provalis	Employee Shareholder	NONE	NONE
<b>Dr R Dearman</b>	Syngenta CTL AstraZeneca	Salary Shareholder	NONE	NONE
<b>Dr P Greaves</b>	Marix Drug Development Ltd Shire Pharmaceutical Development Ltd Experimental Pathology Laboratory USA Sun Coast Tox USA Sanofi-Aventis AstraZeneca	Consultancy	NONE	NONE
<b>Dr J Hinson</b>	GlaxoSmithKline	Shareholder	NONE	NONE
<b>Dr P Jackson</b>	British Heart Foundation Mitchell & Butler Intercontinental Hotels Marks & Spencer	Lecture Fees  Share Ownership	Medtronic AVE	Research Grant
<b>Professor J Lunec</b>	NONE	NONE	Scilucent LLC USA	Funding research group to investigate toxicology of soya-bean oil implants
<b>Professor D Ray</b>	Medical Research Council	Employer	Consortium of: Bayer, DuPont, FMC Syntenta & Valent	Research Grant



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

MEMBER	Personal Interest		Non Personal Interest	
	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 Yamanouchi	Research Grant

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

## Preface



The Committee on Mutagenicity (COM) provides advice on potential mutagenic activity of specific chemicals at the request of UK Government Departments and Agencies. Such requests generally relate to chemicals for which there are incomplete, non-standard or controversial data sets for which independent authoritative advice on potential mutagenic hazards and risks is required. Frequently recommendations for further studies are made.

During 2005, the Committee provided advice on a wide range of chemicals which included furan, halonitromethanes, meglumine, proquinazid perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA).

The assessments of furan, PFOS and PFOA were undertaken in conjunction with its sister Committee on Carcinogenicity (COC). A successful joint meeting between COM and COC was held on the subject of the use of target organ mutagenicity data in carcinogen risk assessment and a publication for a peer reviewed journal is to be submitted soon. The COM also has an ongoing responsibility to provide Government Departments and Regulatory Authorities with advice on developments in procedures for the evaluation and risk assessment of mutagens. In this regard the Committee provided advice on the *cII* transgenic mutation assay and a comparison with data provided by the transgenic *lacI* and *lacZ* assays. A detailed consideration of potential areas of future consideration of mutagen risk assessment was undertaken including developments in the strategy for testing of mutagenicity, the role of methylation in transgenerational effects and evaluation of mixtures of mutagens.

The COM completed a major piece of work on the evaluation of biomonitoring studies of genotoxicity in pesticide applicators. The statement was forwarded to the Advisory Committee on Pesticides and was well received. A peer review publication is in preparation.

Professor P B Farmer Chair  
MA DPhil CChem FRSC

## Biomonitoring studies of genotoxicity in pesticide applicators

- 2.1 The Medical and Toxicology Panel (MTP) of the Advisory Committee on Pesticides (ACP) has asked the COM for advice on the biomonitoring studies of genotoxicity in pesticide applicators. The MTP had considered in April 2004 that there were sufficient numbers of reports of biomonitoring studies in the published literature which had been retrieved and evaluated by MTP to request an independent view from COM on the available studies. It was noted that pesticide active ingredients that are DNA reactive *in-vivo* mutagens are not approved for use in formulated pesticide products in the U.K.
- 2.2 The DH Toxicology Unit at Imperial College in collaboration with the COM secretariat drafted a series of review papers for the COM. The review considered published biomonitoring studies in the scientific literature up to December 2004. A comparison was undertaken with the literature search strategy used by the MTP in order to ascertain whether any published studies had been missed. A very good agreement between the MTP and COM literature searches was attained with only one study identified in the COM literature search which had not already been identified by MTP. The discussion papers can be accessed via the COM internet site under the "papers" section.  
<http://www.advisorybodies.doh.gov.uk/com/>
- 2.3 The COM took its remit from the referral from MTP and agreed to consider data originating from applications of formulated pesticides and pesticide mixtures. COM did not consider studies where a single pesticide active ingredient with known mutagenic potential was used (e.g. methyl bromide fumigation). A total of 70 studies were subject to initial assessment and a review for the adequacy of the genotoxicity assessment. The COM discussed the exclusion/inclusion criteria at the October 2004 and February 2005 meeting and following a postal consultation after the February 2005 meeting. Following this procedure 24 studies selected by COM using the exclusion/inclusion criteria were subjected to an epidemiology overview. The full report of the epidemiology review can be accessed from the COM internet site (<http://www.advisorybodies.doh.gov.uk/pdfs/mut0511.pdf>). One objective of the epidemiology overview was to attempt a quality ranking of studies (for example study design, consideration of confounding, modelling of results and interpretation). The COM considered the epidemiology overview at its 26 May 2005 meeting. Members agreed that the review had highlighted and confirmed their views on the limitations of the data set. However members agreed to review the data in order to reach the most appropriate conclusions possible.
- 2.4 The COM considered the full published reports and a narrative summary of the selected studies.  
<http://www.advisorybodies.doh.gov.uk/pdfs/MUT0419.pdf>  
<http://www.advisorybodies.doh.gov.uk/pdfs/mut051.pdf>
- 2.5 The COM established criteria for assessment. In this regard the publication by Albertini et al (*Mutat Res* **463**, 111-72, 2000) were valuable. The COM also undertook a consideration of the variation in background frequency of genotoxicity indices used in the biomonitoring studies, the magnitude of response seen in studies of patients and nurses exposed to known *in-vivo* mutagenic cytostatic medicines, the potential influence of use of Personal Protective Equipment (PPE), and available exposure information from studies in pesticide applicators. Members agreed that consideration of statistical significance and magnitude of effects from adequately conducted studies was the most appropriate approach to evaluating the available data. Members confirmed that the available information was

severely limited and hence no definite conclusions could be drawn with regard to any U.K. agricultural applications of pesticides or to individual pesticide active ingredients. However the COM agreed that it would be appropriate to consider what conclusions could be derived from the selected studies.

- 2.6 Members considered that the factors which accounted for the variance in the indices of genotoxicity in these biomonitoring studies (chromosome aberrations and micronuclei predominantly in circulating blood lymphocytes) in nurses and cancer patients exposed to cytostatic medicines had not been fully evaluated. The biomonitoring indices of genotoxicity were observed to differ considerably in the control populations of different studies. Members concluded, therefore, that it was not possible to establish a minimum fold increase for biological importance that could be applied to studies of pesticide applicators based on the studies in nurses and cancer patients exposed to cytostatic medicines. Further, it was concluded that factors affecting variance in genotoxicity indices used in the biomonitoring studies of pesticide applicators which had been reviewed were not understood adequately. In this respect members considered it would be very difficult to infer causality for the small magnitude responses seen in the biomonitoring studies of pesticide applicators. There was a need for more data on the background variability in the general population of biomonitoring indices of genotoxicity, and on factors affecting variance, which was required before a proper assessment of studies could be made.
- 2.7 The COM noted that with regard to pesticide active ingredients that were currently approved for use in the U.K. and which were also classified with regard to mutagenicity under EC/67/548, carbendazim was used in 4/11 positive and 1/4 negative studies respectively. This represented a very small and incomplete amount of information. It was noted that benzimidazoles were used in a number of approved pesticide products since a risk assessment could be undertaken for the threshold related effects of these active ingredient on microtubule inhibition. The COM confirmed that the available information was severely limited and hence no definite conclusions could be drawn with regard to any U.K. agricultural applications of pesticides or to individual pesticide active ingredients. The COM concluded;
- i) The COM review was based on 70 retrieved published studies of biomonitoring of genotoxicity in pesticide applicators. The evidence covered a large number of types of applications and a wide diversity of pesticide mixtures. The COM selected 24 studies from which conclusions could be drawn through the application of a quality screen of retrieved studies. An independent epidemiological overview of these 24 studies reported that all had significant design and evaluation faults. The COM agreed that any conclusions reached on this evidence would be limited by the poor quality of the available studies.
  - ii) The COM agreed that following a review of the 24 selected studies there was limited evidence supporting increased biomonitoring indices of genotoxicity in biomonitoring studies of pesticide applicators. The COM agreed that it was not possible to make any conclusions regarding exposure-response from the selected biomonitoring studies of genotoxicity because of the inadequacy and unreliability of exposure measurements and the generally small increases in response. The COM noted that there was no published study of pesticide applicators using pesticide mixtures in the U.K.

- iii) The COM agreed that the factors which accounted for the variance in biomonitoring indices of genotoxicity (chromosome aberrations and micronuclei predominantly in circulating blood lymphocytes) in nurses and cancer patients exposed to cytostatic medicines and in pesticide applicators had not been fully evaluated. It was not possible to define a minimum increase in biomarkers of genotoxicity associated with cytostatic medicines from the available studies on nurses and cancer patients. Based on these observations and the large inter-study variation for the biomonitoring indices of genotoxicity in unexposed populations, the COM concluded that it would be very difficult to infer causality for the small magnitude responses seen in the biomonitoring studies of pesticide applicators. There was a need for more data on the background variability in the general population of biomonitoring indices of genotoxicity, and on factors affecting variance, which was required before a proper assessment of studies could be made.
- iv) The COM agreed that it is very difficult to draw conclusions on what might be the most appropriate biomonitoring study for U.K. pesticide applicators. There was some very limited evidence to suggest that an appropriate study of floriculturalists using benzimidazoles (e.g. carbendazim) might represent a reasonable proposal. The COM concluded that in view of the large numbers of individuals which would be required in order to detect an effect in such a study it was most unlikely that a sufficiently large and appropriate exposure group could be identified.

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

## Furan

2.9 Furan is used industrially primarily as a solvent or in the synthesis of commercial compounds. It is also found in air pollution, tobacco smoke and many foods and beverages. The COM had been asked for advice on the mutagenicity of furan by the COT who were considering research that would be helpful regarding the risk assessment of this contaminant. Members agreed there were no clear structural alerts for mutagenicity but metabolism mediated by CYP2E1 could lead to epoxide formation which was consistent with the finding of cis-2-butene-1,4-dial. It was noted that carbon dioxide was a major metabolite which was indicative that furan ring opening occurred. However there were no quantitative data on metabolism available.

## COM evaluation

2.10 The COM considered that the available *in-vitro* mutagenicity tests of furan in bacteria had been adequately conducted. There was no evidence that furan was mutagenic in *Salmonella typhimurium* strains except for a weak positive response in one study in TA100 in the presence of exogenous metabolising fraction. The positive results in tests using *Salmonella typhimurium TA 104* with cis-2-butene-1,4-dial were consistent with this compound being a reactive aldehyde. Members noted the positive results reported in chromosomal aberrations tests in CHO cells in both the presence and absence of exogenous metabolic activation but commented that the concentrations where positive results had been identified (ca 125 mM and 250 mM) were considerably greater than the OECD upper limit of 10 mM for non cytotoxic test materials. There was no information on cytotoxicity in the NTP CHO test report.

- 2.11 Members considered that the L5178Y mouse lymphoma assay reported by the NTP had been conducted to standards acceptable at the time using plating in soft agar but that a trial had not been conducted in the presence of an exogenous metabolising fraction. Members agreed that in view of the positive results at non-cytotoxic doses this study should be considered as positive and that furan should be regarded as having *in-vitro* mutagenic activity.
- 2.12 The COM considered the available *in-vivo* mutagenicity studies. The key *in-vivo* bone marrow chromosomal aberration assay was undertaken as part of the NTP programme on furan. Members agreed that the data from this study could not be interpreted with certainty because of the confounding effects of toxicity at the dose level used. The COM agreed that negative results had been obtained in *in-vivo* liver UDS studies in rats and mice. Covalent binding studies using oral or intraperitoneal doses of radiolabelled 2,5-<sup>14</sup>C-furan in rats were available but members agreed the data could not be interpreted due to the low specific activity radiolabelled furan used. The results of *in-vitro* studies of the reaction of cis-2-butene-1,4-dial with deoxynucleosides indicated reactions with 2'-deoxyadenosine, 2'-deoxyguanosine and 2'-deoxycytidine but not thymidine. Thus it had been proposed that cis-2-butene-1,4-dial might form DNA cross links. No definite conclusions could be drawn from the available studies of proto-oncogene mutation spectra in hepatocellular adenomas and carcinomas from control and furan treated mice.
- 2.13 The COM concluded that furan should be regarded as an *in-vitro* mutagen but there was insufficient evidence to reach a conclusion on the available *in-vivo* mutagenicity data. Members felt that adequate *in-vivo* mutagenicity testing would be useful. It was suggested this might include a bone marrow micronucleus test or DNA binding studies in cancer target organ tissues. It was noted that the critical carcinogenic effect was cholangiocarcinoma and there would be difficulties in isolating bile duct cells during DNA binding studies.
- 2.14 The COM advice was forwarded to COC and subsequently to COT.

#### Halonitromethanes

- 2.15 Halonitromethanes (HNM's) are a class of compound characterised by the presence of one or more halogen atoms together with a nitro moiety on a single central carbon. They differ from the halomethanes (HM's) by the presence of the nitro moiety. The HNM's had been identified as disinfection by-products (DBP) in drinking water in the US and had received a high priority ranking for investigation by the Environmental Protection Agency [http://www.epa.gov/athens/publications/EPA\\_600\\_R02\\_068.pdf](http://www.epa.gov/athens/publications/EPA_600_R02_068.pdf). The only available published data related to the *in-vitro* studies in Salmonella typhimurium strains and in *in-vitro* Comet studies, conducted using CHO cells. Some preliminary findings had been reported by the EPA to the DH Toxicology Unit regarding *in-vivo* testing of the HNM's in transgenic medaka fish. The COM was also aware of an NTP negative *in-vivo* bone marrow mouse micronuclei test with trichloronitromethane (TCNM; chloropicrin), but that there were no appropriate *in-vivo* mutagenicity data available for other HNMs.



### COM evaluation

- 2.16 The strains tested included TA 98, TA 100, TA 104 and a strain RSJ100 which expresses rat glutathione transferase theta (GSTT1-1) known to activate halomethanes. Members agreed that the standard plate incorporation approach had been undertaken in this study used two plates per dose point (instead of the normal three) but that reproducible small increases in the number of revertant colonies had been reported. It was noted that HNMs might volatilise and therefore procedures to prevent evaporation in the bacterial mutagenicity tests might have been more appropriate. With regard to the Comet studies Members noted that HNMs tested were cytotoxic in CHO cells and that apoptosis induction had not been recorded in these studies. In addition none of the trials had included the addition of an exogenous metabolising fraction. However members agreed that HNMs were genotoxic in this test system.
- 2.17 Overall, in accordance with the COM strategy, HNM's should be regarded as *in-vitro* mutagens and potential *in-vivo* mutagens. It was considered that there was a need for appropriate *in-vivo* testing. The COM suggested:-
- a) *in-vivo* rat liver UDS assay, followed by
  - b) site of contact COMET assay
- If either of these studies yielded positive results, then the chemical under test should be considered to be an *in-vivo* mutagen.
- 2.18 A statement was published and is appended at the end of this report.
- 2.19 The COM was subsequently informed of a personal communication from Dr S Richardson (US EPA) at the October 2005 meeting. It was noted that the preliminary finding of DNA adducts in rat liver following *in-vivo* exposure to dibromonitromethane supported the view that this compound should be considered as having mutagenic potential *in-vivo*.

### Joint COT/COC/COM review of nanomaterials

- 2.20 The risk assessment of nanomaterials was identified by COT/COC/COM as an area of interest during horizon scanning discussions in 2004. The Committee was aware of the publication of a report by the Royal Society and the Royal Academy of Engineering 'Nanoscience and nanotechnologies: opportunities and uncertainties' previously discussed by COT in September 2004. The UK Government's response to this report was published in February 2005. The COT, COC and COM were identified as relevant scientific committees to provide advice on the development of nanotechnology. 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.
- 2.21 The COM considered some specific *in-vitro* mutagenicity data for particulate titanium dioxide and zinc oxide of various sizes including test materials described as being nanomaterials. The COM also considered some specific mutagenicity data on fullerenes. It was agreed that no definite conclusions on the influence of particle size on mutagenicity *in-vitro* could be drawn from the available information.

- 2.22 The COM considered that specific information on particle size was required to assess mutagenicity studies undertaken with nanomaterials. Thus the available information on titanium dioxide did not allow an assessment of the agglomeration/disagglomeration of particles in the vehicles used and it was not possible to conclude what particles had been tested. The COM agreed that it might be appropriate to provide imaging data on particle sizes in order to evaluate *in-vitro* mutagenicity tests nanomaterials.
- 2.23 A statement is appended at the end of the COT section of this report.

### Meglumine

- 2.24 The COM provided advice on the mutagenicity of Flunixin, meglumine and Flunixin meglumine (a non-steroidal anti-inflammatory veterinary medicine)-meglumine (an excipient, exempt from EU regulations) in a statement COM/03/S2 in response to a request for advice from the FSA. The available data on these compounds in 2003 were relatively poor and the COM agreed the best approach was to consider the data on each entity separately. The evaluation of meglumine posed the greatest difficulty in that there were inadequate *in-vitro* data on this compound but some clear positive results had been documented in relatively old *in-vivo* bone-marrow assays using intraperitoneal administration of 2 doses of meglumine followed by harvest 6 hours after the last dose. A number of confirmatory *in-vivo* studies undertaken for the COM had yielded negative/inconclusive findings. The COM had concluded that there was a need for two adequately undertaken *in-vitro* studies (namely a mouse lymphoma assay and an *in-vitro* chromosome aberration assay in human peripheral blood lymphocytes) before conclusions could be reached.
- 2.25 The COM agreed that the two *in-vitro* studies submitted did not provide any evidence for a mutagenic effect and the assays had been adequately conducted.
- 2.26 A revised statement on Flunixin, meglumine and Flunixin meglumine is appended at the end of this report.

### Proquinazid

- 2.27 Proquinazid is a novel fungicide being considered by the ACP under the plant protection Directive (91/414/EC). The data holder is DuPont Chemicals. Proquinazid (6-iodo-2-propoxy-3-propyl-3H-quinazolin-4-one) is intended for use in agriculture and viticulture providing control of powdery mildew in cereals and grapes (*Blumeria graminis*, Class Ascomycetes). It is a novel class of fungicide acting by inhibiting the development of the appressorial germ tube (which is responsible for penetration of the host) but the mechanism of action was unknown. The ACP deferred making a decision pending advice from the COC and COM with regard to the occurrence of cholangiocarcinoma in the rat carcinogenicity bioassay. The COC/COM have not been asked to review any other tumour reported in rodent carcinogenicity bioassays with proquinazid.
- 2.28 Members agreed there were no particular structural alerts for mutagenicity with regard to proquinazid but noted that a possibility for generation of metabolites with a quinone type structure which might potentially alert for free radical generation.

- 2.29 Members heard a presentation from the data holder (Du Pont Chemicals) and discussed a number of aspects of the conduct of the mutagenicity tests which had been submitted. The purpose of the presentation was to provide DuPont's rationale on the adequacy of the genetic toxicology data base for proquinazid and to specifically discuss the results of the mammalian cell *in-vitro* chromosome aberration assay in human lymphocytes and the *in-vitro* mammalian cell gene *hprt* mutation assay in CHO cells. DuPont sought the Committee's agreement that proquinazid is not genotoxic and further testing is unnecessary.
- 2.30 Members considered that the company had provided some relevant information and comments but had not been convinced that the *in-vitro* mutagenicity test package was adequate. Members agreed that a key element of any proposal regarding mechanism of proquinazid induced cholangiocarcinoma in the rat would require an evaluation of mutagenicity and hence it was important to complete the mutagenicity test package. The COM considered that a mouse lymphoma assay should be conducted and that it was advisable to also conduct a continuous 20 h exposure in a mammalian cell chromosomal aberration assay (in human lymphocytes) in the absence of exogenous metabolic activation. Both studies should be conducted to internationally accepted standards. These studies were required to provide full information on the mutagenicity evaluation of proquinazid.

*COM post meeting consideration of additional mouse lymphoma assay*

- 2.31 The data holder submitted on 12 July 2005 the results of a new mouse lymphoma assay undertaken with proquinazid. The assay included an exposure of 3 hour (in presence and absence of exogenous metabolic activation) and a continuous 24 h exposure in the absence of metabolic activation. The results suggested that proquinazid was not mutagenic in this assay. A full report was not available during the postal consultation with COM members. The COM considered the available data from the new mouse lymphoma assay by postal consultation and agreed there was no evidence for a mutagenic effect. It was also agreed that the full report should be considered by COM when available. The final report was submitted to COM members by postal consultation on the 22 August 2005. Members agreed that the submitted mouse lymphoma assay was acceptable and gave negative results.
- 2.32 The COM conclusions were forwarded to the COC. A statement is appended at the end of the COC section of this report.

**Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA)**

- 2.33 These compounds are multifluorinated chemicals based on an eight carbon linear molecule (some branched chain and lower alkane fluorinated carbon impurities may be present at low levels). PFOS and PFOA had unusual physico-chemical properties forming microdispersion micelles in aqueous systems. Members heard that the Environment Agency had recently concluded that PFOS meets the criteria for classification as a Persistent, Bioaccumulative and Toxic (PBT) substance. PFOS and PFOA have the potential to enter the food chain and could have a negative health impact on humans. The COT had concluded that the available toxicology data indicate that PFOS and PFOA do not demonstrate sufficient similarities to be assessed jointly. The COT had asked for advice on mutagenicity and carcinogenicity from the COM.

2.34 There were no apparent structural alerts for mutagenicity and the evidence from animal studies is that absorbed material is not metabolised. However there is evidence for target organ toxicity in repeat dose studies with both PFOS and PFOA in rats and monkeys and evidence for adverse effects on reproduction and the induction of endocrine target organ tumours with PFOS and PFOA and liver tumours in rats with PFOS. The mechanisms for these toxicological effects had yet to be evaluated by COC and COT.

#### PFOS

2.35 Members discussed the mutagenicity data on PFOS and agreed that the physico-chemical properties of PFOS considerably complicated the formulation of appropriate test solutions for mutagenicity studies. The use of dimethyl sulphoxide (DMSO) as a solvent using dose levels up to those which induced cytotoxicity or precipitation was agreed as acceptable. Members agreed that the *in-vitro* plate incorporation test using five strains of *Salmonella typhimurium* and the D4 strain of *Saccharomyces cerevisiae* gave negative results. Members agreed that the reverse mutation assay using *Escherichia coli* gave negative results. The difficulty in formulating an adequate suspension of the test material was noted for the *in-vitro* chromosomal aberration assay in human lymphocytes. Members agreed that this study had yielded negative results. Members agreed that the *in-vitro* UDS assay in rat liver primary hepatocytes gave negative results. Members commented on the difficulty in adequately formulating PFOS for oral dosing of mice in the bone-marrow micronucleus test. Members noted that only 1000 micronuclei had been evaluated at each dose level. However overall the study was considered to be acceptable and provided negative results.

2.36 The COM agreed that the studies undertaken with PFOS were acceptable and that PFOS should be regarded as not mutagenic.

#### PFOA

2.37 The COM discussed the data on PFOA. It was noted that mutagenicity assays had been conducted using aqueous solutions of sodium or ammonium perfluorooctanoate.

2.38 Members agreed that the plate incorporation bacterial mutagenicity tests using strains of *Salmonella typhimurium* and *Escherichia coli* using sodium or ammonium perfluorooctanoate were adequately undertaken and gave negative results. The COM noted that the *in-vitro* hprt assay in CHO cells using ammonium perfluorooctanoate had reported negative results.

2.39 Members considered that a mutagenic response had been documented in the *in-vitro* chromosomal aberration assay of sodium perfluorooctanoate in CHO cells in the presence of exogenous metabolic activation at the two highest concentrations. This finding had been reproduced in a confirmatory assay in the presence of exogenous metabolic activation at the highest concentration tested. No evidence for increased chromosome aberrations had been documented in the absence of exogenous metabolic activation. However it was unclear as to what extent the results reported from this *in vitro* study were due to cytotoxicity. An increase in chromosome aberrations was also documented in an assay using ammonium perfluorooctanoate at the highest dose tested in the presence of exogenous metabolic activation. There was clear evidence of cytotoxicity at this dose level in this particular assay. It was

agreed that sodium perfluorooctanoate, in the absence and presence of metabolic activation, had not induced chromosomal aberrations in cultured human whole blood lymphocytes when tested up to doses that were cytotoxic. Members commented that the available information indicated that absorbed PFOA was not metabolised by mammals.

- 2.40 No evidence for a mutagenic effect was found in a mouse bone marrow micronucleus assay where mice had been given a single oral gavage dose of up to 5000 mg/kg bw sodium perfluorooctanoate or 1990 mg/kg bw ammonium perfluorooctanoate. The test materials had been solubilised in deionised water. There was clear evidence of toxicity (mortality of both males and females) and reduced PCE/NCE ratios in animals dosed with 5000 mg/kg bw sodium perfluorooctanoate. A dose level of 1990 mg/kg bw ammonium perfluorooctanoate did result in one male death but did not have an effect on the PCE/NCE ratio. Members commented that the in-vivo bone marrow mouse micronucleus studies had been adequately conducted although there was no direct measure of exposure of the bone marrow to the test materials and also noted that absorbed PFOA was highly bound to proteins.
- 2.41 The COM discussed what further testing might be appropriate for PFOA salts and considered whether an investigation of in-vivo DNA binding or further evaluation of the mechanism of *in-vitro* chromosomal aberration assays were appropriate. Overall, the COM concluded that the results from the *in-vitro* chromosomal aberration assays with PFOA salts were likely to represent a cytotoxic response. It was agreed that a plausible *in-vitro* mechanism for the positive response in CHO cells was required to reassure the COM of this conclusion.

### Horizon Scanning

2.42 The COM undertakes an annual exercise to identify areas of mutagenicity risk assessment, testing and evaluation which might be the subject of future consideration. The following areas of work were agreed which have been grouped into three areas of priority.

2.43 Areas with high priority were;

- i) COM agreed evaluation of UDS v COMET was useful but noted the amount of data available in contract test houses and in the public domain would be limited. In the first instance the secretariat was asked to identify UDS positives and look for concordance with COMET (liver). It was agreed there might be very few data available. Some wider considerations of usefulness of the comet assay could be considered subsequent to this review.
- ii) Members were very interested in methylation status changes in transgenerational effects and suggested that broadening to effects on histones as potential mechanisms for permanent changes to phenotype needed to be considered. COM agreed this was a major undertaking. (The original request comes from ACP/MTP) COM felt the current mutagenicity tests strategies would not address such compounds, there might be examples from the Medicines and Healthcare products Regulatory Agency (MHRA) (e.g. antisense DNA products).
- iii) COM requested some joint working with COC to improve presentation of advice to general public, to try to get over misconceptions, e.g. all pesticides are genotoxic.

- iv) COM agreed some additional work on the background information on variance of biomarkers of genotoxicity in populations.

2.44 Areas with medium priority were;

- i) COM agreed that consideration of mitochondrial DNA as a cellular target for mutagens was a priority for future work.
- ii) COM agreed that consideration of nitropyrenes and nitro PAHs was a priority for future work.
- iii) COM agreed that a review of aspects of mutagenicity of mixtures would be valuable. After some long consideration of potential mechanisms of interaction, COM agreed to look at modulation of alkylating agent mutagenicity as a first start, to see if dose/effect addition rules were adequate.
- iv) COM asked for a presentation on wider aspects of international validation of mutagenicity testing.

2.45 Areas of low priority were the potency indicator approach suggested by Sanner T and Dybing E (Basics in Clinical Pharmacology and Toxicology, 96, 131-139, 2005) and the proposed evaluation of *hprt* CHO assays held by PSD. It was agreed the review would be of value to PSD in assessing overall performance of this mutagenicity assay.

### Test Strategies and Evaluation

*cII* transgenic mutation assay (comparison with transgenic *lacI* and *lacZ* assays)

2.46 The COM and COC had considered the available mutagenicity and carcinogenicity data on malachite green and leucomalachite green during 2004. (<http://www.advisorybodies.doh.gov.uk/com/mg/mg.htm>) A number of mutagenicity studies using the *lacI* transgene in both rats and mice were reported. However one key piece of evidence came from the finding of an increased mutagen frequency in the liver in an *in-vivo* mutagenicity assay using the *cII* transgene in Big Blue mice fed a diet containing leucomalachite green. An overview of the development and use of *in-vivo* mutagenicity assays using the *cII* transgene was drafted for the February 2005 COM meeting. A general conclusion was reached on the basis of the information reviewed.

2.47 The COM concluded that the *cII* transgene was a useful *in-vivo* mutation assay to investigate potential mutagenic effects in cancer target organs in rodents. It was necessary to routinely sequence mutant colonies in order to evaluate the significance of results obtained with the *cII* transgene in rodents. The COM felt that the currently available data in the published literature did not support the routine use of the *cII* transgene for screening chemicals but suggested it was of value in targeted studies of mutagenicity in specific organs in rodents. The COM agreed to keep the use of the *cII* transgene under review along with other developments of *in-vivo* mutagenicity testing.

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

## Joint meeting of COM/COC on use of target organ mutagenicity assays in carcinogen risk assessment

- 2.49 The COM and COC undertake routine horizon scanning exercises as part of their annual remit (see appended internet links at the end of this statement). The COM identified the use of *in-vivo* target organ mutagenicity studies as a subject for further consideration. The COM and COC agreed to hold a joint meeting on the use of data derived from *in-vivo* target organ mutagenicity studies in carcinogen risk assessment. An open meeting of the committees was held on the 9 June 2005. Attendees included a number of external experts who gave presentations and comments to the committees discussions.
- 2.50 The COM and COC agreed that data from adequately conducted *in-vivo* carcinogen target organ mutagenicity and genotoxicity studies (which included information from investigations using transgenic animals, the comet assay, and approaches to measuring DNA binding (e.g. postlabelling and radiolabel methods) can provide valuable information of use in the mode-of action of carcinogenic responses seen in rodents. Such studies can provide supporting information for use by regulatory authorities in carcinogen risk assessment on a case-by-case basis.
- 2.51 A brief statement is appended at the end of this report. A full write up is being prepared for publication in a peer review journal.

### Ongoing reviews

- 2.52 A number of review papers are in preparation for 2006. These currently include;
- A review of biomonitoring for genotoxicity in patients who have undergone revision arthroplasty for hip replacement.
  - A review of the utility of the rat liver UDS assay and comparison with data from the COMET assay.
  - A review of the factors affecting the background frequency of micronuclei in peripheral blood lymphocytes in biomonitoring studies.

# Statements

## Review of Biomonitoring Studies of Genotoxicity in Pesticide Applicators

### Background

1. The Medical and Toxicology panel of the Advisory Committee on Pesticides has asked the COM for advice on the genotoxicity in pesticide applicators. The referral statement is given below.

*“To review investigations of mutagenicity and DNA adducts in pesticide applicators and workers exposed to pesticides (e.g. handling cut flowers) and factory (manufacturing) staff engaged in pesticide manufacture/formulation and produce a statement for the Advisory Committee on Pesticides and its Medical and Toxicology Panel (MTP). The review should include all studies identified by the MTP and any other relevant studies published, particularly those originating from the UK.*

*The review should consult COC epidemiologists with regard to the rigour of studies evaluated. this should include design, selection of controls, bias, confounding and use of multiple statistical comparisons.*

*The review should be initiated at the October 2004 COM meeting.”*

2. The referral from ACP and the MTP came about through the ongoing routine review of epidemiology literature undertaken by the MTP. The MTP had considered in April 2004 that there were sufficient numbers of reports of biomonitoring studies in the published literature which had been retrieved and evaluated by MTP to request an independent view from COM on the available studies. It is noted that pesticide active ingredients that are DNA reactive *in-vivo* mutagens are not approved for use in formulated pesticide products in the U.K.

### Evidence reviewed

3. The DH Toxicology Unit at Imperial college in collaboration with the COM secretariat drafted a series of review papers for the COM. The review considered published biomonitoring studies in the scientific literature up to December 2004. A comparison was undertaken with the literature search strategy used by the MTP in order to ascertain whether any published studies had been missed. A very good agreement between the MTP and COM literature searches was attained with only one study identified in the COM literature search which had not already been identified by MTP. ( A total of 70 biomonitoring studies of genotoxicity markers in pesticide applicators was identified.<sup>1-70</sup>). A listing of the discussion papers considered by COM during the review period (from the October 2004 to the October 2005 meetings) is given below. All of these review papers are draft discussion papers and do not necessarily represent the views of the COM. A detailed evaluation was undertaken for all of the studies. The discussion papers can be accessed via the COM internet site under the “papers” section. (<http://www.advisorybodies.doh.gov.uk/com/>)



- i) *Biomonitoring studies from EU (MUT/04/19). Annex 1 (overview of literature), Annex 2 Summary of individual studies and IPCS guidelines on biomonitoring studies of genotoxicity. Annex 3 tabular summary according to occupation, Annex 4 tabular summary of statistical approaches used to analysis of data.*
- ii) *Review of biomonitoring studies of pesticide applicators from Croatia (MUT/04/20)*
- iii) *Further information and follow-up of review undertaken in October 2004 (MUT/05/1). (Draft exclusion criteria Annexes I and II, Draft inclusion criteria Annexes III and IV, Magnitude of response Annexes V and VI, Exposure patterns documented in studies).*
- iv) *Submitted published papers (for February 2005 meeting) (MUT/05/6), Addendum 1 MUT/05/6 review of studies from rest of world, Addendum 2 to MUT/05/6, tabulation of rest of world studies by occupation.*
- v) *Cytogenetic changes following cumulative exposure to pesticides (MUT/05/9)*
- vi) *Revised criteria (MUT/05/10)*
- vii) *Epidemiological overview (MUT/05/11)*
- viii) *Discussion paper on evaluation of positive studies and control data (MUT/05/12) (Annex 1 Evaluation of positive response in biomonitoring studies of genotoxicity, Annex 2 Information on pesticide usage in UK).*

*Pesticide applications considered in the review.*

4. The papers retrieved identified a wide diversity of occupational pesticide exposures. The authors described investigations in occupational groups such as floriculturalists, green house workers, agricultural workers and farmers, pesticide sprayers and applicators (which included agricultural/horticultural, amenity, fumigators), production workers (e.g. manufacture of pesticides) and forestry workers. The extent of information provided on occupational exposure to pesticides (e.g. during handling, diluting, applying), the duration of exposure and use and adequacy of personal protective clothing varies considerably between the different accounts. The Committee considered it was difficult to evaluate such a diverse data set. It was agreed that the most appropriate approach would be to assess the adequacy of the studies with regard to investigation and evaluation of genotoxicity indices and with regard to overall adequacy of design, analysis and interpretation of results. With respect to overall adequacy, the COM sought an opinion from an independent epidemiologist.

*Indices of genotoxicity used in the reviewed biomonitoring studies*

5. A short overview of the indices of genotoxicity used in biomonitoring studies is provided to assist in evaluating the significance of findings. Almost all the studies considered in this review provided data for investigations using *in-vitro* culture of peripheral blood lymphocytes derived from blood samples. A

small number of studies used epithelial cells from the buccal cavity. The committee had access to the general guidance published by a WHO IPCS working group on use of genotoxicity indicators in biomonitoring studies.<sup>71</sup> The Committee agreed that in general the genotoxicity indices measured in samples (predominantly peripheral blood lymphocytes) including micronucleus formation, chromosomal aberrations, comet and, <sup>32</sup>P-postlabelled DNA adducts results indicate uptake and exposure to DNA damaging chemicals. The evidence suggested that there may be an increased risk of mutagenicity and also possibly carcinogenicity but it is not possible to be certain that there is a risk or to quantify this risk because of the poor quality of many of the studies and frequent contradictory findings.

#### *Micronucleus frequency*

Micronuclei are small, extranuclear bodies that arise from acentric chromosome fragments or from whole chromosomes that are excluded from the nucleus during mitotic cellular division. They can be a consequence of DNA breakage, replication on a damaged DNA template or inhibition of DNA synthesis, failure of any of the mitotic apparatus or alterations in cellular physiology and mechanical disruption<sup>71</sup>. In most cases, the cytokinesis-block MN method is used, in which scoring only takes place in cells that have only divided once in culture.<sup>16</sup> Micronucleus analysis can be used for a number of cells, both *in vitro* and *in vivo*, including lymphocytes<sup>5,9,72</sup> and buccal epithelial cells.<sup>49-50</sup> Micronucleus induction is an indirect indicator of mutagenicity. It is unclear however, whether MN formation has a specific role in carcinogenesis.<sup>71</sup>

#### *Chromosome aberrations*

Structural chromosome aberrations arise from direct DNA breakage, replication on a damaged DNA template or inhibition of DNA synthesis and may involve both chromatids of the chromosome (chromosome-type CA), or only one chromatid of the chromosome (chromatid-type CA).<sup>71,73</sup> Chromosome aberration analysis has been commonly performed on human peripheral blood lymphocytes to assess DNA damage.<sup>11,14,16,29,42,44</sup> To ensure that only first-generation metaphase cells are scored for CA, bromodeoxyuridine is commonly added to the culture medium prior to DNA replication *in vitro*<sup>29</sup>. Both structural and numerical chromosome aberrations may cause alterations to the structure or arrangement of oncogene and tumour suppressor genes of somatic cells, and hence are involved in the induction of cancer in humans.<sup>74</sup>

#### *Sister chromatid exchange*

Sister chromatid exchanges arise from equal exchange of DNA replication products between two identical sister chromatids of a duplicated chromosome<sup>75</sup> They are thought to arise as a consequence of “error free” homologous recombinational repair or bypass of DNA lesions during replication on a damaged DNA template, possibly at the replication fork.<sup>75</sup> In the most commonly used method of SCE analysis, DNA replication is required for two consecutive cell cycles, hence bromodeoxyuridine is added to the culture medium and cells are scored in the second division metaphase.<sup>14,61</sup> Although the induction of SCE has been widely used as an indicator of DNA damage following exposure to pesticides,<sup>12,26,32,36,45,61</sup> the mechanism of formation and biological significance of SCEs are still unknown.<sup>75</sup> The COM agreed that biomonitoring studies using SCE analysis were not informative with regard to evidence for genotoxicity.

### Comet assay

The comet assay, or single cell gel electrophoresis technique is a more recent technique established as a sensitive method for detecting DNA single strand and double strand breaks, alkali-labile sites, DNA cross linking and incomplete excision repair events.<sup>34,64,76,77</sup> The comet assay can be carried out with a number of cells, both *in vitro* and *in vivo*, including peripheral blood leukocytes, bladder, liver, buccal, gastric and sperm cells. To date, peripheral blood lymphocytes are mainly used for human biomonitoring studies following occupational exposure to an array of chemicals.<sup>34,53,64,77</sup> However, the relevance of the endpoint measured in the comet assay has yet to be established, as it is usually the result of a temporary strand breakage, which is repaired within a few hours under normal circumstances and may or may not become fixed as a mutation.<sup>77</sup>

### DNA adducts

A DNA adduct is a chemical entity covalently bound to DNA<sup>71</sup>, and is usually formed following the interaction of an electrophilic molecule with a nucleophilic site of DNA<sup>78</sup>. They are often the initial DNA lesion following exposure to a genotoxic chemical and may lead to mutation and altered gene function if not repaired. In epidemiological studies DNA adducts are particularly useful as they provide information on the exact chemical exposure of the individual.<sup>78,32</sup> P-DNA postlabelling technique has been widely used to measure non-radioactive carcinogenic large DNA adducts in humans, due to it being a highly sensitive technique.<sup>78</sup>

The COM guidance on a strategy for testing chemicals for mutagenicity recognised that artifactual positives may be obtained in the cell assays that do not reflect intrinsic mutagenic activity. Factors such as hyperthermia, hypothermia or induction of erythropoiesis may produce MN or CA<sup>79</sup> or exercise immediately prior to sampling may lead to increased DNA damage measured by the comet assay.<sup>80,81</sup>

### Overview of approach used by COM

6. A flow diagram outlining the approach used by COM is shown in Figure 1.
7. The COM took its remit from the referral from MTP and agreed to consider data originating from applications of formulated pesticides and pesticide mixtures. COM did not consider studies where a single pesticide active ingredient with known mutagenic potential was used (e.g. methyl bromide fumigation). The COM agreed to eliminate a number of publications arising from studies undertaken in Croatia because of irregularities in the reported data. A number of papers from Zeljezic and Garaj-Vrhovac from the laboratory of Mutagenesis, Institute for Medical Research and Occupational Health, Zagreb, Croatia were published during 2000-2 and were identified during literature searches.<sup>65-70</sup> It was noted that there were apparent discrepancies between the different publications in the reporting of the demographic data on exposed and controls and reporting of results of mutagenicity studies and no confidence could be attached to the results.
8. The remaining 65 studies were undertaken from all parts of the world but the literature search did not find any published study which had evaluated UK pesticide applicators. The 65 studies were subject to a review procedure for the adequacy of the genotoxicity assessment. The COM discussed the exclusion/inclusion criteria at the October 2004 and February 2005 meeting and following a postal consultation after the February 2005 meeting. The criteria and selected studies are outlined in Annex 1 to this statement.

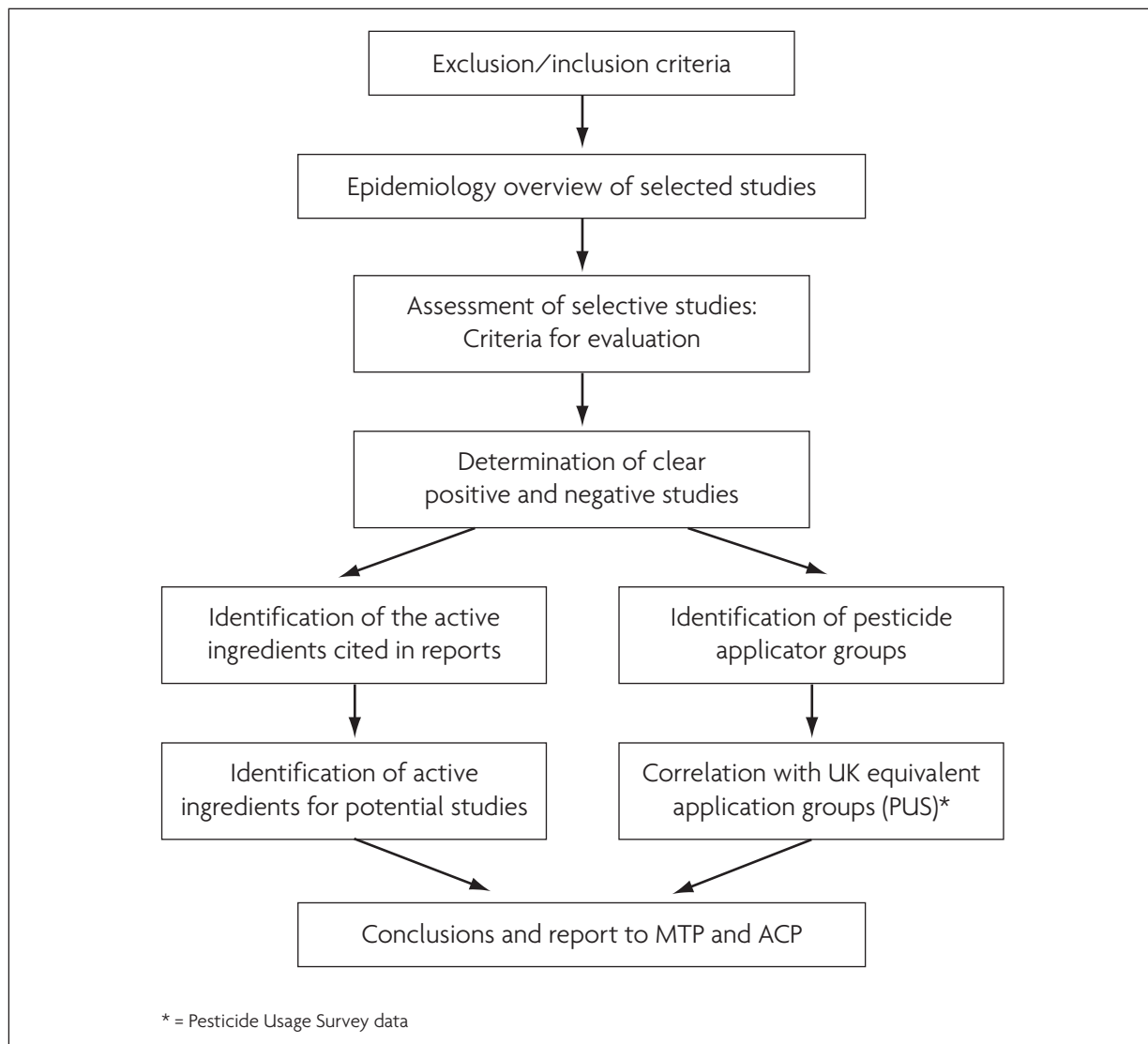


Figure 1: Flow Diagram of approach used by COM.

*Epidemiology overview of biomonitoring studies*

9. The 24 studies selected by COM using the exclusion/inclusion criteria were subjected to an epidemiology overview (see para 3 vii above). The full report can be accessed from the COM internet site (<http://www.advisorybodies.doh.gov.uk/pdfs/mut0511.pdf>). One objective of the epidemiology overview was to attempt a quality ranking of studies.

10. The Committee noted the conclusion reached in the epidemiology overview that all of the studies were limited in design, particularly with regard to measurement of exposure, study size, the assessment of selection and recruitment biases. Many of the studies provided information on demographics, medical history, lifestyle factors, potential occupational exposures to materials other than pesticides (e.g. solvents, radiation), and also information on type of pesticides used, duration and frequency of exposure and use of protective measures. However these data had generally not been used in the analyses reported and the majority of papers did not provide a specific analysis of individual pesticides. It was noted that the majority of studies were not sufficiently large to allow an evaluation of all the variables for which data might be available. Study designs were generally cross sectional, although a few had taken multiple samples (e.g. at different time points in a growing season). The time interval between exposure and sampling thus varied considerably between studies and this might affect the conclusions which could be drawn. There were limitations in the statistical approaches used in many of the studies. Thus for example many did not consider the form of the population distribution and made unsubstantiated assumptions that it was normal. The reporting of modelling was variable and in most cases was not adequate. It was noted that the papers tended to focus on statistical significance even when the absolute difference between groups was tiny. Overall it was not possible to identify any particular study that was clearly better in design and reporting than the other papers in the 24 studies identified by COM.
11. The COM considered the epidemiology overview at its 26 May 2005 meeting. Members agreed that the review had highlighted and confirmed their views on the limitations of the data set. However members agreed to review the data in order to reach the most appropriate conclusions possible.

*COM Review of selected studies.*

12. The COM considered the full published reports and a narrative summary of the selected studies.  
<http://www.advisorybodies.doh.gov.uk/pdfs/MUT0419.pdf>  
<http://www.advisorybodies.doh.gov.uk/pdfs/mut051.pdf>

*Criteria for evaluation*

13. The COM undertook an evaluation of the control data from the 24 studies for micronuclei and chromosomal aberrations in peripheral blood lymphocytes. Such an evaluation might aid in the assessment of the data from studies and help to decide what magnitude of response was suggestive of a positive result. Modelling of the data from the 24 selected studies suggested that MN data were normally distributed whilst there was evidence for a skewed distribution of CA as would be expected. Members felt the available data suggested that the distribution of chromosomal aberrations in human peripheral blood lymphocytes was consistent with an approximately binomial distribution, whilst distribution of micronuclei was much more dispersed than would be expected for binomial/Poisson distributions. It was considered that there might be bimodal or trimodal distributions indicating possible subpopulation effects and that more data would help to resolve the actual distribution of these indices in peripheral blood lymphocytes. The large overall variation in the negative control (reference) data (approximately 16 fold for micronuclei and chromosomal aberrations) suggested that it was not possible to define a single historical control range. Members agreed that statistical significance from adequately

conducted studies in combination with magnitude of response represented the most appropriate approach to evaluating the results of studies. Members agreed that the distribution of data should be assessed prior to consideration of the most appropriate statistical approach to analysis and that the effect of confounding factors should be clearly evaluated.

14. The COM compared the magnitude of response seen in the 24 studies of pesticide applicators with that reported for patients undergoing treatment with cytostatic medicines and nurses occupationally exposed to these medicines. Members were surprised at the small magnitude of response in the biomonitoring studies of nurses or patients exposed to cytostatic medicines. The mean fold increase in nurses (1.8, range 1.5-2.2) and in patients (mean 2.1, range 1.5-2.7) derived from studies for either micronuclei or chromosomal aberrations was similar to that reported for pesticide applicators in the studies reviewed by COM (1.7, range 0.8-5). Members noted that the higher maximum fold increase in pesticide applicators compared to nurses or patients exposed to cytostatic medicines might reflect differences in the extent of control for confounding factors between studies.
15. Members considered that the factors which accounted for the variance in the indices of genotoxicity in these biomonitoring studies (chromosome aberrations and micronuclei predominantly in circulating blood lymphocytes) in nurses and cancer patients exposed to cytostatic medicines had not been fully evaluated. The biomonitoring indices of genotoxicity were observed to differ considerably in the control populations of different studies. Members concluded, therefore, that it was not possible to establish a minimum fold increase for biological importance that could be applied to studies of pesticide applicators based on the studies in nurses and cancer patients exposed to cytostatic medicines. Further, it was concluded that factors affecting variance in genotoxicity indices used in the biomonitoring studies of pesticide applicators which had been reviewed were not understood adequately. In this respect members considered it would be very difficult to infer causality for the small magnitude responses seen in the biomonitoring studies of pesticide applicators. There was a need for more data on the background variability in the general population of biomonitoring indices of genotoxicity, and on factors affecting variance, which was required before a proper assessment of studies could be made.
16. Members agreed that consideration of statistical significance and magnitude of effects from adequately conducted studies was the most appropriate approach to evaluating the available data. Members confirmed that the available information was severely limited and hence no definite conclusions could be drawn with regard to any U.K. agricultural applications of pesticides or to individual pesticide active ingredients. However the COM agreed that it would be appropriate to consider what conclusions could be derived from the selected studies.

#### *Consideration of available data on exposure from selected studies*

17. The Committee noted the limited information on exposure. The only direct exposure measurements were reported in the study by Garry et al<sup>18</sup> for exposure to 2,4-dichlorophenoxyacetic acid. Information had been provided on the identity of pesticides which applicators had used in 15 (11 reporting positive results and 4 reporting negative results) out of the 24 studies reviewed.<sup>5,7,11,14-16,18,20,22,31,33,35,45,48,52</sup> This information had been reviewed in the context of information from the Pesticide Usage Survey regarding information on use over the period from 1993 up to 2002 and also with regard to the available information on classification status under Directive EC/67/548 with regard to mutagenicity which had

been provided by HSE. Members noted that apart from the study published by Garry et al<sup>18</sup> the magnitude of pesticide exposure in these studies had not been recorded and information on use of personal protective clothing had not been documented in many reports.

18. A number of the published papers selected by the COM had reported information which supported the view that the lack of protective clothing used by pesticide applicators/workers was associated with evidence of increased genotoxicity indices in biomonitoring studies.<sup>11,15,22,32,45</sup> It was noted that there was some limited evidence to suggest that work practices in green house (such as avoiding use of protective clothing in humid conditions and on re-entry after pesticide applications) might be potential sources of pesticide exposure.<sup>32,61,82</sup> It was also uncertain to what extent the application practices cited in the published reports were relevant to UK agricultural practice. In addition data from 2003 onwards on pesticide use was not available at the time of the COM consideration.
19. Using information from the Pesticide Usage Survey, the amount of metam sodium and carbendazim increased over part of the period 1993-2001 in floricultural and green house applications. The area of outdoor bulbs and flowers sprayed with carbendazim was reported to increase. Increases in the use of bifenthrin, metam sodium and thiram were reported in agricultural practices. The Committee noted that with regard to pesticide active ingredients that were currently approved for use in the U.K. and which were also classified with regard to mutagenicity under EC/67/548, carbendazim was used in 4/11 positive and 1/4 negative studies respectively.<sup>5,22,31,35,52</sup> This represented a very small and incomplete amount of information. It was noted that one of these studies had used micronucleus or chromosomal aberration analyses which could potentially be affected by spindle inhibitors such as benzimidazoles.<sup>31</sup> However it is unclear whether the results derived from the comet assay<sup>22,35</sup> could be related to benzimidazoles and there is no evidence for direct binding of benzimidazoles to DNA and hence the results of the remaining DNA-adduct study<sup>52</sup> were unlikely to be related to benzimidazole exposure. It was noted that there were a number of other classified mutagens listed in the positive studies which were not approved for use in the U.K.
20. Members considered the Bolognesi et al 2004 study in detail as this was the only available study with provided data on the specific aneuploidy inducing effects of benzimidazoles.<sup>5</sup> Members confirmed that this study should be considered as negative using the criteria agreed by the COM during the review of studies of pesticide applicators. Members agreed this was one of the better studies which had been considered as the investigators had attempted to use a specific index of exposures to tubulin inhibitors such as carbendazim and benomyl, although there were limitations in the dosimetric accuracy of the index of exposure, in the small number of individuals studied and in the lack of correction for multiple comparisons used in the statistical analysis. Members considered that the results of the centromere specific investigations were based on a very small number of individuals and no interpretation of biological significance could be placed on the data. The data provided in this study were consistent with the general conclusions on interpretation given below in paragraphs 22 and 23.

#### *Consideration of data presented on duration of exposure*

21. There were limited data on estimated duration of exposure to pesticides in 6 out of the 24 selected studies.<sup>5,7,22,30,44,45</sup> Four of these studies reported a positive correlation between duration of exposure and increased indices of genotoxicity<sup>7,22,44,45</sup> whereas the remaining two studies reported a negative

correlation.<sup>5,30</sup> The stratification into groups according to exposure was generally based on periods of 10 years or more. No rationale was given in the studies for the stratification of exposure groups according to duration of exposure. The magnitude of increased indices of genotoxicity with duration of exposure was small in all of the studies. The Committee was aware that biomarkers of genotoxicity (such as chromosome aberrations and micronuclei) increase in frequency with age and that this potential confounding factor had not been considered adequately in the analyses reported. The Committee agreed it was not possible to draw any definite conclusions based on these data, although it is noted that three out of four of the studies which reported on duration of exposure and which also reported a positive response had documented evidence for exposure to benzimidazole pesticides.

#### *Consideration of use of Personal Protective Equipment (PPE)*

22. None of the selected studies specifically investigated the effect of use of PPE on biomonitoring indices of genotoxic effects in pesticide applicators. The extent of PPE usage, where reported, varied considerably. Thus in some reports, no PPE was used,<sup>29,30</sup> whilst other reports describe conditions in which most pesticide applicators use PPE.<sup>5,47</sup> Several studies reported significant increases in chromosome aberrations and micronuclei correlated with a lack of PPE use during pesticide application.<sup>11,15,31,33</sup> It is noted that floriculturalists might report using PPE, but some investigators note that due to humid conditions within greenhouses appropriate PPE is not always worn. The Committee noted that a correlation between the lack of use of PPE and increased biomonitoring indices of genotoxicity but concluded that no definite conclusions could be reached with regard to exposure to pesticides based on the available data.

#### *COM Interpretation of available data*

23. The Committee was aware of the guidance available for the conduct on biomonitoring studies of genotoxicity from Albertini et al.<sup>71</sup> but agreed that having considered a large data set of studies on pesticide applicators that more research and guidance on the factors affecting the background variance of biomonitoring indices of genotoxicity was required before such studies could be fully interpreted particularly with regard to the significance of the small magnitude of response seen in the available studies.
24. The COM discussed whether any proposed study should focus on an occupational group (such as floriculture) or on specific pesticides. Members acknowledged that the evidence was limited particularly with regard to the design, conduct, reporting and analysis of the available studies both with respect to identifying either an occupational category or specific pesticides. It was noted that a limitation in the available published literature concerned relevant information on the mutagenicity of mixtures of pesticides. Members were aware of the COT report on mixtures of pesticides (the WIGRAMP report <http://www.food.gov.uk/science/ouradvisors/toxicity/COTwg/wigramp/>).<sup>83</sup> The COT working group had identified benzimidazoles as a possible common mechanism group of compounds for further evaluation. The Committee considered that although a UK study based on use of carbendazim in floriculture represented a reasonable proposal, there were considerable difficulties in undertaking and interpreting such a study. In particular it was agreed that more background information on the factors affecting the variance of biomonitoring indices of genotoxicity in unexposed populations would be required before a study of specific pesticide exposures was undertaken. If appropriate



background information were available, then it was agreed that a longitudinal study where individuals acted as their own controls would be most appropriate. It was agreed that there was supporting evidence that biomonitoring for urinary excretion of 5-hydroxy-2-benzimidazole (5HBC) could be used to assess exposure and uptake of carbendazim.<sup>84</sup> Members commented that the application of Personal Protective Equipment (PPE) specified for use of carbendazim products (such as in floriculture) in the U.K. meant that it was unlikely that any increase in genotoxicity could be detected with any reliability. Any such study would have to be very large and there were doubts as to whether an appropriate exposure group could be identified.

### COM conclusions

25. The Committee was aware that no DNA reactive *in-vivo* mutagens were used as active ingredients in approved formulated pesticide products in the U.K. It was noted that benzimidazoles were used in a number of approved pesticide products since a risk assessment could be undertaken for the threshold related effects of these active ingredient on microtubule inhibition. The Committee confirmed that the available information was severely limited and hence no definite conclusions could be drawn with regard to any U.K. agricultural applications of pesticides or to individual pesticide active ingredients. The COM concluded;
- i) The COM review was based on 70 retrieved published studies of biomonitoring of genotoxicity in pesticide applicators. The evidence covered a large number of types of applications and a wide diversity of pesticide mixtures. The COM selected 24 studies from which conclusions could be drawn through the application of a quality screen of retrieved studies. An independent epidemiological overview of these 24 studies reported that all had significant design and evaluation faults. The COM agreed that any conclusions reached on this evidence would be limited by the poor quality of the available studies.
  - ii) The COM agreed that following a review of the 24 selected studies there was limited evidence supporting increased biomonitoring indices of genotoxicity in biomonitoring studies of pesticide applicators. The Committee agreed that it was not possible to make any conclusions regarding exposure-response from the selected biomonitoring studies of genotoxicity because of the inadequacy and unreliability of exposure measurements and the generally small increases in response. The Committee noted that there was no published study of pesticide applicators using pesticide mixtures in the U.K.
  - iii) The Committee agreed that the factors which accounted for the variance in biomonitoring indices of genotoxicity (chromosome aberrations and micronuclei predominantly in circulating blood lymphocytes) in nurses and cancer patients exposed to cytostatic medicines and in pesticide applicators had not been fully evaluated. It was not possible to define a minimum increase in biomonitoring indices of genotoxicity associated with cytostatic medicines from the available studies on nurses and cancer patients. Based on these observations and the large inter-study variation for the biomonitoring indices of genotoxicity in unexposed populations, the Committee concluded that it would be very difficult to infer causality for the small magnitude responses seen in the biomonitoring studies of pesticide applicators. There was a need for more data on the background variability in the general population of biomonitoring indices of genotoxicity, and on factors affecting variance, which was required before a proper assessment of studies could be made.

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- iv) The COM agreed that it is very difficult to draw conclusions on what might be the most appropriate biomonitoring study for U.K. pesticide applicators. There was some very limited evidence to suggest that an appropriate study of floriculturalists using benzimidazoles (e.g. carbendazim) might represent a reasonable proposal. The Committee concluded that in view of the large numbers of individuals which would be required in order to detect an effect in such a study it was most unlikely that a sufficiently large and appropriate exposure group could be identified.

October 2005.

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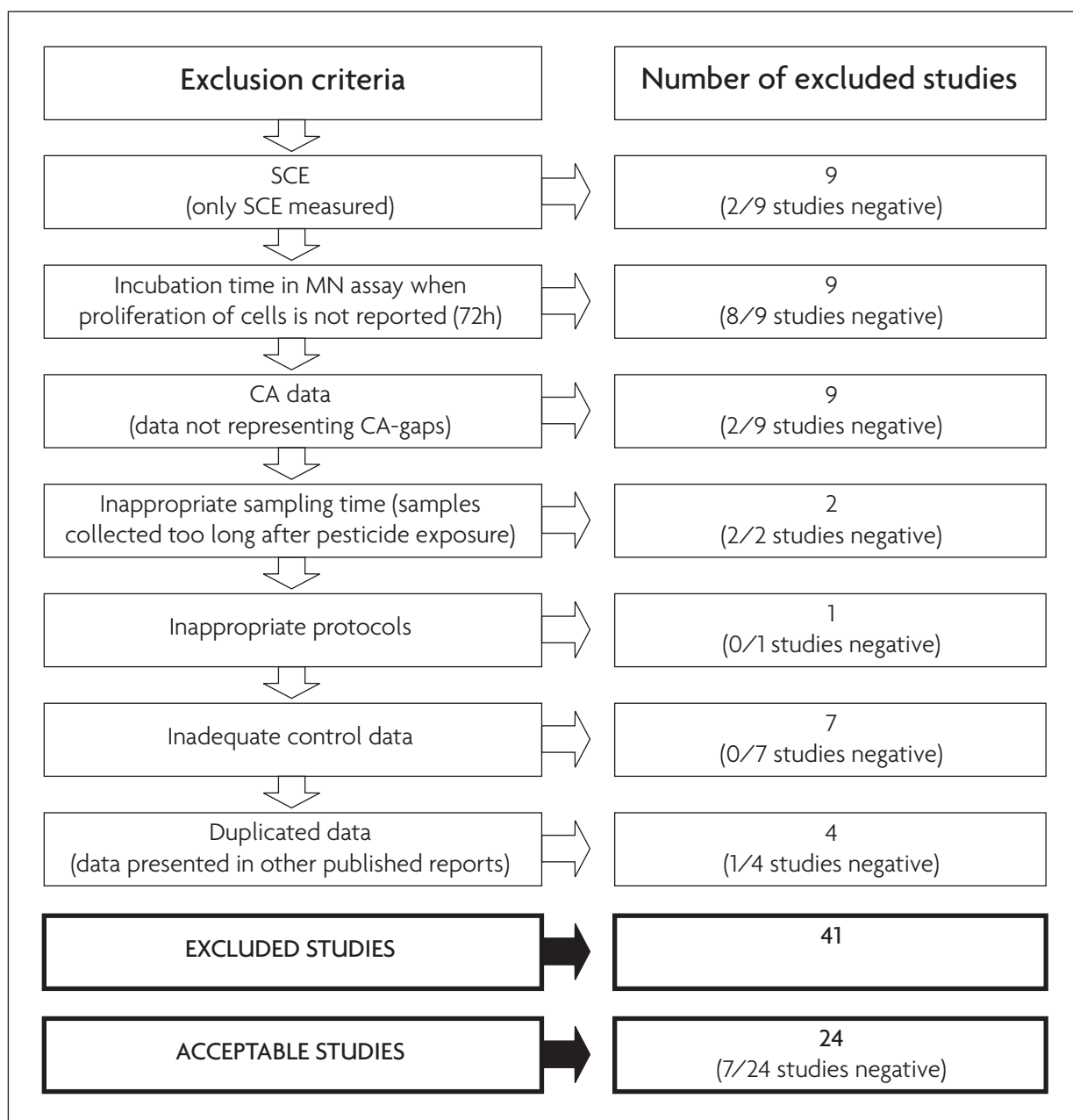


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# Annex 1 to Draft Working Paper on Biomonitoring Studies of Genotoxicity in Pesticide Applicators July 2005

Flow chart of excluded studies.

The flow chart demonstrates how many studies were excluded from further analysis due to various selection criteria, and the data reported in such studies.



## Annex 2 to COM Statement on Biomonitoring Studies of Genotoxicity in Pesticide Applicators

Data reported in selected studies

Author	Results	Data for controls	Data for exposed subjects	Fold increase over controls
<sup>a</sup> Bolognesi <i>et al.</i> , 1993b	+ MN	MN frequency = $6.67 \pm 3.12$	MN frequency = $8.57 \pm 5.02$ (mean / 1000 cells $\pm$ SD) RR = 1.25; 95 % CI = 1.11 – 1.41	<b>1.3-fold increase</b>
<sup>a</sup> Bolognesi <i>et al.</i> , 2004	– MN	Total MN (C+MN) = $2.18 \pm 6.31$ Total MN (C-MN) = $1.32 \pm 3.38$	Total MN (C+MN) = $2.79 \pm 12.21$ Total MN (C-MN) = $1.56 \pm 6.00$ (mean / 1000 cells $\pm$ SD )	<b>1.2-fold increase</b> <b>1.3-fold increase</b>
<sup>a</sup> Carbonell <i>et al.</i> , 1995	+ CA	Spring/summer Cells with aberrations = $4.56 \pm 2.53\%$ Chromatid-type aberrations = $3.14 \pm 2.76\%$ Chromosome-type aberrations = $1.90$ ffl $1.51\%$ Total aberrations = $5.04 \pm 2.85\%$ Autumn/winter Cells with aberrations = $3.39 \pm 2.4\%$ chromatid-type aberrations = $2.57 \pm 2.0\%$ Chromosome-type aberrations = $1.32 \pm 1.96\%$ Total aberrations = $3.90 \pm 3.23\%$	Spring/summer Cells with aberrations = $6.27 \pm 2.96\%$ Chromatid-type aberrations = $5.31 \pm 3.12\%$ Chromosome-type aberrations = $1.63$ ffl $1.56\%$ Total aberrations = $6.93 \pm 3.5\%$ Autumn/winter Cells with aberrations = $3.69 \pm 2.14\%$ chromatid-type aberrations = $2.49 \pm 0.56\%$ Chromosome-type aberrations = $1.21 \pm 1.83\%$ Total aberrations = $3.70 \pm 2.15\%$ (mean $\pm$ SD)	<b>1.4-fold increase</b> <b>1.7-fold increase</b> <b>0.9-fold increase</b> <b>1.4-fold increase</b> <b>1.1-fold increase</b> <b>1.0-fold increase</b> <b>0.9-fold increase</b> <b>1.0-fold increase</b>
<sup>a</sup> De Ferrari <i>et al.</i> , 1991	+ CA + SCE	Chromatid-type aberrations = $4.44 \pm 3.06$ Chromosome-type aberrations = $1.08 \pm 1.28$ Complex rearrangements = $<0.02$ Total aberrations = $5.52 \pm 4.12$	Exposed subjects Chromatid-type aberrations = $7.46 \pm 6.22$ Chromosome-type aberrations = $2.72 \pm 1.58$ Complex rearrangements = $0.12 \pm 0.12$ Total aberrations = $10.30 \pm 7.18$ Exposed subjects with bladder cancer; Chromatid-type aberrations = $5.07 \pm 3.90$ Chromosome-type aberrations = $2.65 \pm 0.26$ Complex rearrangements = $0.30 \pm 0.62$ Total aberrations = $8.02 \pm 4.98$ (Mean / 100 metaphases $\pm$ SD)	<b>1.7-fold increase</b> <b>2.5-fold increase</b> <b>6.0-fold increase</b> <b>1.9-fold increase</b> <b>1.1-fold increase</b> <b>2.5-fold increase</b> <b>15.0-fold increase</b> <b>1.5-fold increase</b>

Author	Results	Data for controls	Data for exposed subjects	Fold increase over controls
<sup>b</sup> Dulout <i>et al.</i> , 1985	+ CA	Abnormal cells = 2.65 ± 1.01 % Gaps = 1.56 ± 2.09 Chromatid breaks = 1.70 ± 0.74 Chromosome breaks = 0.54 ± 0.62 Dicentric chromosome and ring chromosome = 0.10 ± 0.21	Abnormal cells = 2.71 ± 0.36% Gaps = 2.43 ± 1.62 Chromatid breaks = 1.51 ± 1.26 Chromosome breaks = 0.95 ± 1.08 Dicentric chromosome and ring chromosome = 0.43 ± 0.84 (CA / 100 cells – gaps ± SD)	1.0-fold increase 1.6-fold increase 0.9-fold increase 1.8-fold increase 4.3-fold increase
<sup>a</sup> Falck <i>et al.</i> , 1999	+ MN	MN frequency 0.5 µg/ml BrdU = 7.4 ± 3.1 1 µg/ml BrdU = 7.4 ± 3.1	0.5 µg/ml BrdU = 7.8 ± 2.4 1 µg/ml BrdU = 8.0 ± 2.7 (mean / 1000 cells ± SD)	1.1-fold increase 1.1-fold increase
<sup>b</sup> Garry <i>et al.</i> , 1996	+ CA	Rearrangement frequency = 0.4 ± 0.57	Rearrangement frequency Fumigant = 1.4 ± 1.44 Insecticide = 1.4 ± 1.28 Herbicide = 1.0 ± 1.34 (mean ± SD)	3.5-fold increase 3.5-fold increase 2.5-fold increase
<sup>b</sup> Garry <i>et al.</i> , 2001	+ CA	Translocations/inversions/deletions = 0.65 ± 1.12	Translocations/inversions/deletions Low volume (1-100 gall) = 1.20 ± 1.13 Mid-range (100-1000 gall)= 1.00 ± 1.13 Heavy (>1000 gall) = 2.22 ± 1.14	1.9-fold increase 1.5-fold increase 3.4-fold increase
<sup>b</sup> Gomez-Arroyo <i>et al.</i> , 2000	+ MN	MN frequency = 0.38 ± 0.021	MN frequency = 1.01 ± 0.03 (mean / 100 cells ± SD)	2.7-fold increase
<sup>b</sup> Grover, <i>et al.</i> , 2003	+ comet	Smokers Comet tail length = 7.03 ± 11.46 Non-smokers Comet tail length = 10.34 ± 13.25	Smokers Comet tail length = 18.26 ± 9.76 Non-smokers Comet tail length = 19.75 ± 14.48	2.6-fold increase 1.9-fold increase
<sup>b</sup> Hogstedt <i>et al.</i> , 1980	- CA	Cell with aberrations = 4.6%	Cell with aberrations = 4.2%	0.9-fold increase
<sup>a</sup> Kourakis <i>et al.</i> , 1992	+ CA	Chromosome-type aberrations = 0.2 ± 0.37 chromatid-type aberrations = 0.34 ± 0.60 Total aberrations = 0.54 ± 0.90%	Chromosome-type aberrations = 1.34 ± 1.62 chromatid-type aberrations = 0.80 ± 0.81 Total aberrations = 2.14 ± 1.62 % (mean / 100 metaphases ± SD)	4.6-fold increase 6.1-fold increase 5.0-fold increase

Author	Results	Data for controls	Data for exposed subjects	Fold increase over controls
<sup>a</sup> Lander <i>et al.</i> , 2000	+ CA	Pre-season Chromatid-type aberrations-gaps = $1.03 \pm 0.82$ Chromosome-type aberrations-gaps = $0.28 \pm 0.45$ Total aberrations-gaps = $1.31 \pm 0.85\%$	Pre-season; Chromatid-type aberrations-gaps = $0.87 \pm 0.95$ Chromosome-type aberrations-gaps = $0.45 \pm 0.74$ Total aberrations-gaps = $1.32 \pm 1.23\%$  Post-season Chromatid-type aberrations-gaps = $1.04 \pm 0.99$ Chromosome-type aberrations-gaps = $0.34 \pm 0.56$ Total aberrations-gaps = $1.37 \pm 1.20\%$ (Mean / 100 metaphases $\pm$ SD)	0.84-fold increase 1.6-fold increase <b>1.0-fold increase</b>  1.2 (1.0)-fold increase 0.8 (1.2)-fold increase <b>1.0 (1.0) -fold increase</b> compared with pre-season (compared with controls)
<sup>a</sup> Lebailly <i>et al.</i> , 1998	+ comet	Beginning of spraying season = 30 Beginning of spraying season = 30	Middle of spraying season = 43 End of spraying season = 36	<b>1.4-fold increase</b> <b>1.2-fold increase</b>
<sup>a</sup> Lebailly <i>et al.</i> , 1998b	+ comet	DNA damage Before spraying Mixture of pesticides = 48% Herbicides on wheat = 30% Fungicides on wheat = 43% Fungicides & insecticides on peas = 36%  Tail moment Before spraying Mixture of pesticides = 3.21 Herbicides on wheat = 2.30 Fungicides on wheat = 3.64 Fungicides & insecticides on peas = 2.39	DNA damage After spraying Mixture of pesticides = 56% Herbicides on wheat = 28% Fungicides on wheat = 35% Fungicides & insecticides on peas = 39%  Tail moment After spraying Mixture of pesticides = 3.92 Herbicides on wheat = 1.93 Fungicides on wheat = 3.58 Fungicides & insecticides on peas = 3.16 (mean)	<b>1.2-fold increase</b> <b>0.9-fold increase</b> <b>0.8-fold increase</b> <b>1.1-fold increase</b>  <b>1.2-fold increase</b> <b>0.8-fold increase</b> <b>0.9-fold increase</b> <b>1.3-fold increase</b>
<sup>a</sup> Lebailly <i>et al.</i> , 2003	- comet	DNA damage Morning before pesticide use = 10% (2-21%)  Tail moment Morning before pesticide use = $4.35 \pm 1.11$ (2.16-5.85)	DNA damage Evening after pesticide use = not measured Following morning = 13% (5-49%)  Tail moment Evening after pesticide use = not measured Following morning = $4.80 \pm 2.57$ (3.18-12.76) (Mean $\pm$ SD)	<b>1.3-fold increase</b> (compared to before pesticide use)  <b>1.1-fold increase</b> (compared to before pesticide use)

Author	Results	Data for controls	Data for exposed subjects	Fold increase over controls
<sup>a</sup> Munnia <i>et al.</i> , 1999	+ DNA adducts	DNA adducts = $2.17 \times 10^9 \pm 5.75$ RAL	DNA adducts = $8.50 \times 10^9 \pm 14.95$ RAL (Mean $\pm$ SD)	<b>3.9-fold increase</b>
<sup>a</sup> Mustonen <i>et al.</i> , 1986	- CA	Aberrant metaphases-gaps Non-smokers; $1.5 \pm 0.73$ Smokers; $1.9 \pm 1.2$	Aberrant metaphases-gaps Non-smokers; $1.2 \pm 1.5$ Smokers; $1.8 \pm 1.26$ (Mean $\pm$ SD)	<b>0.8-fold increase</b> <b>1.0-fold increase</b>
<sup>b</sup> Paldy <i>et al.</i> , 1987	+ CA	Chromosome aberrations = $1.1 \pm 0.36$	Years of exposure Chromosome aberrations 0-5 years = $2.96 \pm 0.36$ 6-10 years = $3.55 \pm 0.75$ 11-15 years = $4.28 \pm 0.76$ (Sum of aberrations / 100 cells without gaps $\pm$ SD)	<b>2.7-fold increase</b> <b>3.2-fold increase</b> <b>3.9-fold increase</b>
<sup>a</sup> Pasquini <i>et al.</i> , 1996	+ MN	MN frequency = $13.30 \pm 5.35$ Overall MN frequency = $13.30 \pm 5.35$	MN frequency (>19 year) = $18.30 \pm 7.22$ Overall MN frequency = $15.98 \pm 7.65$ (Mean / 1000 cells $\pm$ SD)	<b>1.37-fold increase</b> <b>1.2-fold increase</b>
<sup>a</sup> Pastor <i>et al.</i> , 2001b	- MN	MN frequency = $16.38 \pm 12.19$	MN frequency = $12.20 \pm 6.58$	<b>0.7-fold increase</b>
<sup>a</sup> Pastor <i>et al.</i> , 2002a	- MN	MN frequency = $10.3 \pm 7.06$	MN frequency = $10.22 \pm 7.06$	<b>1.0-fold increase</b>
<sup>a</sup> Peluso <i>et al.</i> , 1996	+ DNA adducts	DNA adducts = 9	DNA adducts = 42 <sup>44</sup>	<b>4.7-fold increase</b>
<sup>a</sup> Piperakis <i>et al.</i> , 2003	- comet	DNA damage Male non-smokers = $82.3 \pm 14.1$ Female non-smokers = $81.1 \pm 16.12$	DNA damage Male non-smokers = $83.2 \pm 14.02$ Female non-smokers = $82.1 \pm 13.14$ (mean $\pm$ SD)	<b>1.0-fold increase</b> <b>1.0-fold increase</b>
<sup>a</sup> Studies from EU <sup>b</sup> Studies from rest of world				
Mean fold increase of positive studies over controls $\pm$ SD (SE) = $1.73 \pm 1.07$ (0.15)				
Figures in bold denote the total or mean fold increase of the study.				

# Halnitromethanes (HNMs)

## COM/05/S3 – June 2005

### Background to COM review

1. Halnitromethanes (HNM's) are a class of compound characterised by the presence of one or more halogen atoms together with a nitro moiety on a single central carbon. They differ from the Halomethanes (HM's) by the presence of the nitro moiety. The HNM's have recently been identified as disinfection by-products (DBP) in drinking water in the US (Richardson *et al* 1999) and had received a high priority ranking for investigation by the Environmental Protection Agency (Weinberg *et al* 2002 [http://www.epa.gov/athens/publications/EPA\\_600\\_R02\\_068.pdf](http://www.epa.gov/athens/publications/EPA_600_R02_068.pdf)). The current data review was initiated following the submission of one of papers published on the HNM's by a member of the COM.
2. Members were told that the only available published data related to the *in-vitro* studies in the three publications (Plewa *et al* 2004, Kundu *et al* 2004a, b). Some preliminary findings had been reported by the EPA to the DH Toxicology Unit regarding *in-vivo* testing of the HNM's in transgenic medaka fish. Members noted the availability of an NTP negative *in-vivo* bone marrow mouse micronuclei test with trichloronitromethane (TCNM; chloropicrin), but that there were no appropriate *in-vivo* mutagenicity data available for other HNMs.

### Data considered by the COM

3. The COM discussed the *in-vitro* COMET studies, conducted using CHO cells (Plewa *et al* 2004). Members noted that HNMs tested were cytotoxic in CHO cells and that apoptosis induction had not been recorded in these studies. In addition none of the trials had included the addition of an exogenous metabolising fraction. However members agreed that HNMs were genotoxic in this test system and that the rank order reported by the study authors was a reasonable guide to relative potency for *in-vitro* DNA damage. It was noted that the data precluded a determination of absolute potency of HNMs compared to the positive controls used in the study but the data were consistent with HNMs being more potent in the test system than ethyl methanesulphonate.
4. The COM reviewed the available bacterial mutagenicity data obtained from plate incorporation trials using *Salmonella typhimurium* strains both in the presence and absence of an exogenous metabolising fraction (Kundu *et al* 2004a). The strains tested included TA 98, TA 100, TA 104 and a strain RSJ100 which expresses rat glutathione transferase theta (GSTT1-1) known to activate halomethanes. Members agreed that the standard plate incorporation approach had been undertaken in this study used two plates per dose point (instead of the normal three) but that reproducible small increases in the number of revertant colonies had been reported. It was noted that HNMs might volatilise and therefore procedures to prevent evaporation might have been more appropriate. In a further study (Kundu *et al* 2004b) trials had been undertaken using pre-incubation in screw-capped glass vials using *Salmonella typhimurium* strain TA 100 at 37°C for 30 minutes followed by plate incorporation assessment of revertant colonies. Members felt that some residual loss of HNMs could still have occurred and that it

would have been appropriate to undertake pre-incubation at a lower temperature of 30°C. Overall members considered that the estimates of relative potency for *in-vitro* mutagenesis in *Salmonella* should be interpreted with caution.

#### COM evaluation of the data

5. Members considered the potential mechanisms of HNM induced mutagenicity in *Salmonella typhimurium* strains and agreed this included the potential for both direct acting and metabolically activated mutagenic responses. This might include an oxidative pathway but it was evident that there were possible differences between the nine chemicals in the HNM group. Whether direct activity was due to HNM carbocation formation (loss of the halogen resulting in a reactive, positively charged carbon) and DNA alkylation in an SN1 reaction or alternatively SN2 substitution (in which nucleophilic attack precedes halogen loss), was not clear. Members felt that, unlike the halomethane group of compounds, there was no convincing evidence for a glutathione mediated pathway with HNMs. Members observed that it was difficult to derive clear conclusions regarding mechanism and potency in the bacterial mutagenicity tests, but overall brominated HNMs appeared from the limited data to be more potent *in-vitro* mutagens than chlorinated HNMs.
6. The COM discussed the potential testing strategy that might be applied to HNMs and agreed that each individual HNM needed to be tested. It was agreed that the standard COM strategy was appropriate in this instance but should be modified so that the *in-vivo* rat liver UDS assay was the first *in-vivo* test. Members felt that a liver UDS assay would be more appropriate than the more usual *in-vivo* micronucleus test because of the likelihood that potential direct acting chemicals or reactive intermediates would not reach the bone marrow intact. Also, due to the presence of potentially direct acting mutagens a site of contact assay was considered appropriate (for example the COMET assay in the stomach). Members felt that an *in-vitro* liver UDS assay would be a useful pre-screen.

#### COM overall conclusions

7. Overall, in accordance with the COM strategy, HNM's should be regarded as *in-vitro* mutagens and potential *in-vivo* mutagens. It was considered that there was a need for appropriate *in-vivo* testing. The COM suggested:-
  - a) *in-vivo* rat liver UDS assay, followed by
  - b) site of contact COMET assay
8. If either of these studies yielded positive results, then the chemical under test should be considered to be an *in-vivo* mutagen.

This resulting statement could be shared with the EPA.



## References

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# Flunixin, Meglumine and Flunixin Meglumine

## COM/05/S1 – March 2005

### Background to COM review

1. Flunixin in the form of the meglumine salt is a non-steroidal anti-inflammatory drug (NSAID) and a non-narcotic analgesic drug with antipyretic activities. It is used in veterinary medicine (including food-producing animals) but is not used in human medicine. Flunixin meglumine dissociates *in vivo* to Flunixin and meglumine. In 1997, the European Medicine Evaluation Agency's (EMEA) Committee on Veterinary Medicinal Products (CVMP) considered the safety of Flunixin meglumine as part of its review of old veterinary medicinal products, operating under Council Regulation 2377/90, which covers the marketing authorisation of pharmacologically active substances used in veterinary medicines.
2. With regard to meglumine, the CVMP considered that it could be regarded as an excipient that had the purpose of increasing the solubility of Flunixin. The CVMP concluded that "meglumine used as an excipient at up to 1.5 mg/kg bw does not fall within the scope of Council Regulation (EEC) number 2377/90".
3. The Food Standards Agency is concerned about the possible mutagenicity of Flunixin meglumine, meglumine and Flunixin. The Food Standards Agency considered that it would be useful to seek the opinion of the COM.
4. The COM undertook an initial review of the mutagenicity data on Flunixin meglumine, Flunixin and meglumine during 2002/2003. A statement was published in March 2003 ([www.advisorybodies.doh.gov.uk/com/flunmeg](http://www.advisorybodies.doh.gov.uk/com/flunmeg)). The COM requested additional *in-vitro* mutagenicity data on meglumine, which is considered in this statement.

### Assessment of Mutagenicity data – Flunixin

5. In the statement COM/03/S2, the COM agreed that there was limited evidence that Flunixin was mutagenic *in-vitro*, as data were inadequate, but there was no evidence to suggest that Flunixin had mutagenic potential *in vivo*, as a bone-marrow assay was carried out in mice that was considered adequate by the COM. Members, however, considered that the issue of the inadequate *in-vitro* data should be raised with the CVMP.

### Assessment of Mutagenicity data – Meglumine

6. In the statement COM/03/S2, negative results were reported in an old plate incorporation assay in a limited number of *Salmonella typhimurium* strains. A positive result had been reported in a bone-marrow micronucleus assay in B6 mice using intraperitoneal administration of 500 or 1000 mg/kg bw suspended in 0.25% methylcellulose (two doses given 24 hours apart and harvest 6 hours after last dose). A repeat test using 1000 mg/kg bw was also positive. Members queried how the repeat test could

have been undertaken on the same day as the initial test. It was noted that negative results were obtained in a separate *in-vivo* micronucleus assay using intraperitoneal administration of two doses given 24 hours apart at up to 600 mg/kg bw of meglumine to CD1 mice. Members noted that a clear positive control had only been seen in males and not females in this latter assay and that the sampling regimen (24 and 48 hours after last dose) differed from the study in B6 mice. A number of *in-vivo* bone marrow micronucleus assays were undertaken for the Committee using a variety of single/double intraperitoneal dosing regimens at dose levels of 500 mg/kg bw and 1000 mg/kg bw and sampling for micronuclei at 6 or 24 h after the last dose. There was some evidence for an effect 6 h after the second dose in one study using Alpk:ApfCD-1 mice, but no definite conclusions could be reached as the result was not repeatable, there was considerable individual animal variation and the observed effect could have been complicated by toxicity.

#### Assessment of Mutagenicity data – Flunixin meglumine

7. In the statement COM/03/2 the Committee agreed that there was limited evidence that Flunixin meglumine was an *in-vitro* mutagen. However, although negative results had been documented in an *in-vivo* micronucleus assay in B6 mice, members were concerned about adequacy of the selection of dose levels. Therefore the inconclusive *in-vivo* data on meglumine suggested that a definite conclusion regarding Flunixin meglumine could not be reached.

#### COM Evaluation

8. The Committee noted the poor quality of mutagenicity data on flunixin, meglumine and Flunixin meglumine. Members noted that while negative results obtained in carcinogenicity bioassays with Flunixin meglumine in the rat and the mouse provided some reassurance with regard to flunixin, they were not informative with regard to meglumine.
9. The Committee was aware of several *in-vivo* bone-marrow micronucleus assays using meglumine but noted that the results were inconsistent. Two studies using B6 and Alpk:ApfCD-1 mice reported a mutagenic effect following a treatment regime of two doses of meglumine (24 hour apart) with a sampling time of 6 hours after the second dose (i.e. 30 hours after the first dose). However this positive result was not repeated in two bone marrow micronucleus assays in mice using an equivalent treatment regime.
10. The Committee requested additional *in-vitro* assays with meglumine to properly assess whether meglumine had mutagenic potential *in vitro* before considering the need for any further *in-vivo* mutagenicity studies. Thus data were required from an *in-vitro* chromosomal aberration assay and a mouse lymphoma assay, which together with the data currently available for a test in bacteria using Salmonella, would complete the *in-vitro* package to modern standards given in the COM guidance. If these were negative, there would be no concerns regarding meglumine.

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## Data submitted in February 2005

### Mouse Lymphoma Assay

11. A Mouse Lymphoma Assay was undertaken that conformed to the OECD Guideline 476<sup>1</sup> and ICH S2B Guideline.<sup>2,3</sup>
12. Meglumine was not mutagenic, at the concentrations tested in L5178Y mouse lymphoma cells, either with or without exogenous metabolic activation.

### Chromosome Aberration Assay

13. A cytogenetics test was undertaken which conformed to OECD Guideline 473.<sup>4,5</sup>
14. Meglumine, at the concentrations tested, did not significantly increase chromosome aberrations or decrease the mitotic index in human blood lymphocytes *in vitro*, either with or without exogenous metabolic activation.

### COM conclusion and recommendations

15. The COM confirmed the following general conclusions on the mutagenicity of flunixin, Flunixin meglumine and meglumine
  - i) The mutagenicity data previously submitted to the Committee on flunixin, Flunixin meglumine and meglumine are relatively old and the studies have not been conducted to contemporary standards.
16. The COM reaffirmed that it is difficult to draw any definite conclusions on the mutagenicity of these chemicals. The prudent conclusions reached for flunixin and Flunixin meglumine were reaffirmed.
  - ii) For Flunixin (a non-steroidal anti-inflammatory veterinary medicine) there was limited evidence that Flunixin was mutagenic *in-vitro* but there was no evidence to suggest that flunixin had mutagenic potential *in-vivo*.
  - iii) For Flunixin meglumine, (Flunixin in the form of the meglumine salt) there is limited evidence for a mutagenic effect *in-vitro*. In addition the inconclusive *in-vivo* data on meglumine indicate that a definite conclusion regarding Flunixin meglumine cannot be reached.
17. The COM agreed a revised conclusion for meglumine using the new information from the *in-vitro* mutagenicity studies considered in this statement.

- iv) For meglumine, the available *in-vivo* mutagenicity data are inconsistent with some positive and negative results. The new *in-vitro* mutagenicity tests (namely a mouse lymphoma assay and a chromosome aberration assay using human blood lymphocytes) were conducted to internationally accepted standards. There was no evidence for a mutagenic effect in either of these two assays either in presence of absence of exogenous metabolic activation. The COM concluded that there was no further requirement for testing of meglumine.

March 2005  
COM/05/S1

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## References

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# STATEMENT ON JOINT COC AND COM MEETING 9 June 2005

## Use of target organ mutagenicity data in carcinogen risk assessment

### COM/05/S6 AND COC/05/S3 – October 2005

#### Background

1. The COM and COC undertake routine horizon scanning exercises as part of their annual remit (see appended internet links at the end of this statement). The COM identified the use of *in-vivo* target organ mutagenicity studies as a subject for further consideration. The COM and COC agreed to hold a joint meeting on the use of data derived from *in-vivo* target organ mutagenicity studies in carcinogen risk assessment. An open meeting of the committees was held on the 9 June 2005. Attendees included a number of external experts who gave presentations and comments to the committees discussions. This brief statement has been drafted to record the main outcomes of the meeting. A full write up is being prepared for publication in a peer review journal.

#### Introduction to current review

2. The interpretation and consequent risk assessment of rodent carcinogenicity data is currently aided by the evaluation of a battery of mutagenicity data.<sup>1</sup> However there are examples of compounds for which equivocal data, or lack of concordance between mutagenicity and carcinogenicity data make it difficult to complete the risk assessment. This is of particular importance when the organ in which tumours are found is not one of those assessed during *in vivo* genotoxicity tests. Recent developments have facilitated the identification of target organ mutagenicity thus offering the potential to more closely define whether tumours seen are attributable to specific mutagenic events.<sup>2</sup>

#### Muta<sup>®</sup>mouse and Big Blue transgenic rodent assay systems

3. An increasing number of rodent carcinogens are being investigated using the Big Blue or Muta<sup>™</sup>Mouse transgenic systems. (For example the COM and COC have recently evaluated transgenic mutation assays as part of the evaluation of malachite green and leucomalachite green.<sup>2</sup> An important contribution of these assays is that any tissue can be evaluated for the presence of mutations following the administration of a chemical by any exposure route. By demonstrating carcinogen target organ mutagenesis, it can then be inferred that conditions are favourable for DNA reactivity therein (e.g. the occurrence of site-specific metabolism). Site of contact mutagenesis can also be readily studied.<sup>4</sup> Additionally, sequencing of both the lac genes and cII is now commonplace and this provides information on the more precise nature of the induced mutations. It is considered that these analyses will contribute to the understanding of target organ tumourigenesis and subsequent risk assessment.<sup>5</sup> There are several examples in the published literature of how data from Big Blue or Muta<sup>™</sup>Mouse have aided carcinogen risk assessment.<sup>6-8</sup>

## The Comet assay

4. The comet assay is now well established as a supplementary assay to the standard battery of genotoxicity tests and can be used to assist in evaluating chemical which have given equivocal results in other *in-vivo* mutagenicity tests or to investigate the potential mechanisms of tumourigenic responses.<sup>9,10</sup> Guidelines and recommendations for performing the assay have recently been developed.<sup>11</sup> The principle concerns that may arise in respect of the use of the comet assay are the relevance of the measured endpoint to the carcinogenic process, and the robustness and sensitivity of the method.

## DNA binding approaches

5. The COM considered the measurement of DNA binding by a number of methods (e.g. postlabelling, radioimmunochemical and HPLC/mass spectrometry) in 1996.<sup>12</sup> The COM agreed that these methods could provide useful data on exposure to and uptake of DNA reactive chemicals and metabolites. More recently the COC has considered the application of Accelerator Mass Spectrometry (AMS) in the detection of DNA binding in biological samples.<sup>13</sup> AMS is the most sensitive technique for measuring the formation of adducts with DNA. AMS technology allows the accurate measurement of very low levels of radiolabelled chemicals (particularly <sup>14</sup>C) in biological samples at around 10<sup>-21</sup> to 10<sup>-18</sup> mole. The COC considered that one potential application of AMS was in hazard identification. AMS has provided evidence for a lack of DNA binding of 2-phenylphenol and its metabolites in rat bladder which has been important information in concluding a non-genotoxic mechanism for the carcinogenic effect of 2-phenylphenol and its sodium salt in rat bladder.<sup>14</sup>

## Overview of joint COM/COC meeting on target organ mutagenicity studies

6. The symposium was attended by Committee members, relevant officials from government agencies and delegates from industry and academia, took the form of introductory presentations followed by round-table discussion groups. A programme was published.<sup>15</sup>

## Overview of presentations

7. Professor John Heddle (York University, Toronto, Canada) gave the first presentation and provided a comprehensive overview of the usefulness of the transgenic mouse and rat mutation assays in carcinogenicity risk assessment. His principal observations were that the transgenes lac I and lac Z, are neutral genes which persist and accumulate mutations linearly over time, ensuring consistency in response which has allowed for the development of optimum protocols, notably the establishment of expression periods. The Committee noted the relevance of persistent mutations in transgenes to the initiation-promotion model of carcinogenesis.
8. Professor David Phillips (Institute of Cancer Research, Sutton) gave a brief synopsis on the use of DNA adduct detection methodologies in providing evidence of genotoxic mechanisms of action. These techniques are constantly being improved. DNA adduct formation represents mutagenic and carcinogenic potential, but it is recognised that the presence of DNA adducts in a particular tissue does not necessarily correlate with tumour induction.



9. Dr Brian Burlinson (HLS) summarised recent developments in the use of the COMET assay, which was gaining in popularity as a second tissue in vivo mutagenicity assay, to supplement the in-vivo bone marrow assay for chromosomal aberrations or micronuclei. One advantage was that a number of tissues including any tumour target organ could be included in any study. It was noted that a draft OECD guideline was currently under consideration.
10. Dr Phil Carthew (Unilever) presented the pathologists view of the usefulness of target organ mutagenicity data. He pointed out that lack of concordance between mutation frequency, DNA adducts and tumour burden indicate a need to understand more definitively the importance of other steps in the carcinogenic process. However, it is anticipated that data from transgenic mutation assays will be able to provide answers to critical questions, principally with view to hypothesis testing and mode of action evaluation.

### Group discussions

11. Delegates then split into two groups for the ensuing discussions. Group 1 considered methodological developments, robustness, sensitivity and target organ specificity of the assays under scrutiny, whilst Group 2 addressed specifically the use of target organ mutagenicity data in carcinogenicity risk assessment through questions such as 'how can these data be used to understand the aetiology/pathogenesis of rodent tumours?' and 'are these data likely to be more useful for the evaluation of some tumours types and/or target organs?'

### Conclusions from Group discussions

12. Group 1 (methods) derived conclusions on the use of the assays considered during the symposium with regard to the screening of chemicals for potential in-vivo mutagenesis. It was concluded that the transgenic mutation assays were sufficiently robust for general use and that moves should be made to optimise assay conditions and develop protocol guidelines. There were some concerns regards overall sensitivity and this may be particularly relevant when using unusual tissues or chemicals about which little is known (e.g. nature of DNA damage, ADME profile). Nevertheless, it was felt that the limitations of transgenic mutation assays were fairly well understood and this added to a general feeling of confidence in their use. However, it was recognised that the expense of conducting the transgenic assays meant that it was likely that the COMET assay would be used more frequently, even though this was more suited to detecting clastogens and potentially less sensitive at identifying point mutagens. The COMET assay was considered to be satisfactorily validated in most tissues. DNA adducts were not considered useful in a risk assessment scenario, and more likely to be of value for weight of evidence approaches to understanding carcinogenic mechanisms.
13. Group 2 (risk assessment) derived conclusions on the use of target organ mutation assays in carcinogen risk assessment, i.e. as part of mode-of-action assessments. It was concluded that the in-vivo target organ mutagenesis/ genotoxicity assays provided important information for the mode-of-action evaluations for rodent cancer target organs and thus contribute to carcinogen risk assessment. It was considered important to ensure adequate conduct of studies particularly with regard to information on target cell exposure and potential modes of action. In general, positive results which are not due to high dose cytotoxicity, inflammation or reactive oxygen DNA damage support a non-threshold genotoxic

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mode of action and that negative results need to be interpreted with regard to the sensitivity of the study used. Clearly, concordant results for several in-vivo approaches increase the confidence of conclusions reached.

### Overall conclusion

14. The COM and COC agreed that data from adequately conducted in-vivo carcinogen target organ mutagenicity and genotoxicity studies (which included information from investigations using transgenic animals, the comet assay, and approaches to measuring DNA binding (e.g. postlabelling and radiolabel methods) can provide valuable information of use in the mode-of action of carcinogenic responses seen in rodents. Such studies can provide supporting information for use by regulatory authorities in carcinogen risk assessment on a case-by-case basis.

October 2005

COM/05/S6

COC/05/S3

(COM/ COC horizon scanning papers for 2004;  
<http://www.advisorybodies.doh.gov.uk/pdfs/MUT0422.pdf>  
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# 2005 Membership of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

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**Dr Gillian Clare** BSc PhD  
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*Professor of Environmental Carcinogenesis, Institute of Cancer Research*

## SECRETARIAT

Dr R Fielder BSc PhD Dip RCPATH Scientific Secretary(until August 2004)

Dr D Benford BSc PhD Scientific Secretary – Food Standards Agency

Mr K Mistry Administrative Secretary

Mr J Battershill BSc MSc

## Declaration of COM members interests during the period of this report

Member	Personal interest		Non personal interest	
	Company	Interest	Company	Interest
Prof P B Farmer (Chairman)	Abbey National	Shareholder	American Chemistry	Research Support
	Bradford & Bingley	Shareholder	Council	Research support
	Celltech	Shareholder		
	Foreign & Colonial	Shareholder	CEFIC	
	Friends Provident	Shareholder		
	Health Effects Institute	Research Committee Member		
	Torotrak	Shareholder		
Dr C Allen	NONE	NONE	NONE	NONE
Dr B Burlinson	Huntingdon Life Sciences	Salary Employee Share Option Holder	NONE	NONE
Dr G Clare	Covance	Salary	NONE	NONE
	Allied Domecq	Shareholder		
	AstraZeneca	Shareholder		
	Diageo	Shareholder		
	HBOS	Shareholder		
	Marks & Spencer	Shareholder		
Dr J Clements	Covance	Salary Share Option Shareholder	NONE	NONE
Dr D Gatehouse	Covance	Salary Consultant	NONE	NONE
	Friends Provident	Shareholder		
	GlaxoSmithKline	Pension Share Option Holder		
		Shareholder		

Member	Personal interest		Non personal interest				
	Company	Interest	Company	Interest			
Mrs R Glazebrook	Dr Foster Ltd	Salary	NONE	NONE			
	BT Group	Shareholder					
	Lloyds TSB	Shareholder					
	National Grid	Shareholder					
Dr N Gooderham	Abbey National	Shareholder	GlaxoSmithKline	BBSRC Collaborative Studentship			
	Friends Provident	Shareholder					
	Game	Shareholder					
	ML Laboratories	Shareholder					
	Northern Rock	Shareholder					
	Proctor & Gamble	Consultant					
	Protherics	Shareholder					
	Sunderland AFC	Shareholder					
Dr I Mitchell	Kelvin Associates	Associate Consultant	NONE	NONE			
	IM Enterprises	Director/Creditor					
	GlaxoSmithKline	Pensioner Option and Shareholder Consultant					
	Bass	Shareholder					
	Cable & Wireless	Shareholder					
	Cadbury Schweppes	Shareholder					
	Renishaw	Shareholder					
	Pfizer	Shareholder					
	RTZ	Shareholder					
	Shell	Shareholder					
	Unilever	Shareholder					
	Vodafone	Shareholder					
	Whitbread	Shareholder					
	British Telecom	PEP Holder					
	Centrica	PEP Holder					
	Scottish Power	PEP Holder					
	Dr E M Parry	Invesco			PEP Holder	NONE	NONE
		Fleming			PEP Holder		
Legal & General		PEP Holder					
Prof D Phillips	Aviva	Shareholder	NONE	NONE			
	Banco Santander	Shareholder					
	BG Group	Shareholder					
	Bradford & Bingley	Shareholder					
	Centrica	Shareholder					
	Lattice Group	Shareholder					
	National Grid	Shareholder					
	Takeda	Consultant					

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

## Preface



The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) evaluates chemicals for their human carcinogenic potential at the request of UK Government Departments and Agencies. The membership of the committee, agendas and minutes of meetings, and statements are all published on the internet (<http://www.advisorybodies.doh.gov.uk/coc/index.htm>).

During 2005, the Committee provided advice on a wide range of topics, including the chemicals furan, proquinazid, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), and the potential carcinogenicity of nanomaterials. It also completed its review of the potential chemical aetiology of childhood cancer, in particular, the possible association between childhood leukaemia and living near sources of traffic exhaust and petrol fumes.

The assessments of furan, proquinazid, PFOS and PFOA were undertaken in conjunction with its sister committee, the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM). The assessment of nanomaterial toxicology was undertaken jointly with the COM and the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT).

The COC gives advice on generic issues and on new developments in chemical carcinogenicity. The COC held a successful joint meeting with the COM in June on the use of target organ mutagenicity data in carcinogen risk assessment. During the course of the year, the COC also considered the development by the International Life Sciences Institute (ILSI) of the mode of action approach for chemical carcinogens to generate guidance on how to assess the relevance of an animal carcinogens to humans.

This was my last full year as chairman of the committee, as I stand down in March 2006. It has been a valuable and rewarding experience. I would like to record my thanks and appreciation of the work carried out by members of the committee during my 10 year tenure as chairman and of the support of the scientific and administrative secretariat. My successor as chairman is David Phillips, who is Professor of Environmental Carcinogenesis at the Institute of Cancer Research.

I wish him well and am sure he will lead the committee successfully through new challenges in the future.

Professor PG Blain CBE (Chairman)  
BMedSci MB PhD FRCP(Lond) FRCP (Edin) FFOM CBiol FIBiol



## Carcinogenesis mode of action and human relevance framework

- 3.1 The International Programme on Chemical Safety (IPCS) has developed a framework for evaluating modes of action (MOA) of chemical carcinogens. The COC reviewed the IPCS MOA framework in 1999 and endorsed the proposed approach. Since then, the initial framework has been used by a working group under the sponsorship of the US Environmental Protection Agency (EPA) and ILSI Risk Science Institute (RSI) to develop guidance on how to assess the relevance of an animal carcinogen to humans, based on MOA data. This 'Human Relevance Framework' (HRF) is based on the premise that, once an MOA has been established for the tumourigenic effects of a chemical in experimental animals, a systematic analysis of the individual key events comprising that MOA should enable its relevance to humans to be determined.
- 3.2 At its April 2005 meeting, the COC considered a paper published in 2003 which describes the HRF and includes seven case studies. A further three evaluations of anonymised pesticides carried out by Defra's Pesticides Safety Directorate were also considered.
- 3.3 Members noted that the HRF approach extended the MOA approach by considering whether the key events in the MOA are plausible in humans, taking into account kinetic and dynamic factors. They also noted the advantages of collaborative working to enable a consensus view to be reached where there was both conflicting evidence and conflicting views in the published literature about the mechanism of carcinogenesis of a chemical. One particular value of the HRF approach was considered to be that data were presented in a structured format which allowed gaps in the data to be readily identified. It was hoped that completion of the case studies would provide generic evaluations, limiting the need for duplication of effort. The COC concluded that both the MOA and HRF approaches provided a logical framework in which to set the information needed when assessing the relevance of chemical induced animal tumours to humans.

## Childhood cancer

### *Overview*

- 3.4 The COC began a review of childhood cancer in 2004. It was asked to evaluate the published literature on whether there was an increased incidence of childhood cancer in the UK and to address whether evidence from epidemiological studies suggests a possible chemical aetiology. Based on the available epidemiological data, four childhood tumours were identified as being relevant for further consideration: CNS tumours; acute lymphocytic leukaemia (ALL); germ cell tumours (GCT) and neuroblastomas (NBT).
- 3.5 The COC obtained expert advice from the UK Childhood Cancer Group (UKCCRG) and the Newcastle Childhood Cancer Group on whether the reported increased incidences in these tumours was accounted for by better ascertainment. UKCCRG data confirmed an upward trend in the incidence of all four tumours. The increase in ALL was considered to be to some extent a true increase in incidence. However, chemical pollution was unlikely to be a significant risk factor. It was not clear if the upward trends in the incidence of childhood CNS tumours and germ cell tumours were real or due to better diagnosis. Advice was received that there is an increase in neuroblastomas but probably largely in benign tumours, under 1 year of age, and due to better diagnosis.

- 3.6 Overall, Members considered the evidence for a chemical aetiology for these tumours to be weak. The COC discussed the possible role of transplacental carcinogens and paternal exposure, and the significance of animal models in the risk assessment of childhood cancer. It was pointed out that any risk assessment modelling of transplacental carcinogenesis needed to separate genotoxic compounds and non-genotoxic compounds.

The possible association between childhood leukaemia and residence near sources of traffic exhaust and petrol fumes

- 3.7 The COC was asked by the Department of Health Air Pollution Unit to advise on three epidemiology studies which had investigated whether there was an association between residence near to petrol stations, garages, and road traffic exhaust fumes and the occurrence of childhood leukaemia. The COC subsequently conducted a full review of the epidemiological literature on this topic. It concluded that the available evidence provided insufficient evidence from which to conclude that there was an association between risk of childhood leukaemia and proximity to petrol stations, garages and road traffic. A full statement can be found at the end of this report.

Consideration of paper by Knox et al on "Childhood cancers and atmospheric carcinogens (Journal of Epidemiology and Community Health (2005), volume 59, pp 101-105)

- 3.8 The COC was asked to consider this paper by the Department of Health. The paper reports an investigation of the relation between major sources ("hotspots") of emissions of a range of pollutants, taken from Internet maps for 2001 compiled from the National Atmospheric Emissions Inventory, and birth and death addresses of children dying from cancer between 1966 and 1980. It reports a positive association between deaths from cancer and hotspots for a number of air pollutants.
- 3.9 The COC noted a number of problems with the paper and concluded that they were unable to draw any conclusions from the study, because of flawed methodology and the inadequate information provided.

## Furan

- 3.10 Furan is used as an industrial chemical or in the synthesis of commercial compounds. It is also present as a contaminant in air, tobacco smoke and many foods and beverages. The FSA sought the advice of the COT on the toxicity of furan during 2005 and the COT referred it to the COC for advice on carcinogenicity.
- 3.11 The COC noted that furan is rapidly and extensively absorbed and distributed following administration to rats. A key reactive metabolite is cis-2-butene-1,4-dial. Two NTP studies have indicated that furan increases the incidence of cholangiocarcinoma in rats, hepatocellular tumours in rats and mice, pheochromocytoma of the adrenal gland in mice, and mononuclear cell leukaemia in rats. At least two possible modes of action for furan-induced liver changes have been described: altered cell proliferation and oxidative stress mechanisms.

- 3.12 The COC heard that the COM had concluded that furan should be regarded as an *in vitro* mutagen, but that there was insufficient evidence to reach a conclusion on the available *in vivo* mutagenicity data.
- 3.13 Recent work conducted by a group from the University of Birmingham in collaboration with AstraZeneca had examined the role of oxidative stress in the induction of cholangiocarcinoma in the rat. A representative from AstraZeneca gave a presentation of the findings to the COC. He outlined what is known about cholangiocarcinomas in humans, specifically, that many oncogenes, including p53, are up regulated. In rats, these tumours are induced by potent genotoxins and also by proposed non-genotoxic mechanisms (coumarin), when the same oncogenic changes are apparent. The 'furan' model of tumour formation is the best characterised. In this model, administration of furan results in characteristic morphological changes in the liver: intestinal metaplasia, diffuse centrilobular toxicity and a distinct focal response in the portal tracts. Notably, only particular lobes are affected. Administration of furan for one month is sufficient to cause tumours by one year. Areas expressing the enzyme CYP2E1 correlate with areas of necrosis and p53 activation, although it is noted that CYP2E1 is down regulated on continued administration. In areas where the periportal damage occurs, ductal cells develop as early as day 3, although only in the target lobes. This lesion spreads, becomes surrounded by basement membrane, and is infiltrated by intestinal metaplasia and capillarisation of the damaged area. If furan administration is discontinued, the properties of intestinal metaplasia are maintained. High, cytotoxic doses are required to elicit this response.
- 3.14 Members proposed the following mode of action: the cytotoxic intermediate generated by CYP2E1 was stimulating the regenerative response via oval cell proliferation and/or biliary progenitor cells. The intestinal infiltration seen may be a secondary response. Phenotypic changes were evidently being induced by the chronic inflammatory response, although it was not clear how furan was activating the mechanism leading to the response. It was unfortunate that no data were available on the response at low doses i.e. below those used in the NTP study, where cholangiocarcinoma occurred at all dose levels.
- 3.15 In conclusion, the COC considered that it was reasonable to hypothesise that there was a threshold for the mechanism for induction of cholangiocarcinoma but this needed further investigation before a decision could be made. It was suggested that the following were required: carcinogenicity studies with lower doses of furan, *in vivo* mutagenicity data, and information on metabolite production and the contribution of metabolites to an oxidative stress mechanism. The COC noted that the mode of action for cholangiocarcinoma may not be applicable to the other tumours induced by furan but commented that the strain of rat used in the NTP study was known to have a high incidence of mononuclear cell leukaemia and that the pheochromocytomas in the mouse may arise from a neuroendocrinological mechanism.

#### Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA)

- 3.16 Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are multifluorinated chemicals based on an eight carbon linear molecule (some branched chain and lower alkane fluorinated carbon impurities may be present at low levels). PFOS and PFOA have unusual physico-chemical properties, forming microdispersion micelles in aqueous systems. PFOS and related compounds are used as in industrial and household applications. PFOA is used in industrial applications and is also produced by

environmental degradation of some PFOS precursors and fluorotelomer alcohols. The PFOS salt in most widespread use is ammonium perfluorooctanoate (APFO). PFOS and PFOA are persistent and bioaccumulative and could potentially enter the food chain. The FSA sought the advice of the COT on the toxicity of furan during 2005 and the COT sought the advice of the COC on the carcinogenicity of these chemicals.

#### PFOS

- 3.17 The COC considered the one dietary carcinogenicity study on PFOS, in Sprague-Dawley rats. Non-neoplastic effects reported in the liver included increased absolute and relative liver weight, hepatocellular cystic degeneration and hepatocellular hypertrophy (often associated with vacuolation), with a NOAEL of 2 parts PFOS per million parts diet (2 ppm). No signs of hepatotoxicity were evident 52 weeks after cessation of administration of the highest dose for 52 weeks. The incidence of hepatocellular adenomas was significantly increased at the 20 ppm level in both male and female rats. There was a single hepatocellular carcinoma in the female high dose group. The incidence of thyroid follicular cell adenoma was significantly increased in the male high dose recovery group, but not in the male and female high dose groups fed PFOS for 104 weeks. The study had investigated treatment-related effects on hepatic palmitoyl-CoA oxidase activity, but the COC considered that the time points at which activity was measured were not optimal for this endpoint.
- 3.18 The COC also reviewed the results of a dietary carcinogenicity study in Sprague Dawley rats in which *N*-ethylperfluorooctanesulfonamido ethanol (*N*-EtFOSE) was administered at dose levels of 3, 30 and 100 ppm in the diet for 104 weeks. This compound has been shown to degrade to PFOS. No significant treatment-related effects were observed at 2 years. However, survival in all groups, including the controls, was relatively poor. There was evidence of hepatocellular hypertrophy in high dose males and females. The incidence of hepatocellular adenomas was slightly higher in high dose male and female groups than in controls. A single hepatocellular carcinoma was observed in a high dose female.
- 3.19 Two limited epidemiological studies (a retrospective mortality study and an 'episodes of care' analysis) had been conducted in occupationally exposed populations. Cohorts were relatively small and also relatively young. The COC noted that, in the retrospective cohort mortality study, standardised mortality ratios were below one for all causes of death and all malignant neoplasms when analyses were restricted to workers with at least one year of employment and high exposure to PFOS. There was a statistically significant increase in deaths from malignant neoplasms of the bladder in males with over 5 years in high exposure jobs. Members questioned the adequacy of exposure assessment by using job categories and noted that there had been potential exposure of the workers to benzidine, a known bladder carcinogen. The COC advised that, overall, it was not possible to draw definite conclusions from this study. The episode of care analysis was considered unusual in design and uninformative.
- 3.20 In conclusion, the COC concluded that there was equivocal evidence for carcinogenicity in the animal studies, limited to hepatocellular adenoma, and that the NOAEL was 5 ppm. It further concluded that a threshold approach could be used for the risk assessment of PFOS. The COC noted that a mode of action involving peroxisome proliferation had been proposed but was not convinced that adequate evidence had been provided to support this.

## PFOA

- 3.21 The carcinogenicity of PFOA has been investigated in two dietary exposure studies in Sprague-Dawley rats. In the first, ammonium perfluorooctanoate (APFO) was administered at levels of 0, 30, or 300 ppm in the diet for 104 weeks. Dose-related non-neoplastic liver effects included megalocytosis, cystoid degeneration and portal mononuclear infiltration. A significant increase in the incidence of mammary fibroadenoma in female rats was considered by the study authors not to be related to treatment on the basis of comparisons with the incidence in historical control data. Similarly, a dose-related increase in the incidence of Leydig cell adenomas was not significant compared to the historical control incidence. The COC noted that there were at least two viral infections in the rats used in this study and considered that this limited its value. In the second study, a single high dietary dose of PFOA (300 ppm) was administered for 24 months. Increased incidences of hepatocellular adenoma, Leydig cell adenoma and pancreatic acinar cell adenoma were reported. Serum estradiol concentrations were significantly increased in treated animals.
- 3.22 The COC noted that a hypothesis had been put forward which proposed that PFOA induces liver, Leydig cell and pancreatic acinar cell tumours via PPAR-alpha activation and that, therefore, these tumours are unlikely to be induced in humans. However, Members noted that PFOA showed indications of oestrogenicity, possibly via aromatase induction, and thus it was not possible to be certain that all toxic effects were mediated via peroxisome proliferation. They were informed of some recently retrieved research which indicated that PFOA had effects on liver weight in PPAR-alpha null mice. The committee agreed with a proposal that the observed increases in serum estradiol levels suggested a mode of action for the Leydig cell tumours. However, it considered that it was not possible to propose modes of action for the liver and pancreatic tumours observed in the second study. However, the committee concluded that it would be acceptable to use a threshold approach for the risk assessment of PFOA i.e. to select an appropriate NOAEL for a precursor event for the most sensitive tumour and to apply an uncertainty factor of 100.
- 3.23 In two linked, retrospective cohort studies of mortality in an occupationally exposed population, small increases were reported in death from cancer of the large intestine and from cancer of the prostate in employees with over 1 year definite exposure to PFOA. The COC considered that none of the effects reported were significant for the risk assessment of PFOA.

## Proquinazid

- 3.24 Proquinazid (6-iodo-2-propoxy-3-propyl-3H-quinazoli-4-one) is a novel fungicide under consideration by the Advisory Committee on Pesticides (ACP). It is intended for use in agriculture and viticulture, providing control of powdery mildew in cereals and grapes. The ACP sought advice from the COC and COM on an increased incidence of cholangiocarcinoma in female rats in a carcinogenicity study with proquinazid.
- 3.25 At its meeting on 14 July 2005, the COC heard a presentation from representatives of the data holders, Du Pont, on the interpretation of the cholangiocarcinomas. Du Pont representatives noted that proquinazid was not genotoxic, that cholangiocarcinomas of the "intestinal" type occurred only in female rats fed 600 ppm or more at the end of the two-year study, the tumour type was linked to

chronic hepatocellular injury with “cholangiofibrosis” and there was no risk of the lesion occurring in the absence of severe hepatic toxicity. A number of representative sections were shown to COC members. It was noted that the lesions termed “cholangiocarcinoma” were quite unlike the usual pattern seen in human cholangiocarcinoma. COC members supported the view that the lesions were not neoplastic but were a florid inflammatory response to severe acute and chronic hepatocellular damage.

3.26 The COC concluded that it was uncertain that the lesions termed “cholangiocarcinomas” were truly neoplastic but accepted the conventionality, used by the study pathologist in the report of the carcinogenicity study, of classifying them as neoplastic. Members considered that a satisfactory non-genotoxic Mode of Action explanation had been submitted. The COC agreed that a threshold approach to risk assessment could be used and agreed that there was a satisfactory margin of safety between the NOAEL for putative cholangiocarcinoma and the Acceptable Daily Intake proposed by the Pesticide Safety Directorate. A full statement of the committees’ assessment of proquinazid is attached at the end of this report.

#### Review of the quantitative relationship between alcohol consumption and squamous cell carcinoma

3.27 The COC reviewed the relationship between alcohol and oesophageal cancer in 1995 as part of the review of alcohol and cancer. Several studies indicated that there was a quantitative relationship between alcohol intake and squamous cell carcinoma (SCC) of the oesophagus but a threshold level could not be defined. Therefore, the COC had estimated levels of alcohol intake for which there was convincing evidence of an increased risk of SCC. In 2004, a paper on oesophageal cancer had suggested that further consideration of alcohol-induced SCC was necessary. Following consultation, it was decided that a review should be conducted to review the post 1995 data on the quantitative relationship between alcohol and SCC. The COC also reviewed data on the risk in smokers who drink heavily and the possible existence of any susceptible groups.

3.28 Twenty-four new epidemiological studies were retrieved for review by the COC. It was noted that the data suffer from the same limitations as were noted in 1995 including a lack of data in women, poor study design, and imprecise estimates of alcohol intake. The studies rarely considered confounding by other risk factors, such as dietary intake, lack of vitamins and, possibly, Barrett’s oesophagus. Also, members did not consider that the studies were easily comparable and suggested that it may only be necessary to consider the meta-analysis data, as these give the best estimates. Nevertheless, it was considered that they strengthened the overall picture. An increased risk was apparent at intakes above 30 g alcohol per day. However, the COC could not identify a level of consumption below which there is no increase in risk, due to both lack of data and the intrinsic weakness of the data (in particular, the fact that the ‘non-drinkers’ group may include light drinkers).

3.29 The COC considered that the data provided evidence of potentially susceptible groups. It noted the multiplicative effects of alcohol and smoking on the incidence of SCC and that smoking increased the risk of SCC at even moderate doses of alcohol. However, gender and ethnic differences were considered possibly to reflect temporal differences in drinking habits rather than a real difference in susceptibility.

### Single or short term exposure to carcinogens

- 3.30 Carcinogenicity is usually evaluated in the context of long term exposure to a chemical. In carcinogenicity studies, animals are subject to medium to long-term exposure to a test substance with the aim of establishing whether it has any carcinogenic potential. This information, together with any available epidemiological or other data, is used to characterise the risk to humans from prolonged exposure to the chemical. In the case of genotoxic carcinogens it is assumed that there is no threshold and that any level of exposure carries a theoretical risk. The risk from non-genotoxic carcinogens is characterised using a NOAEL and uncertainty factor to estimate a level of exposure at which no risk is anticipated.
- 3.31 It may be necessary to provide advice on whether there is a risk associated with acute exposure to a carcinogen, e.g. in the case of a chemical accident or a food contamination incident. This would entail the characterisation of cancer risk in later life following a single or short-term exposure some time earlier. At its horizon scanning exercise in 2004, the COC agreed to review this issue and it did so at its April 2005 meeting.
- 3.32 In 1999, Calabrese and Blain published information about a database of data from approximately 5500 studies which assess whether a single dose of a chemical (or physical) agent can cause tumour development in animals, without exogenous promotional stimuli. The database contained information on approximately 800 chemicals, of which 426 caused a positive response. The authors claim that it reveals that a single dose of an agent can cause tumours to develop in males and females of numerous animal models in all principal age groups. The COC reviewed this paper and an overview of studies relating to limited exposure (less than one year) to occupational carcinogens, also cited in the paper.
- 3.33 Calabrese and Blain had described the period of less than one year as relevant to the identification of potential single/short-term exposure to carcinogens but the COC considered that there was no rationale for this criterion. Members considered that exposure to single doses of certain cytotoxic medicines or genotoxic carcinogens, or possibly asbestos, might be associated with an increased risk of cancer. It was possible that the persistence of a carcinogen after exposure was a potentially important factor in the assessment of risk associated with a single exposure: beryllium was cited as an example of a persistent carcinogen. The COC cautioned that care was needed when interpreting the data cited in the Calabrese and Blain paper; for example, single parenteral exposure of experimental animals to chemicals in pellets might be associated with carcinogenicity in animals but such data were unlikely to be relevant to single exposures of humans to carcinogens. It considered that there was no evidence to support the view proposed by Calabrese and Blain that risks associated with single exposures to carcinogens varied with life stages. It was suggested that a more detailed review may be required of the information cited in the Calabrese and Blain database.

## Advice to the European Food Safety Authority (EFSA)

- 3.34 The committee submitted a response as part of the consultation on the 'Draft Opinion of the EFSA Scientific Committee on a Harmonised Approach for Risk Assessment of Compounds which are both Genotoxic and Carcinogenic'.

## Horizon scanning

- 3.35 The COC undertakes "horizon scanning" exercises at regular intervals to identify new and emerging issues which have the potential to impact on public health. At the 2005 exercise, Members considered that the following topics should be considered further.

### *Age-related differences in risk of carcinogenesis*

- 3.36 Conventional animal cancer studies start treatment with the test chemical in early adulthood – usually 6-8 weeks of age in rats and mice. However, after reviewing an extensive set of nonconventional animal carcinogenicity study results, the US Environmental Protection Agency (EPA) concluded in 2003 that there was appreciable evidence that juvenile exposures to genotoxic carcinogens conferred greater risks per day of dosing than do exposures during adulthood. The EPA proposed additional uncertainty factors for individuals aged under 2 years and aged 2-15 years. Recently, an improved analysis of the data for 9 chemicals suggested that the proposed additional factors may not be appropriate in all cases. This improved analysis proposed that the estimated population mean risk from lifetime exposures to a generic genotoxic carcinogen is about 2.8 fold larger than expected from adult-only exposure. The COC decided that it should review this work, and the topic should have high priority. The project should revisit the information which was examined when the committee reviewed the EPA guidelines for cancer risk assessment but should also look independently at the original data. It was noted that there is considerable discussion at present as to whether animal carcinogenicity data applied to children.
- 3.37 The COC also agreed to review data on *in-utero* exposures to carcinogens. This topic should have high priority.

### *Trends in cancer incidence*

- 3.38 Members noted that the incidence of both testicular cancer and of non-Hodgkin's lymphoma were increasing and considered that the possible chemical aetiology of both should be reviewed. It was considered that these topics should both be given high priority. The committee also noted that the incidence of upper outer quadrant breast cancer was increasing and that there is interest in the media in the proposal that this cancer may be caused by carcinogens, e.g. parabens, absorbed through the armpit. The secretariat was asked to come forward with a proposal for work on breast cancer. This topic was also deemed to be high priority.



### *Comparative risk assessment*

- 3.39 The committee supported a proposal to explore whether it was possible to compare risks from environmental carcinogens with risks from other activities. It also agreed with the view of the COM that more should be done on how to get the work of the committees across to the public. Both topics were deemed to be high priority.

### *Hormesis*

- 3.40 Members agreed that the topic of hormesis should be discussed further, including a review of recent publications. This topic was considered to be of medium priority.

### *Paper by Jacobs (2005) on prediction of carcinogenicity results from the results of short-term studies*

- 3.41 It has been proposed that the results of 2-year carcinogenicity studies could be predicted from certain short-term data, structural analyses or omics information. The committee considered a paper which concluded that such data are not currently sufficient to accurately and reliably predict the outcome of long-term carcinogenicity studies. It suggested that the committee should keep a watching brief on this issue.

### *Computational systems biology*

- 3.42 It was also agreed that the committee should consider whether computational systems biology could be applied to its work. This topic was deemed medium priority.

## Ongoing reviews

### Aspartame

- 3.43 The COC provided *ad hoc* advice to the FSA on the results of a rat carcinogenicity study with aspartame published by the European Ramazzini Foundation. The committee expressed a number of reservations about the study and noted that it was due to be formally evaluated by EFSA.

# Statements of the COC

## Review of the possible associations between childhood leukaemia and residence near sources of traffic exhaust and petrol fumes

### Background to review

1. Vehicle-related air pollution has increased considerably over the last 45 years, due to the increased numbers of vehicles on the roads (IEH, 1999). Traffic exhaust contains a complex mixture of many chemicals, of which several are known or suspected to be carcinogens. These include benzene, 1,3-butadiene and diesel-particulate matter. Diesel engine exhaust has been classified by IARC as Group 2A (i.e. probable human carcinogen) and gasoline<sup>†</sup> engine exhaust as Group 2B (i.e. possible human carcinogen) (IARC, 1989). The nature of traffic exhaust has, however, changed over the years following legislation about the composition of fuel.
2. Evaporative fuel emissions also contribute to vehicle-related air pollution. High airborne concentrations of fuel vapour are present in petrol stations and garages. Given that benzene is a component of both petrol/diesel exhaust and petrol vapour and is well documented as a cause of leukaemia in adults<sup>‡</sup>, there has been some concern as to whether residence near petrol stations/garages could be associated with an increased incidence of childhood leukaemia (petrol stations/garages are unlikely to generate sufficient traffic on their own to cause a substantial increase in pollutants from vehicle exhaust).
3. The Committee was asked by the Department of Health Air Pollution Unit to review three recent epidemiological studies which report an association between residence near to petrol stations and garages and/or road traffic exhaust fumes and the occurrence of childhood leukaemia. The Committee considered these studies (<http://www.advisorybodies.doh.gov.uk/pdfs/cc0437.pdf>) at the November 2004 meeting, but were unable to reach definitive conclusions on whether the studies indicated a causative link. The Committee therefore conducted a full review of the epidemiology literature on this issue, including studies reporting on benzene exposure and leukaemia in children.

### Information reviewed

#### *Childhood leukaemia: current perspectives on incidence trends and aetiology*

1. The Committee first reviewed the incidence trends and potential chemical causes of childhood leukaemia in 2004, as part of a horizon scanning exercise to examine evidence for a possible role for chemicals in childhood cancer (<http://www.advisorybodies.doh.gov.uk/pdfs/cc0431.pdf>).

<sup>†</sup> Gasoline is the US term for petrol

<sup>‡</sup> Benzene has been reported to cause Acute Myelogenous Leukaemia in occupationally-exposed adults

2. Of the two main types of leukaemia that occur in children<sup>§</sup>, Acute Lymphoblastic Leukaemia (ALL) is the most common. There is evidence suggesting that the incidence of childhood ALL in the UK is increasing but members questioned whether this increase was real or an artefact resulting from better ascertainment of cases. An expert opinion was sought from the UK Childhood Cancer Research Group (UKCCRG) and the Newcastle Childhood Cancer Group who advised that the increase in ALL was, at least in part, real.
3. Only two non-hereditary risk factors are clearly associated with the development of leukaemia in children: intrauterine exposure to diagnostic X-rays and postnatal exposure to therapeutic doses of ionising radiation. In recent years, the use of these medical procedures in pregnant women and children has been extremely rare, and so it is likely that these aetiological factors now account for only a small percentage of leukaemia cases. The Committee noted that there is strong evidence for the involvement of non-chemical factors in the aetiology of childhood leukaemia (IARC, 1999). However, this does not preclude the possibility that there may be chemical risk factors, as childhood leukaemia is a biologically and clinically diverse disease and is likely to arise via a number of aetiological pathways (Greaves & Alexander, 1993).

*Exposure to vehicular emissions as a possible cause of childhood leukaemia*

4. The Committee considered that, because benzene is a well established leukaemogen in adults, benzene in vehicle exhaust and/or fuel vapour could represent a potential risk factor in the development of childhood leukaemia. Both vehicle exhaust and fuel vapour also contain several other carcinogenic compounds<sup>\*\*</sup>, although there are differences in the profile of these carcinogenic constituents (and thus the carcinogenic potential). The Committee decided that there are differences in the hazards presented by these two types of emissions and, therefore, considered that information on exposure to fuel vapour should be discussed separately from information on exposure to vehicle exhaust.

*(a) Exposure to fuel vapour*

Exposure to fuel vapour arises largely from the evaporation of fuel from the tanks and engines of parked vehicles. Petrol evaporates more readily than diesel because diesel has a lower vapour pressure. Therefore, there are only a limited number of studies examining the risk of inhalation exposure to diesel vapour.

The Committee reviewed a draft report by the Oil Companies' European Organisation for Environment, Health and Safety (CONCAWE) on the risk assessment of inhalation exposure to 'gasoline'<sup>††</sup> (petrol) and benzene vapour by the general public (CONCAWE, 2004). Members commented on CONCAWE's main findings in relation to childhood exposure as described below.

§ Acute leukaemia tends to affect younger people with symptoms developing rapidly. The most common types are Acute Lymphoblastic Leukaemia (ALL) and Acute Myeloid Leukaemia (AML; also termed Acute Non-Lymphoblastic leukaemia (ANLL)). Chronic leukaemia tends to occur in older people and has a slow progression rate.

\*\* Most tailpipe emissions consist of many hydrocarbons, including benzene, which itself is likely to derive from a mixture of unburnt benzene and benzene formed from the combustion of other aromatic petrol components.

†† CONCAWE referred to petrol as gasoline in their report.

1. CONCAWE assessment of exposure to petrol vapour

CONCAWE prepared separate risk assessments for three child-relevant scenarios:

- visiting service stations
- travelling in cars
- residing indoors at locations near petrol vapour sources

The report calculated typical and reasonable worst case (RWC) airborne concentrations ( $\text{mg}/\text{m}^3$ ) and average daily doses (ADD) ( $\text{mg}/\text{kg}/\text{day}$ ) for inhalation exposure to petrol for each of the above scenarios as shown in Table 1 below. (See <http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf> for rationale)

Scenario	Airborne Concentration ( $\text{mg}/\text{m}^3$ )		Estimated Inhalation Intake ( $\text{mg}/\text{kg}/\text{day}$ )	
	Typical	RWC	Typical	RWC
Visiting service stations	2.7	3.83	0.0005	0.0006
	Adults* Children**	3.83	0.0011	0.0015
Travelling in cars	3.15	3.94	0.04	0.06
	Adults Children	3.94	0.12	0.15
Staying indoors (residence)	2.07	7.16	0.25	0.87
	Adults Children	7.16	1.66	5.71

\*Body weight used for adults = 70 kg

\*\*Body weight used for children = 9 kg

**Table 1. Summary of the exposure results for petrol vapour inhalation for several consumer scenarios (CONCAWE, 2004)**

The Committee noted that children had considerably higher estimated inhalation intakes than adults. It was further noted that children staying in their homes received the highest estimated airborne exposures to petrol vapour (largely due to the amount of time spent indoors) with an ADD (RWC) value approximately ten-fold greater than that estimated for a service station attendant working a full shift (data not shown). However, it was further noted that much of the difference was due to variations in the duration of exposure represented by the three scenarios. The Committee accepted that these estimates were very conservative<sup>††</sup> and so were likely to have overestimated true exposures.

## 2. CONCAWE assessment of exposure to benzene vapour

The Committee also considered data from the CONCAWE report, as presented in Table 2, on airborne concentrations and estimated ADD for inhalation exposure to benzene vapour. Similar trends to those seen in the petrol vapour exposure assessment were apparent, i.e. indoor rather than outdoor exposures to benzene from petrol fumes appear to be of more significance. Again, the duration of exposure represented by the three scenarios accounted for much of the difference in intake between them.

Scenario	Airborne Concentration ( $\mu\text{g}/\text{m}^3$ )		Estimated Inhalation Intake ( $\mu\text{g}/\text{kg}/\text{day}$ )	
	Typical	RWC	Typical	RWC
Visiting service stations	12	17	2.0E-03	2.9E-03
Adults*	12	17	4.7E-03	6.7E-03
Children**				
Travelling in cars	14	18	0.2	0.3
Adults	14	18	0.5	0.7
Children				
Staying indoors (residence)	10.7	26	1	3
Adults	10.7	26	9	20
Children				

\*Body weight used for adults = 70 kg

\*\*Body weight used for children = 9 kg

*NB. The current levels of exposure to benzene from gasoline are likely to be lower than those estimated above. This is because the data was obtained from studies conducted prior to the 2000 European EU directive 98/70/EC, (in which levels of benzene in gasoline was reduced from 5% to 1%)*

Table 2. Summary of the exposure results for benzene vapour inhalation for several consumer scenarios (CONCAWE, 2004)

*(Figures have been converted into  $\mu\text{g}/\text{m}^3$  and  $\mu\text{g}/\text{kg}/\text{day}$  for ease of reference)*

‡ Many of the underlying assumptions used to derive these estimates were based on the regulatory requirements of the risk assessment process. For example, CONCAWE used guidance provided in the EU Existing Substances Risk Assessment Technical Guidance Document to convert air concentrations of chemicals into daily doses. CONCAWE states that these estimates represent industry obligations in the process rather than the organisation's view of the current situation.

A substantial amount of information was available to the Committee from the published literature on levels of benzene inside and outside the home. This enabled the Committee to obtain a greater understanding of the issues relating to inhalation exposure to benzene vapour, and to generate a more considered view on the above exposure assessment.

- i) Work by the Building Research Establishment showed that the mean indoor benzene concentrations in 174 homes within the Avon area (UK) were higher than outdoor levels, especially in those households with an attached garage (Duarte-Davidson et al., 2001). Furthermore, benzene concentrations in rooms directly above an integral garage have been reported to be 2.5 times the ambient air standard (Mann et al., 2001). See <http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf> for further details.
- ii) Studies providing data on mean air concentrations of benzene outdoors showed that UK levels were consistent with levels in the US and Canada. These levels varied depending on the proximity of parked or moving traffic – the highest levels of benzene were documented to be within the cars [up to 2527.2  $\mu\text{g}/\text{m}^3$  (780 ppb)<sup>§§</sup>] and may even reach the US Occupational Exposure Limit of 3240  $\mu\text{g}/\text{m}^3$  (1000 ppb), although Wolff (1992) reports that the typical range is in the order 32.4 – 64.8  $\mu\text{g}/\text{m}^3$  (10-20 ppb). Table 3 provides a summary of the range of benzene levels detected inside and outside the home.
- iii) Members noted that there were a number of national and international initiatives intended to reduce population exposure to benzene. In a personal communication to the Committee, CONCAWE identified four important technological developments designed to reduce current levels of benzene emissions from automobiles (see <http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf>). Air Quality Objectives in the UK are for an annual running mean standard<sup>\*\*\*</sup> for benzene of 5  $\mu\text{g}/\text{m}^3$  (1.54 ppb) in England and Wales, and 3.25<sup>†††</sup>  $\mu\text{g}/\text{m}^3$  (1 ppb) for Scotland and Northern Ireland by 31 December 2010 ([http://www.ehsni.gov.uk/pubs/publications/AQS\\_addendum\\_web.pdf](http://www.ehsni.gov.uk/pubs/publications/AQS_addendum_web.pdf)). In Europe, the ambient air limit value<sup>†††</sup> for benzene is 5  $\mu\text{g}/\text{m}^3$  (to be met by 1 January 2010, as set by the European Community Directive 2000/69/EC) (<http://europa.eu.int/comm/environment/air/pdf/ppbenzene.pdf>).

§§ 1 ppb = 1 part by volume, in one thousand million i.e. 1 in 10<sup>9</sup>; 1 ppb of benzene  $\equiv$  3.24  $\mu\text{g}/\text{m}^3$  at 20°C and 1013 millibars (<http://www.defra.gov.uk/environment/airquality/aqs/benzene/4.htm>)

\*\*\* the annual running mean standard refers to a value calculated from data collated for the 12 months prior to the quoted date

††† 3.246  $\mu\text{g}/\text{m}^3$  rounded to nearest 2 decimal places

††† the air limit value is a legislative standard, which takes cost and benefit into account and provides a date for when the legislation must be effected. The long term policy aim for England and Wales is to achieve an Air Quality Objective of 1 ppb annual mean concentration.

	[Benzene]/( $\mu\text{g}/\text{m}^3$ )
Air Quality Objective	5
Outdoor*†	1 – 8
Indoor	4 – 40
Outdoor (20m busy road)*	10 – 45
Outdoor (kerbside)*	118
Cars (inside)*	32 – 2527
US Occupational Limit	3240

\* It would be difficult to determine the contribution of evaporative emissions or vehicle exhaust to the benzene levels detected.

† Levels of benzene in outdoor air during winter can be three times the level detected in summer.

**Table 3. Ambient air concentrations of benzene**  
(taken from Duarte-Davidson et al., 2001; Mann et al., 2001 and Wolff, 1992)

- iv) The Committee was also reminded of the findings of the Working Group on Benzene (1998), which conducted a similar assessment of the potential risks from benzene exposure to the general population as part of a process to deal with “Ambient Air Quality Assessment and Management”. These also suggested that children received much higher intakes of benzene than adults.

On the basis of the available information on levels of evaporative emissions inside and outside the home, the Committee concluded that exposures of children to benzene and petrol vapour inside the home could be greater than outside the home and that the significance of indoor exposures to petrol vapour and benzene should be considered further.

(a) Exposure to vehicle exhaust

One of the major challenges in epidemiological studies investigating the effects of exposure to vehicle exhaust is quantifying the exposure. This is because vehicle exhaust is a chemically complex mixture and its components derive from multiple sources (see Table 4 for a list of compounds present in vehicle exhaust). It was noted by the Committee that most epidemiological studies examining the effects of vehicular emissions have focussed on the combustion of fuel rather than on fuel evaporation. Therefore, the subject of exposure to vehicle exhaust is discussed further in the following section.

Gas phase	Particulate phase***
Acrolein	Heterocyclics and derivatives
Ammonia	Hydrocarbons (C14-C35) and derivatives
Benzene	Inorganic sulphates and nitrates
<i>1,3-Butadiene*</i>	Metals (e.g., lead and platinum)
<i>Formaldehyde</i>	<b>Polycyclic aromatic hydrocarbons and derivatives</b>
Formic acid	
Heterocyclics and derivatives* *	
<u>Hydrocarbons (C1-C18) and derivatives* *</u>	
Hydrogen cyanide	
Hydrogen sulphide	
Methane	
Methanol	
Nitric acid	
Nitrous acid	
Oxides of nitrogen	
<i>Polycyclic aromatic hydrocarbons and derivatives* *</i>	
Sulphur dioxide	
Toluene	

(Source: IARC, 1989)

\* compounds in italics have been classified by IARC as either carcinogenic to humans, probably carcinogenic to humans or possibly carcinogenic to humans (Groups I, IIA or IIB, respectively)

\*\* derivatives include acids, alcohols, aldehydes, anhydrides, esters, ketones, nitriles, quinines, sulphonates and halogenated and nitrated compounds and multifunctional derivatives

\*\*\* diesel exhaust particulates are reasonably anticipated to be human carcinogens according to the National Toxicology Program 10th Report on Carcinogens

NB. Nitroarenes are also present in engine exhaust and are produced in large amounts from diesel engines. They are formed when nitric acid reacts with PAHs to form nitrated PAHs.

**Table 4. Some compounds and classes of compounds in vehicle engine exhaust (IARC, 1989)**

*Epidemiological studies of exposure to traffic-derived pollutants and childhood leukaemia*

The Committee was provided with information from 17 studies (sixteen<sup>§§§</sup> published as peer-reviewed papers), which have investigated the possible association between proximity of residence to road traffic exhaust fumes, and/or petrol stations and garages, and either childhood cancer or childhood leukaemia. Table 5 provides a summary of the 17 studies reviewed by the Committee. Three of the studies were from the UK and only nine specifically investigated childhood leukaemia <http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf>. Two studies examined exposures to vehicle evaporative emissions; vehicle exhaust was the predominant chemical exposure evaluated.

Ten of the 17 studies reported some positive significant associations, although three authors concluded that their positive findings were only suggestive of an association due to the qualitative limitations of the study.

§§§ The unpublished results of a study by Urayama et al. (2004) were presented as a poster at an international conference on 'Children with Leukaemia' in London (2004).



### *Evaluation of epidemiological evidence*

The Committee reviewed the methodology used in the studies to help formulate its conclusions and proposed that, based on the quality of the study designs, the actual number from which conclusions could be drawn was much lower than 17. Only two studies were considered to have adopted an adequate method for the assessment of exposure to petrol fumes and exhaust (i.e. Raaschou-Nielsen et al., 2001, and Reynolds et al., 2002). Both of these studies reported null results. However, Members considered it would be inappropriate to dismiss completely the 10 studies reporting positive findings.

Most of the studies were population-based, case-control studies where cases were identified from cancer registries. One study identified cases and controls from hospitals (Steffen et al., 2004) and five were geographical studies. Controls for population based studies were mostly selected from registers of the general population or via random digit dialling (RDD) – the latter method was considered to be a source of selection bias. In Steffen et al. (2004), controls were selected from children in the same hospital receiving treatment for acute pathologies such as trauma or orthopaedic diseases. The majority of studies matched controls for age, sex and area (for more information on control selection see <http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf>).

In general, researchers based exposure assessments on objective measurements such as residence, traffic counts and/or measurements of concentrations of air pollutants, which are not affected by recall bias. However, Steffen et al. (2004) cited possible recall bias in that the mother of cases may have over-declared previous exposures to sources of hydrocarbons; Crosignani et al. (2004) suggested that the method used by his group to sample controls could be a source of selection bias.

Few of the 17 studies obtained comprehensive information on the child's or parent's past exposures to chemicals and other potential confounders.

Members considered a suggestion that a meta-analysis be carried out on the studies but concluded that the methodologies used in the studies were too divergent for this to be feasible.

Members made the following observations and suggestions:

#### *(a) Sample size*

With the exception of Reynolds et al. (2003), most of the papers reporting a positive association were based on much smaller sample sizes (the average number of leukaemia cases = 394) than those reporting a null association (average no.= 861). The two negative studies with adequate exposure assessment referred to above Raaschou-Nielsen et al. (2001) and Reynolds et al. (2002) were noted to be among the largest.

*(b) Exposure Assessment*

Most of the studies used area-based measures as proxies for traffic-exhaust exposures (See <http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf> for further details). The Committee suggested that, although the methods currently used to assess exposure to petrol fumes/exhaust have generally improved with time, there is still potential for further development/refinement. However, useful information can be obtained from existing studies provided it is clear what the exposure metric represents. It was suggested that the distance-weighted traffic density metric was a useful approach although it should be made more specific for each pollutant to give greater utility in the ensuing risk estimation (<http://www.advisorybodies.doh.gov.uk/pdfs/cc058.pdf>).

*(c) Case-control distance comparison*

The Committee also considered the distance between the source of traffic exhaust/petrol fumes and the index<sup>\*\*\*\*</sup> child's house. The impact of main roads on air quality is not considered to be detectable above local background air pollution more than 100-200m away. Therefore, those studies in which concentrations of pollutants were measured or estimated in homes that were located more than 200m away from traffic roads or petrol stations and garages were considered to provide little useful relevant information.

*(d) Age-related factors and residential history*

The Committee noted that most studies of traffic and childhood leukaemia examined exposure potential based on a child's residence at either diagnosis or birth. Only two investigations obtained information on residence of the parent prior to birth, usually due to limitations in the recorded information available at the time, although the Committee noted that the study by Raaschou-Nielsen et al (2001) collated data spanning a child's complete residential history and encompassed an exposure window from 9 months before birth through to the child's date of diagnosis. The Committee suggested that, ideally, future studies should take into account a child's complete residential history and not just residence at diagnosis, although it was recognised that this would be difficult.

*(e) Genetic susceptibility*

Finally, the Committee considered the question of genetic susceptibility to possible traffic fumes/fuel vapour related childhood leukaemia, in the context of the study by Urayama, 2004. This investigated the combined effects of genetic polymorphisms and traffic exposure on risk of childhood leukaemia and found an association between leukaemia and traffic only in those with the CYP1A1 m2 allele. Interestingly, without accounting for individual genetic susceptibility, the authors found no association between risk of childhood leukaemia and traffic density. The Committee noted that the study was not in line with the COC criteria for the design of gene-environment studies because it lacked a prior hypothesis and hence the power of the study was lower than that assumed in the analysis.

\*\*\*\* child diagnosed with cancer of interest

## Conclusion

The Committee concluded that the available evidence provided insufficient evidence from which to conclude that there was an association between risk of childhood leukaemia and proximity to petrol stations, garages and road traffic.

The Committee considered that further studies to examine the possibility of an association between residence near traffic exhaust/petrol fumes and leukaemia would be valuable. The Committee concluded that there are differences in hazard between vehicle exhaust and evaporative emissions. Therefore, it considered that information on exposure to fuel vapour should always be discussed separately from exposure to vehicle exhaust. It requested that indoor exposures of children to petrol vapour and to benzene should be considered in a separate review.

It was recommended that future studies should also investigate leukaemia on a subtype basis (ALL, ANLL) rather than leukaemia as a whole, and any evaluation of genetic susceptibility should include the examination of a comprehensive range of Phase I and II enzymes involved in the metabolism of compounds deriving from traffic.

COC/05/S2  
October 2005

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# Mutagenicity and Carcinogenicity of Proquinazid (Cholangiocarcinoma in the rat)

## Background

1. Proquinazid (6-iodo-2-propoxy-3-propyl-3*H*-quinazolin-4-one) is a new fungicide intended for use in agriculture and viticulture providing control of powdery mildew in cereals and grapes. It is a novel class of fungicide acting by inhibiting the development of the appressorial germ tube. An application for first inclusion of proquinazid in Annex 1 of 91/414/EEC and for UK provisional approval (PPPR), in the product 'Proquinazid 200g/L EC', formulated as an emulsifiable concentrate containing 200g/L proquinazid was discussed at the 311<sup>th</sup> meeting of the Advisory Committee on Pesticides on 13 January 2005. The ACP deferred making a decision pending advice from the COC and COM with regard to the occurrence of cholangiocarcinoma in the rat carcinogenicity bioassay. The COC and COM have not been asked to review any other tumour reported in rodent carcinogenicity bioassays with proquinazid.
2. Proquinazid has slight solubility in water but is soluble in a range of organic solvents (Log  $K_{ow}$  is 5.5 at 25°C). It is slightly volatile. A number of batches were produced and used in the toxicology tests between 1997 and 2001 ranging from 96.4% w/w up to 97.9% w/w. Proquinazid is extensively absorbed from the gastrointestinal tract of rats and widely distributed in both single and repeated dose oral studies. It is noted that levels of radiolabel in bone marrow were reported to be equivalent to plasma and blood. Absorbed proquinazid was extensively metabolised and excreted via the bile and urine. Metabolism comprised predominantly of phenyl ring hydroxylation and hydroxylation at the propyl and propoxy side chains, as well as hydrolysis of some side chains. Major excretory metabolites included conjugates of these metabolites. One minor de-iodinated metabolite was identified.

## Introduction to COM/COC review

3. The COM considered proquinazid at its meeting of the 26 May 2005. The COM had access to the individual study reports<sup>17</sup>, the evaluation prepared by PSD for the ACP<sup>8</sup> and some additional information from the data holder provided in a power point presentation document. The data holder made a presentation to COM.<sup>9-10</sup>
4. The COC considered proquinazid at its meeting of the 14 July 2005. The COC had access to the summary evaluation prepared by PSD for the ACP<sup>8</sup>, the draft COM conclusions on proquinazid, detailed information on survival and pathology from the rat and mouse carcinogenicity bioassays<sup>11,12</sup> information from a one year study in dogs<sup>13</sup>, the report of the Scientific Advisory Panel review of liver pathology from the rat and mouse carcinogenicity bioassays commissioned by the data holder<sup>14</sup>, a mode of action assessment document for intestinal-type cholangiocarcinomas in the two year feeding study with proquinazid submitted by the data holder<sup>15</sup>, and information from an internal memorandum on the hepatic pathology in the 2 year feeding study with proquinazid in rats<sup>16</sup>. The data holder made a presentation to COC, which included photomicrographs of a number of histological sections of liver tissue from control and proquinazid treated rats.<sup>17</sup> (An information paper on the pathogenesis of cholangiocarcinoma in humans and rats was drafted by the secretariat <http://www.advisorybodies.doh.gov.uk/pdfs/cc0510.pdf>)

## COM review of Mutagenicity (26 May 2005)

### Presentation by data holder

5. The COM heard a presentation from DuPont on the mutagenicity of proquinazid. The purpose of the presentation was to provide DuPont's rationale on the adequacy of the genetic toxicology data base for proquinazid and to specifically discuss the results of the mammalian cell *in-vitro* chromosome aberration assay in human lymphocytes and the *in-vitro* mammalian cell gene *hprt* mutation assay in CHO cells. DuPont sought the committee's agreement that proquinazid is not genotoxic and further testing is unnecessary.
6. DuPont was aware of minor limitations in the *in-vitro* chromosomal assay and *in-vitro* gene mutation assay in mammalian cells. A battery of seven genotoxicity studies had been conducted with technical grade material during the period 1996-1998 (in-life dates of studies). The studies complied with the EU directive 91/414/EEC, the COM guidance of 1989, and OECD test guidelines (current at the time of testing). Two batches of technical material were evaluated (which had been produced by different manufacturing methods, the earlier batch DPX-KQ926-45 was 97% pure and the latter batch DPX-KQ926-75 was 97.9% pure). Tests undertaken with the 45 batch included the Ames test, the *in-vitro* chromosomal aberration assay, the *in-vitro* gene mutation assay in CHO cells (*hprt*), the *in-vitro* UDS assay using primary rat hepatocytes, and an *in-vivo* bone marrow micronucleus (MN) assay. Tests undertaken with the 75 batch included an Ames test and an *in-vivo* bone marrow MN assay.
7. The presentation then focused on the two *in-vitro* mutagenicity studies where limitations had been identified. The design of and results obtained in the *in-vitro* chromosomal mutation assay in human lymphocytes were reviewed. The negative results and adequate performance of the positive control trials were noted. Some additional historical positive control data were presented (additional slide not in information tabled for members). COM members asked for further information on these additional positive control data. The information was intended to show the lower limit of positive control responses in the test facility both before and after the in-life phase of the *in-vitro* chromosomal aberration assay. The data were consistent with those already provided to members and were generated from a number of tests with cyclophosphamide and MMC. DuPont noted that the study complied with an early proposed revision of the OECD guideline (ca 1996) and included a delayed harvest and that marked cytotoxicity was seen at the 24 h and 48 h harvests. In addition the consistent negative findings in this assay were supported by negative findings in two *in-vivo* bone marrow MN assays in mice in which oral dose levels up to 2g/kg bw had been tested with the occurrence of clinical signs of toxicity and/or mortality in mice and evidence of bone marrow exposure in the rat metabolism study.
8. The design of and results obtained in the *in-vitro* mammalian cell gene mutation assay (CHO/*hprt*) were reviewed. Two independent assays had been conducted for each treatment and a third trial had been performed with activation due to a lack of toxicity in the second trial. The study was a qualitative assessment of mutagenic potential. A positive response was obtained if the mutation frequency was  $> 40 \times 10^6$  cells at two or more consecutive concentrations. Plating of  $10^6$  cells was sufficient to detect a positive response. There was no evidence for a positive response in the assay. Mutation frequencies obtained with the test material were within the negative historical control range and there was a lack of reproducible dose-dependent increases. Positive controls produced marked increases in mutation

frequency in all trials and it was noted that there was comparatively little variance in the positive control data between trials using the same treatment condition. DuPont had commented that all negative control mutation frequencies were within the range of historical controls for the test laboratory, the positive controls exhibited consistent responses despite the fluctuation in negative control frequencies and the negative findings were supported by a lack of genotoxicity in the battery of other *in-vitro* and *in-vivo* genotoxicity studies.

9. DuPont concluded that proquinazid does not pose a mutagenic concern. There were negative findings in a battery of seven *in-vitro* and *in-vivo* studies. There was no result to suggest a positive or weak positive response. Neither proquinazid, nor metabolites formed in rats, contain structural alerts for DNA reactivity. The studies were conducted to the prevailing guidelines at the time. The limitations noted by PSD with regard to the *in-vitro* chromosomal aberration assay and the mammalian cell gene mutation assay (CHO/hprt) did not impact on the overall genotoxic assessment of proquinazid. Thus further testing was unnecessary to conclude that proquinazid is not genotoxic.

#### COM consideration of presentation and mutagenicity data

10. The COM held a detailed discussion with the representatives of DuPont regarding the mutagenicity data and in particular the adequacy and conduct of the *in-vitro* chromosomal aberration assay in human lymphocytes and the *in-vitro* mammalian *hprt* gene mutation assay in CHO cells. The Committee agreed that as the COM and COC had been asked to evaluate the potential mechanism of cholangiocarcinomas seen in the rat long-term carcinogenicity bioassay with proquinazid, there was a need to have a high level of confidence in the mutagenicity data on proquinazid. Whilst members agreed that there was no convincing evidence for positive results in any of the tests, it was not possible to conclude that the *in-vitro* cytogenetic assay in human lymphocytes and the *in-vitro hprt* mammalian gene mutation assay in CHO cells were negative in view of the limitations. Members accepted that some reassurance was gained from the *in-vivo* bone marrow micronuclei assays in mice.
11. The COM discussed and reached the following conclusions with regard to the mutagenicity data on proquinazid.

*Bacterial reverse mutation assay with batch DPX-KQ926-75 (97.9% pure). Date 18/11/98<sup>1</sup>*

12. Members agreed that this study had been adequately conducted and no further information was required.

*Bacterial reverse mutation assay with batch DPX-KQ926-45 (97% pure). Date 28/2/97<sup>2</sup>*

13. Members agreed that this study had been adequately conducted and no further information was required.



*In-vitro mammalian cytogenicity test with batch DPX-KQ926-45 (97% pure). Date 26/2/97<sup>3</sup>*

14. Members agreed that some aspects of the *in-vitro* chromosome aberration assay in human lymphocytes had been conducted in excess of requirements but considered that a prolonged exposure assay was required so that cells were exposed over the whole cell cycle. Members did not consider that the test had adequately covered this aspect or that the potential for aneugenicity had been examined. Members observed that the dose selection had resulted in high levels of cytotoxicity and that the positive control response reported (towards the lower limit of a very wide range, ca 6-80% cells with aberrations) limited the value of this assay. The Committee agreed that it would be advisable to request a continuous 20 h exposure in a mammalian cell chromosomal aberration assay (in human lymphocytes) in the absence of exogenous metabolic activation. This would ensure that all stages of the cell cycle were exposed. It would be appropriate to include estimation of polyploidy as an indicator of potential for aneugenicity. The study should be conducted to internationally accepted standards. The Committee was aware of the need to adequately explain the mode of action of the cholangiocarcinomas reported in the long-term bioassay of proquinazid in rats and agreed that this information would help to complete the *in-vitro* mutagenicity evaluation of proquinazid which was an important part of the carcinogenicity risk assessment.

*In-vitro gene mutation in mammalian cells with batch DPX-KQ926-45. (97% pure). Date 22/5/97<sup>4</sup>*

15. Members commented that the data from the *in-vitro* mammalian cell *hprt* gene mutation assay in CHO cells were consistent with the conclusions COM had previously published on practical difficulties in undertaking this assay (<http://www.advisorybodies.doh.gov.uk/com/mut033.htm>). There was considerable inter-trial variance in mutation frequency in the proquinazid assay and the finding of very low mutation frequencies in some trials (<0.6 x 10<sup>6</sup> cells) limited the conclusions that could be drawn. Thus it was noted that the mutation frequency in the third trial in the acetone control and test material experiments was low at all dose levels. Members concluded that the data were not interpretable. The Committee agreed that a mouse lymphoma assay conducted to internationally accepted standards should be requested. The Committee was aware of the need to adequately explain the mode of action of the cholangiocarcinomas reported in the long-term bioassay of proquinazid in rats and agreed that this information was essential to complete the *in-vitro* mutagenicity evaluation of proquinazid which was an important part of the carcinogenicity risk assessment.

*In-vitro unscheduled DNA synthesis with batch DPX-KQ926-45 (97% pure). Date 21/5/97. Minor editorial revision to report 22/2/99<sup>5</sup>*

16. Members agreed that this study had been adequately conducted and no further information was required.

*In-vivo bone marrow micronucleus in mice with batch DPX-KQ926-75 (97.9% pure) Date 14/1/99<sup>6</sup>*

*In-vivo bone marrow micronucleus in mice with batch DPX-KQ926-45 (97% pure) Date 22/2/99<sup>7</sup>*

17. Members agreed that the data provided were indicative of negative findings in these assays. Members agreed the evidence for small increases in percentage micronucleated polychromatic erythrocytes seen at some doses most probably represented fluctuation in the background incidence of micronuclei. Members accepted the evidence of toxicity in these studies and information from the toxicological and toxicokinetic studies with proquinazid suggested that the bone-marrow would have been exposed to proquinazid and metabolites in these two studies.

#### COM conclusions from May 2005 meeting

18. Members considered that the company had provided some relevant information and comments but had not been convinced that the *in-vitro* mutagenicity test package was adequate. Members agreed that a key element of any proposal regarding mechanism of proquinazid induced cholangiocarcinoma in the rat would require an evaluation of mutagenicity and hence it was important to complete the mutagenicity test package.
19. The Committee considered that a mouse lymphoma assay should be conducted and that it was advisable to also conduct a continuous 20 h exposure in a mammalian cell chromosomal aberration assay (in human lymphocytes) in the absence of exogenous metabolic activation. Both studies should be conducted to internationally accepted standards. These studies were required to provide full information on the mutagenicity evaluation of proquinazid.

#### COM post meeting consideration of additional mouse lymphoma assay

20. The data holder submitted on 12 July 2005 the results of a new mouse lymphoma assay undertaken with proquinazid.<sup>18</sup> The assay included an exposure of 3 hour (in presence and absence of exogenous metabolic activation) and a continuous 24 h exposure in the absence of metabolic activation. The results suggested that proquinazid was not mutagenic in this assay. A full report was not available during the postal consultation with COM members. The Committee considered the available data from the new mouse lymphoma assay by postal consultation and agreed there was no evidence for a mutagenic effect. It was also agreed that the full report should be considered by COM when available. The final report was submitted to COM members by postal consultation on the 22 August 2005. Members agreed that the submitted mouse lymphoma assay was acceptable and gave negative results.<sup>19</sup>

#### COC evaluation of cholangiocarcinoma in the rat (14 July 2005)

21. The COM heard a presentation from DuPont on the interpretation of cholangiocarcinoma reported in female rats fed a diet containing proquinazid as part of a long term carcinogenicity bioassay.<sup>15,16</sup> In brief, groups of 80 male and 80 female Cr1:CD<sup>®</sup>(SD) BR rats were fed diets containing 0, 10, 30, 300, 600 (females), 1000 (males), 1200 (females) or 2000 ppm (males) for 104 weeks. Groups of five animals/sex were subject to interim haematology, clinical chemistry necropsy and histopathological evaluation at 1 week and 1 year of feeding. Survival at termination was increased at dose levels of 600 ppm and above. A statistically significant increase in intestinal-type cholangiocarcinoma was reported in female rats (8/60 at 600 ppm and 12/61 at 1200ppm, cf none in concurrent control).<sup>11</sup>

22. The representatives from DuPont were accompanied by Dr P Greaves (University of Leicester) who had been a member of a Scientific Advisory Panel (sponsored by DuPont). The Scientific Advisory Panel had reviewed the liver slides from the rat and mouse carcinogenicity bioassays. COC members questioned the representatives from DuPont and Dr Greaves during the presentation.
23. The Scientific Advisory Panel (SAP), had reviewed aspects of the liver pathology and a report had been finalised in November 2003. Dr Greaves had reviewed the slides again recently before the submission for the COC consideration was compiled.<sup>16</sup> The SAP considered that the lesions seen in the female rats were not indicative of a carcinogenic risk for humans. The Panel's rationale had been that proquinazid was not genotoxic, that cholangiocarcinomas of the "intestinal" type occurred only in female rats fed 600 ppm or more at the end of the two-year study, the tumour type was linked to chronic hepatocellular injury with "cholangiofibrosis" and there was no risk of the lesion occurring in the absence of severe hepatic toxicity. A number of representative sections were shown to COC members. Features of the histopathology of the lesions seen at 1200 ppm included: cellular degeneration, inflammatory infiltrate, extensive necrosis, giant nuclei and fibrotic changes termed "cholangiofibrosis" by the original reporting pathologists. The cells within the lesions showed goblet cell differentiation and were therefore termed "intestinal-type". The cholangiofibrosis was not necessarily related to the portal tract and the lesions termed "cholangiocarcinoma" were quite unlike the usual pattern seen in human cholangiocarcinoma. COC members supported the view that the lesions were not neoplastic but were a florid inflammatory response to severe acute and chronic hepatocellular damage with marked reactive epithelial changes including cytoplasmic alterations, nuclear pleomorphism, and intestinal metaplasia. COC pathologists noted that there was little evidence of increased mitotic activity in the slides shown and aberrant mitoses were not a feature.
24. Dr Greaves confirmed that the SAP had reviewed virtually all the lesions diagnosed as cholangiocarcinomas and he had reviewed a proportion of them again recently. In answer to specific questioning, he agreed that, if chronic exposure to proquinazid was sufficiently high to induce similar severe liver toxicity in humans, there was a risk that similar lesions to that identified in rats could occur. He noted that liver toxicity was seen in female rats receiving 300 ppm proquinazid but was less severe than at 600 ppm. There had been individual variation in severity at 600 ppm. In answer to another question, DuPont representatives noted that the pigment seen in the liver sections had been shown to be PAS positive and was therefore likely to be lipofuscin as expected in the setting of cell degeneration and increased turnover. There was minimal deposition of stainable iron.
25. The Committee raised a question as to whether the role of oval cells in liver regeneration, as proposed in the MOA<sup>15</sup>, is hypothetical or is more generally accepted. It was agreed that oval cell proliferation was seen in rodents when a regenerative stimulus was supplied but hepatocyte proliferation inhibited, for example by 2AAF.

#### COC consideration and conclusions

26. The committee concluded that it was uncertain that the lesions termed "cholangiocarcinomas" were truly neoplastic but accepted the conventionality of the term used by the study pathologist. However, it was noted that the pathology seen with proquinazid lacked the expected characteristics of that caused by a genotoxic carcinogen.

27. Members agreed that the MOA proposed by DuPont for the putative cholangiocarcinomas was plausible. They noted that the lesions were only seen at dose levels which caused severe liver toxicity and which exceeded the MTD. They further agreed that the lesions were relevant to humans in that they were a potential hazard. However, whether they were a risk in humans depended on the level of exposure.
28. Members agreed that the No Observed Adverse Effect Level (NOAEL) for putative cholangiocarcinoma was 16 mg/kg bw/day (300 ppm in female rats). They were informed that PSD had proposed an acceptable daily intake for proquinazid of 0.01 mg/kg bw, based on the overall No Observed Adverse Effect Level of 1.2 mg/kg bw/day derived from all the toxicology data on proquinazid and a safety factor of 100. Members confirmed that this would give an adequate margin of safety (1600) for cholangiocarcinoma.

#### Overall discussion of COM/COC on proquinazid

29. The COM has reviewed the available mutagenicity data on proquinazid (26/5/05). All of the studies were considered acceptable with the exception of the *in-vitro* mammalian mutagenicity assay in CHO cells (*hprt* locus) and it was also noted that an extended exposure of human lymphocytes in the absence of exogenous metabolic activation had not been undertaken as part of the assay for chromosomal aberrations. The COM was aware of a recently submitted *in-vitro* mouse lymphoma assay and agreed by postal consultation that the data were suggestive of a negative response (results submitted 12/7/05).<sup>18</sup> The final report was submitted to COM members by postal consultation on the 22 August 2005. Members agreed that the submitted mouse lymphoma assay was acceptable and gave negative results. The COM agreed that it would not be necessary to complete the *in-vitro* mutagenicity test package by undertaking an extended exposure treatment of human lymphocytes for chromosomal aberrations in view of the totality of negative *in-vitro* and *in-vivo* mutagenicity data available on proquinazid if the MOA assessment for the cholangiocarcinoma which had been submitted to COC was deemed acceptable.
30. The COC considered the available carcinogenicity data and additional information on histopathology of the liver from the carcinogenicity bioassay in rats on 14/7/05. Members were unconvinced that the lesions reported as cholangiocarcinomas were truly neoplastic, but accepted that the study pathologists' diagnosis could be used for risk assessment. The COC agreed that a threshold approach to risk assessment could be used and agreed that there was a satisfactory margin of safety between the NOAEL for putative cholangiocarcinoma and the ADI proposed by PSD.

#### Overall conclusions of COM/COC on proquinazid

31. The COM concluded that the mutagenicity data submitted provided evidence that proquinazid is not an *in-vitro* or *in-vivo* mutagen. There were limitations in the adequacy of the submitted *in-vitro* gene mutation assay in mammalian cells (CHO, *hprt*) and the submitted *in-vitro* assay in human lymphocytes for chromosomal aberrations. The data holder submitted negative results from a mouse lymphoma assay which included both short-term (3h) and extended exposure (24 h) treatments after the COM meeting (26/5/05). The full report of this study would need to be considered by COM when available. The final report was submitted to COM members by postal consultation on the 22 August 2005.

Members agreed that the submitted mouse lymphoma assay was acceptable and gave negative results. The COM agreed that the advice for a further *in-vitro* study to examine chromosomal aberrations in human lymphocytes study would not be a requirement in view of the totality of negative mutagenicity data on proquinazid and if the MOA assessment for the cholangiocarcinoma which had been submitted to COC was deemed acceptable (see para 32).

32. The COC concluded that it was uncertain that the lesions termed “cholangiocarcinomas” were truly neoplastic but accepted the conventionality, used by the study pathologist in the report of the carcinogenicity bioassay of proquinazid in rats, of classifying them as neoplastic. A satisfactory non-genotoxic Mode of Action explanation for the cholangiocarcinomas in rats had been submitted. The COC agreed that a threshold approach could be used for the risk assessment of cholangiocarcinoma reported in the rat.

COM/05/S4, COC/05/S1  
September 2005

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# 2005 Membership of the Committee on Carcinogenicity of Chemicals in food, Consumer Products and the Environment

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*Non-specialist Member*

**Professor Alan Boobis** OBE BSc PhD CBIol FIBiol  
*Section of Experimental Medicine and Toxicology, Division of Medicine,  
Imperial College London (Hammersmith Campus)*

**Dr Philip Carthew** BSc MSc PhD FRCPath  
*Senior Pathologist, SEAC Toxicology Unit, Unilever*

**Professor Peter B Farmer** MA DPhil CChem FRSC  
*Professor of Biochemistry, Cancer Studies and Molecular Medicine,  
Cancer Biomarkers and Prevention Group, Biocentre, University of Leicester*

**Professor David Forman** BA PhD FFPHM  
*Professor of Cancer Epidemiology, Unit of Epidemiology and Health Services Research, School of Medicine,  
University of Leeds*

**Mrs Rosie Glazebrook**  
*Non-specialist member*

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

**Ms Denise M Howel** BSc MSc CStat FIS  
*Senior Lecturer in Epidemiological Statistics, School of Population and Health Sciences, University of  
Newcastle*

**Dr Sandra Jane Kennedy** BSc PhD FRCPath CBIol FIBiol  
*Vice-President of Safety Assessment UK, GlaxoSmithKline*



**Professor David H Phillips** BA PhD DSc FRCPath

*Professor of Environmental Carcinogenesis, Institute of Cancer Research*

**Dr Ruth Roberts** BSc PhD

*Director of Toxicology, Safety Assessment, AstraZeneca*

**Professor David E G Shuker** BSc ARCS PhD DIC CChem FRSC

*Professor of Organic Chemistry and Head of Department of Chemistry,  
The Open University*

**Dr Nicola Wallis** BSc MBChB MRCPATH MFPM

*Safety and Risk Management, Pfizer Global Research & Development*

#### SECRETARIAT

**Mr J Battershill** BSc MSc Scientific Secretary

**Dr D Benford** BSc PhD Scientific Secretary – Food Standards Agency

**Mr K Mistry** Administrative Secretary

**Ms F Pollitt** MA Dip RCPATH

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

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
<b>Professor P G Blain (Chairman)</b>	NONE	NONE	NONE	NONE
<b>Dr C Allen</b>	NONE	NONE	NONE	NONE
<b>Professor A Boobis</b>	Abbey Life Abbey National Barclays Bank BG Group BT Group Centrica HBOS Marks and Spencer National Grid Transco Scottish Power OSI Pharmaceuticals	223 Shareholder 875 Shareholder 112 Shareholder 94 Shareholder 110 Shareholder 99 Shareholder 80 Shareholder 102 Shareholder Shareholder 37 Consultancy	Servier	Research support
<b>Dr P Carthew</b>	Provalis Unilever	Shareholder Salary	NONE	NONE
<b>Professor P B Farmer</b>	Abbey National Bradford & Bingley Celltech Foreign & Colonial Friends Provident Health Effects Institute Torotrak	Shareholder Shareholder Shareholder Shareholder Research Committee Member Shareholder	American Chemistry Council CEFIC	Research Support Research Support
<b>Professor D Forman</b>	Barclays Friends Provident HBOS Woolwich	Shareholder Shareholder Shareholder Shareholder	NONE	NONE
<b>Mrs R Glazebrook</b>	Dr Foster Ltd BT Group Lloyds TSB National Grid	Salary Shareholder Shareholder Shareholder	NONE	NONE
<b>Professor D Harrison</b>	Medical Solutions Qunitiles Scottish Medicine	Shareholder Consultant Consultant	NONE	NONE
<b>Ms D Howel</b>	NONE	NONE	NONE	NONE
<b>Dr S J Kennedy</b>	Unilever	Shareholder	NONE	NONE

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor D Phillips	Aviva	Shareholder	NONE	NONE
	Banco Santander	Shareholder		
	BG Group	Shareholder		
	Bradford & Bingley	Shareholder		
	Centrica	Shareholder		
	Lattice Group	Shareholder		
	National Grid	Shareholder		
	Takeda	Consultancy		
Dr R Roberts	AstraZeneca	Salary	NONE	NONE
	P&O	Shareholder		
		Shareholder		
Professor D Shuker	NONE	NONE	NONE	NONE
Dr N Wallis	Pfizer	Salary	NONE	NONE
		Shareholder		

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# ANNEX 1 – Terms of Reference

To advise at the request of:

Department of Health  
Food Standards Agency  
Department for the Environment, Food and Rural Affairs  
Department of Transport, Local Government and the Regions  
Department of Trade and Industry  
Health and Safety Executive  
Health Protection Agency  
Pesticide Safety Directorate  
Veterinary Medicines Directorate  
Medicines and Healthcare products Regulatory Agency  
Home Office  
Scottish Executive  
National Assembly for Wales  
Northern Ireland Executive  
Other Government Departments and Agencies

To assess and advise on the toxic risk to man of substances which are:

- a. used or proposed to be used as food additives, or used in such a way that they might contaminate food through their use or natural occurrence in agriculture, including horticulture and veterinary practice or in the distribution, storage, preparation, processing or packaging of food;
  - b. used or proposed to be used or manufactured or produced in industry, agriculture, food storage or any other workplace;
  - c. used or proposed to be used as household goods or toilet goods and preparations;
  - d. used or proposed to be used as drugs, when advice is requested by the Medicines and Healthcare products Regulatory Agency, Section 4 Committee or the Licensing Authority;
  - e. used or proposed to be used or disposed of in such a way as to result in pollution of the environment.
2. To advise on important general principles or new scientific discoveries in connection with toxic risks, to co-ordinate with other bodies concerned with the assessment of toxic risks and to present recommendations for toxicity testing.

## ANNEX 2 – Code of conduct for members of advisory committees

### Public service values

Members must at all times:

- observe the highest standards of impartiality, integrity and objectivity in relation to the advice they provide and the management of this Committee;
- be accountable, through the Chairman of the Food Standards Agency, the Chief Medical Officer, to Ministers, Parliament and the public for its activities and for the standard of advice it provides.

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

### Standards in Public Life

All Committee members must:

- follow the Seven Principles of Public Life set out by the Committee on Standards in Public Life (see page 173);
- comply with this Code, and ensure they understand their duties, rights and responsibilities, and that they are familiar with the function and role of this Committee and any relevant statements of Government policy. If necessary members should consider undertaking relevant training to assist them in carrying out their role;
- not misuse information gained in the course of their public service for personal gain or for political purpose, nor seek to use the opportunity of public service to promote their private interests or those of connected persons, firms, businesses or other organisations; and
- not hold any paid or high profile unpaid posts in a political party, and not engage in specific political activities on matters directly affecting the work of this Committee. When engaging in other political activities, Committee members should be conscious of their public role and exercise proper discretion. These restrictions do not apply to MPs (in those cases where MPs are eligible to be appointed), to local councillors, or to Peers in relation to their conduct in the House of Lords.

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## Role of Committee members

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

- engage fully in collective consideration of the issues, taking account of the full range of relevant factors, including any guidance issued by the Food Standards Agency; the Department of Health and sponsor departments or the responsible Minister;
- in accordance with Government policy on openness, ensure that they adhere to the Code of Practice on Access to Government Information (including prompt responses to public requests for information); agree an Annual Report; and, where practicable and appropriate, provide suitable opportunities to open up the work of the Committee to public scrutiny;
- not divulge any information which is provided to the Committee in confidence;
- ensure that an appropriate response is provided to complaints and other correspondence, if necessary with reference to the sponsor department; and;
- ensure that the Committee does not exceed its powers or functions.

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

Communications between the Committee and the Food Standards Agency (FSA) Board and/or Ministers will generally be through the Chairman except where the Committee has agreed that an individual member should act on its behalf. Nevertheless, any member has the right of access to the FSA Board and/or Ministers on any matter that he or she believes raises important issues relating to his or her duties as a Committee member. In such cases the agreement of the rest of the Committee should normally be sought.

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

## The role of the Chairman

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

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

## Handling conflicts of interests

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

### (i) Declaration of Interests to the Secretariat

Members of the Committee should inform the Secretariat in writing of their current personal and non-personal interests, when they are appointed, including the principal position(s) held. Only the name of the company and the nature of the interest are required; the amount of any salary etc. need not be disclosed. An interest is current if the member has an on-going financial involvement with industry, e.g. if he or she holds shares in industry, has a consultancy contract, or if the member or the department for which he or she is responsible is in the process of carrying out work for industry. Members are asked to inform the Secretariat at any time of any change of their personal interests and will be invited to complete a declaration form once a year. It is sufficient if changes in non-personal interests are reported in the annual declaration form following the change. (Non-personal interests involving less than £1,000 from a particular company in the previous year need not be declared to the Secretariat).

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

### (ii) Declaration of Interest and Participation at Meetings

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

## Personal liability of Committee members

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

<sup>1</sup>Close family members include personal partners, parents, children, brothers, sisters and the personal partners of any of these.

## THE SEVEN PRINCIPLES OF PUBLIC LIFE

### **Selflessness**

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

### **Integrity**

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

### **Objectivity**

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

### **Accountability**

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

### **Openness**

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

### **Honesty**

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

### **Leadership**

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



## DIFFERENT TYPES OF INTEREST

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

### Personal Interests

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

- **Consultancies and/or direct employment:** any consultancy, directorship, position in or work for industry which attracts regular or occasional payments in cash or kind;
- **Fee-Paid Work:** any commissioned work by industry for which the member is paid in cash or kind;
- **Shareholdings:** any shareholding or other beneficial interest in shares of industry. This does not include shareholdings through unit trusts or similar arrangements where the member has no influence on financial management;

### Non-Personal Interests

A non-personal interest involves payment which benefits a department for which a member is responsible, but is not received by the member personally. The main examples are:

- **Fellowships:** the holding of a fellowship endowed by industry;
- **Support by Industry:** any payment, other support or sponsorship which does not convey any pecuniary or material benefit to a member personally, but which does benefit their position or department e.g.
  - i) a grant for the running of a unit or department for which a member is responsible;
  - ii) a grant or fellowship or other payment to sponsor a post or a member of staff or a post graduate research programme in the unit for which a member is responsible. This does not include financial assistance for students;
  - iii) the commissioning of research or other work by, or advice from, staff who work in a unit for which the member is responsible.

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

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

## DEFINITIONS

In this Code, 'the industry' means:

- Companies, partnerships or individuals who are involved with the production, manufacture, sale or supply of products subject to the following legislation;

The Food Safety Act 1990

The Medicines Acts 1968 and 1971

The Food and Environmental Protection Act 1985

The Consumer Protection Act 1987

The Cosmetic (Safety) (Amendment) Regulations 1987

The Notification of New Substances Regulations 1982

- Trade associations representing companies involved with such products;
- Companies, partnerships or individuals who are directly concerned with research, development or marketing of a product which is being considered by the Committees on Toxicity, Mutagenicity, or Carcinogenicity of Chemicals in Food, Consumer Products and the Environment.

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

## ANNEX 3 – Openness

### Introduction

1. The Committee on Toxicity (COT) and its sister committees the Committee on Mutagenicity (COM) and Committee on Carcinogenicity (COC) are non-statutory independent advisory committees who advise the Chairman of the Food Standards Agency and the CMO and, through them, the Government on a wide range of matters concerning chemicals in food, consumer products and the environment.
2. The Government is committed to make the operation of advisory committees such as the COT/COM/COC more open and to increase accountability. Proposals have been published in “Quangos-Opening the Doors” (Cabinet Office, July 1998). The COT/COM/COC have recently considered a number of options for greater openness of Committee business. There was a high level of agreement between the COT/COM/COC regarding the adoption of proposals for greater openness.
3. In discussing these proposals (during the course of 1999) the Committees were aware that the disclosure of information which is of a confidential nature and was communicated in circumstances importing an obligation of confidence is subject to the common law of confidentiality. Guidance is set out in the Code of Practice on Access to Government Information (second edition, 1997). Thus an important aspect of implementing initiatives for greater openness of Committee business concerns setting out clear guidelines for the handling of information submitted on a confidential basis.

### *General procedures for openness*

4. The Committees agreed that the publication of agendas, finalised minutes, agreed conclusions and statements (subject to the adoption of appropriate procedures for handling commercially sensitive information) and appointment of a lay/public interest member to each Committee would help to increase public scrutiny of Committee business. The Committees also agreed that additional open meetings on specific topics where interest groups, consumer organisations etc could attend and participate should be held.
5. A summary of the proposals is tabulated. A more detailed outline of procedures regarding products where confidential data has been reviewed is given in paragraphs 11-13.
6. The Committees stressed that, in view of the highly technical nature of the discussions, there was a need for all documents released to be finalised and agreed by the Committee, i.e. any necessary consultation with Members and Chairman should be completed before disclosure.
7. Statements and conclusions should summarise all the relevant data, such as information regarding potential hazards/risks for human health in respect of the use of products and chemicals, and any recommendations for further research.

8. The Committees will be asked for an opinion based on the data available at the time of consideration. It is recognised that, for many chemicals, the toxicological information is incomplete and that recommendations for further research to address these gaps will form part of the Committee's advice.
9. The release of documents (papers, minutes, conclusions and statements) where the COT/COM/COC has agreed an opinion on the available data but where further additional information is required in order to finalise the Committee's conclusions, needs to be considered on a case-by case basis. The relevant considerations include the likelihood that such additional data would alter the Committee's conclusion, any representations made by a company about, for example, commercial harm that early disclosure could cause and also the public interest in disclosure.

ISSUE	PROPOSALS	COMMENT
Open meetings on specified topics (eg invited audience, interest groups, consumer organisations, professional societies).	Agreed. Suggestions include meeting at time of release of Annual Report. External consultation on identifying topics for such meetings.	Meetings would be on generic issues in chemical toxicology, carcinogenicity, mutagenicity and risk assessment. There would be no discussion of individual commercial products.
Agenda	Agreed	Made publicly available via Internet site prior to meeting.
Papers	Agreed	Finalised papers to be made available upon request. Confidential information/annexes to be removed.
Minutes*	Agreed	Anonymised minutes made available upon request and on Internet site after appropriate consultation with members and agreement by the full committee.
Conclusions/statements*	Agreed	Agreed conclusions/statements published as appropriate including via the Internet and also made available on request.
Annual Report*	Agreed	Publish in accordance with procedures for previous years.

\* (Procedures for handling confidential information outlined in paragraphs 11-13 below).

- 10 In the event that the Committees need to consider an item over several meetings, it might be necessary to keep relevant documents (e.g. papers and minutes) confidential until an agreed opinion (e.g. statement) is available.

# Openness

## Procedures for handling confidential information

### Background

- 1 COT/COM/COC quite often consider information which has been supplied in confidence. For the most part this comprises information which is commercially sensitive. For example, this could include product formulations/specifications, methods of manufacture, and reports of toxicological investigations and company evaluations and safety assessment.
- 2 Normal procedure in the past has been to publish a summary of the Committee's advice in the Annual Report and to ask companies to release full copies of submitted reports for retention by the British Library at the completion of a review. Given the clear Ministerial commitment to the publication of detailed information regarding the activities of advisory committees, and in particular following the assessment of products which are already available to the general public, the COT/COM/COC have begun to adopt where possible a more open style of business where detailed statements have been published via the Internet soon after they have been finalised.
- 3 Except in cases where there is legislation under which information has been submitted and which deals with disclosure and non-disclosure, the general principle of the common law duty of confidentiality will apply. This means that any information which is of a confidential character and has been obtained in circumstances importing a duty of confidence may not be disclosed unless consent has been given or there is an overriding public interest in disclosure (such as the prevention of harm to others). The following procedure will be adopted which allows confidential information to be identified, assessed and appropriate conclusions/statements to be drafted and published on the basis of a prior mutual understanding with the companies. There is scope for companies to make representations also after submission of the information and prior to publication regarding the commercial sensitivity of data supplied and to comment on the text of statements which are to be published. However, companies would not have a right of veto in respect of such statements.

### *Procedures prior to committee consideration*

#### *Initial discussions*

4. Upon referral to COT/COM/COC the Secretariat will liaise with the relevant company supplying the product in the UK to:
  - i) Clearly state the policy of Committee openness (as summarised above).
  - ii) To identify and request the information needed by the COT/COM/COC (e.g. test reports, publications etc).

### *Confidential data*

- iii) The company will be asked to clearly identify any confidential data and the reason for confidentiality.

### *Handling confidential data*

- iv) The procedures by which the COT/COM/COC will handle confidential data and the public availability of papers, minutes, conclusions and statements where reference is made to such data will be discussed with the company prior to submission of papers to the Committee(s). The general procedures for handling documents are outlined in paragraphs 4-10 above. Companies will be informed that confidential annexes to Committee papers (e.g. where detailed information supplied in confidence such as individual patient information and full study reports of toxicological studies) will not be disclosed but that other information will be disclosed unless agreed otherwise with an individual company.
- v) The following is a suggested list of information which might be disclosed in COT/COM/COC documents (papers, minutes, conclusions and statements). The list is not exhaustive and is presented as a guide:
  - a) name of product (or substance/chemical under consideration),
  - b) information on physico-chemical properties,
  - c) methods of rendering harmless,
  - d) a summary of the results and evaluation of the results of tests to establish harmlessness to humans,
  - e) methods of analysis,
  - f) first aid and medical treatment to be given in the case of injury to persons,
  - g) surveillance data (e.g. monitoring for levels in food, air, or water).

## **Procedures during and after Committee consideration**

- 5. The timing of release of Committee documents (papers, minutes, conclusions and statements) where the item of business involved the consideration of confidential data would be subject to the general provisions outlined in paragraphs 4-10 above. Documents would not be released until a Committee – agreed conclusion or statement was available.
- 6. The most important outcome of the Committee consideration is likely to be the agreed statement. Companies will be given an opportunity to comment on the statement prior to publication and to make representations (for example, as to commercial sensitivities in the statement). The Chairman would be asked to consider any comments provided, but companies would not be able to veto the publication of a statement or any part of it. Companies will continue to be asked to release full copies of submitted reports for retention by the British Library at the completion of a review.

## ANNEX 4 – Glossary of Terms

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

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

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

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

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

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

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

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

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

**Allele:** Alternative form of a gene.

**Allergen:** Substance capable of stimulating an allergic reaction.

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

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

**Ames test:** *In vitro* (qv) assay for bacterial gene mutations (qv) using strains of *Salmonella typhimurium* developed by Ames and his colleagues.

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**Aneugenic:** Inducing aneuploidy (qv).

**Aneuploidy:** The circumstances in which the total number of chromosomes within a cell is not an exact multiple of the normal haploid (see 'polyploidy') number. Chromosomes may be lost or gained during cell division.

**Apoptosis:** A form of active cell death resulting in fragmentation of the cell into membrane-bound fragments (apoptotic bodies). These are usually rapidly removed *in vivo* by engulfment by phagocytic cells. Apoptosis can occur normally during development, but is often triggered by toxic stimuli.

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

**Bias:** In the context of epidemiological studies, an interference which at any stage of an investigation tends to produce results that depart systematically from the true values (to be distinguished from random error). The term does not necessarily carry an imputation of prejudice or any other subjective factor such as the experimenter's desire for a particular outcome.

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

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

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

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

**Bradford Hill Criteria:** Sir Austin Bradford-Hill established criteria that may be used to assist in the interpretation of associations reported from epidemiological studies:-

- Strength – The stronger the association the more likely it is causal. The COC has previously noted that the relative risks of <3 need careful assessment for effects of bias or confounding.
- Consistency – The association has been consistently identified by studies using different approaches and is also seen in different populations with exposure to the chemical under consideration.
- Specificity – Limitation of the association to specific exposure groups or to specific types of disease increases likelihood that the association is causal.
- Temporality – The association must demonstrate that exposure leads to disease. The relationship of time since first exposure, duration of exposure and time since last exposure are all important in assessing causality.



- Biological gradient – If an association reveals a biological gradient or dose-response curve, then this evidence is of particular importance in assessing causality.
- Plausibility – Is there appropriate data to suggest a mechanism by which exposure could lead to concern? However, even if an observed association may be new to science or medicine it should not be dismissed.
- Coherence – Cause and effect interpretation of data should not seriously conflict with generally known facts.
- Experiment – Can the association be demonstrated? Evidence from experimental animals may assist in some cases. Evidence that removal of the exposure leads to a decrease in risk may be relevant.
- Analogy – Have other closely related chemicals been associated with the disease?

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

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

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

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

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

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

**Carcinogens:** The causal agents which induce tumours. They include external factors (chemicals, physical agents, viruses) and internal factors such as hormones. Chemical carcinogens are structurally diverse and include naturally-occurring substances as well as synthetic compounds. An important distinction can be drawn between *genotoxic* (qv) carcinogens which have been shown to react with and mutate DNA, and *non-genotoxic* carcinogens which act through other mechanisms. The activity of genotoxic carcinogens can often be predicted from their chemical structure – either of the parent compound or of active metabolites (qv). Most chemical carcinogens exert their effects after prolonged exposure, show a dose-response relationship and tend to act on a limited range of susceptible target tissues. Carcinogens are sometimes species- or sex-specific and the term should be qualified by the appropriate descriptive adjectives to aid clarity. Several different chemical and other carcinogens may interact, and constitutional factors (genetic susceptibility, hormonal status) may also contribute, emphasising the multifactorial nature of the carcinogenic process.

**Carcinoma:** Malignant tumour arising from epithelial cells lining, for example, the alimentary, respiratory and urogenital tracts and from epidermis, also from solid viscera such as the liver, pancreas, kidneys and some endocrine glands. (See also 'tumour').

**Case-control study:** (Synonyms – case comparison study, case referent study, retrospective study) A comparison is made of the proportion of cases who have been exposed to a particular hazard (e.g. a carcinogen) with the proportion of controls who have been exposed to the hazard.

**Cell transformation:** The process by which a normal cell acquires the capacity for neoplastic growth. Complete transformation occurs in several stages both *in vitro* and *in vivo*. One step which has been identified *in vitro* is 'immortalisation' by which a cell acquires the ability to divide indefinitely in culture. Such cells do not have the capacity to form tumours in animals, but can be induced to do so by extended passage *in vitro*, by treatment with chemicals, or by transfection with oncogene DNA. The transformed phenotype so generated is usually, but not always, associated with the ability of the cells to grow in soft agar and to form tumours when transplanted into animals. It should be noted that each of these stages of transformation can involve multiple events which may or may not be genetic. The order in which these events take place, if they occur at all, *in vivo* is not known.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

**DNA (Deoxyribonucleic Acid):** The carrier of genetic information for all living organisms except the group of

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RNA viruses. Each of the 46 chromosomes in normal human cells consists of 2 strands of DNA containing up to 100,000 nucleotides, specific sequences of which make up genes (qv). DNA itself is composed of two interwound chains of linked nucleotides (qv).

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

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

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

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

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

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

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

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

**Epithelium:** The tissue covering the outer surface of the body, the mucous membranes and cavities of the body.

**Erythema:** Reddening of the skin due to congestion of blood or increased blood flow in the skin.

**Erythrocyte:** Red blood cell.

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

**Exogenous:** Arising outside the body.

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

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

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

**Forestomach:** (See glandular stomach).

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

**Gavage:** Administration of a liquid via a stomach tube, commonly used as a dosing method in toxicity studies.

**Gene:** The functional unit of inheritance: a specific sequence of nucleotides along the DNA molecule, forming part of a chromosome (qv).

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

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

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

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

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

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

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

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

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

**Genomics:** The study of genes and their function.

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

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

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Compare this with phenotype.

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

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

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

**Hepatic:** Pertaining to the liver.

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

**Hepatotoxic:** Causing toxicity to the liver.

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

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

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

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

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

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

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

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

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

**Intraperitoneal:** Within the abdominal cavity.

**Isomer:** Isomers are two or more chemical compounds with the same molecular formula but having different properties owing to a different arrangement of atoms within the molecule. The  $\beta$ -isomer of alitame is formed when the compound degrades and the atoms within the molecule are rearranged.

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

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

**LD50:** The dose of a toxic compound that causes death in 50% of a group of experimental animals to which it is administered. It can be used to assess the acute toxicity of a compound, but is being superseded by more refined methods.

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

**Ligand:** A molecule which binds to a receptor.

**Lipids:** Fats, substances containing a fatty acid and soluble in alcohols or ether, but insoluble in water.

**Lipophilic:** 'Lipid liking' – a substance which has a tendency to partition into fatty materials.

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

**Lymphoma:** Malignant tumours arising from lymphoid tissues. They are usually multifocal, involving lymph nodes, spleen, thymus and sometimes bone marrow, and other sites outside the anatomically defined lymphoid system. (See also 'tumour').

**Malignancy:** See 'tumour'.

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

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

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

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

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

**Metabolite:** Product formed by metabolism of a compound.

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

**Metaphase:** Stage of cell division (mitosis and meiosis) during which the chromosomes are arranged on the equator of the nuclear spindle (the collection of microtubule filaments which are responsible for the movement of chromosomes during cell division). As the chromosomes are most easily examined in metaphase, cells are arrested at this stage for microscopical examination for chromosomal aberrations (qv) – known as metaphase analysis.

**Metastasis:** The process whereby malignant cells become detached from the primary tumour mass, disseminate (mainly in the blood stream or in lymph vessels) and ‘seed out’ in distant sites where they form secondary or metastatic tumours. Such tumours tend to develop at specific sites and their anatomical distribution is often characteristic; it is non-random.

**Micronuclei:** Isolated or broken chromosome fragments which are not expelled when the nucleus is lost during cell division, but remain in the body of the cell forming micronuclei. Centromere positive micronuclei contain DNA and/or protein material derived from the centromere. The presence of centromere positive micronuclei following exposure to chemicals can be used to evaluate the aneugenic (qv) potential of chemicals.

**Micronucleus test:** See Micronuclei.

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

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

**Mouse lymphoma assay:** An *in vitro* assay for gene mutation in mammalian cells using a mouse lymphoma cell line L5178Y, which is heterozygous for the gene (carries only one functional gene rather than a pair) for the enzyme thymidine kinase (TK<sup>+/−</sup>). Mutation of that single gene is measured by resistance to toxic trifluorothymidine. Mutant cells produce two forms of colony – large, which represent mutations within the gene and small, which represent large genetic changes in the chromosome such as chromosome aberrations. Thus this assay can provide additional information about the type of mutation which has occurred if colony size is scored.

**Mouse spot test:** An *in vivo* test for mutation, in which pregnant mice are dosed with the test compound and mutations are detected by changes (spots) in coat colour of the offspring. Mutations in the melanocytes (skin pigment cells) of the developing fetus are measured.



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

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

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

**Mycotoxin:** Toxic compound produced by a fungus.

**Neoplasm:** See ‘tumour’.

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

**Nephrotoxicity:** Toxicity to the kidney.

**Neurobehavioural:** Of behaviour determined by the nervous system.

**Neurotoxicity:** Toxicity to the nervous system.

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

**Non-genotoxic:** See ‘carcinogens’.

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

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

**Null allele:** inactive form of a gene.

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

**Oedema:** Excessive accumulation of fluid in body tissues.

**Oestrogen:** (See estrogen)

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

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

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

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

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

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

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

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

**Plasticiser:** A substance which increases the flexibility of certain plastics.

**Polymer:** A very large molecule comprising a chain of many similar or identical molecular sub units (monomers) joined together (polymerised). An example is the polymer glycogen, formed from linked molecules of the monomer glucose.

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

**Polymorphism:** (see genetic polymorphism)

**<sup>32</sup>P postlabelling:** A sensitive experimental method designed to measure low levels of DNA adducts induced by chemical treatment.

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

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

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

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

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

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

**Reference nutrient intake (RNI):** An amount of the nutrient that is enough, or more than enough, for most (usually at least 97%) of people in a group. If the average intake of a group is at the RNI, then the risk of deficiency in the group is very small.

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

**Relative risk:** A measure of the association between exposure and outcome. The rate of disease in the exposed population divided by the rate of disease among the unexposed population in a cohort study or a population-based case control study. A relative risk of 2 means that the exposed group has twice the disease risk compared to the unexposed group.

**Renal:** Relating to the kidney.

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

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

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

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

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

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

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

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

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

**TDI:** See 'Tolerable Daily Intake'.

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

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

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

**Toxic Equivalency Factor (TEF):** A measure of relative toxicological potency of a chemical compared to a well characterised reference compound. TEFs can be used to sum the toxicological potency of a mixture of chemicals which are all members of the same chemical class, having common structural, toxicological and biochemical properties. TEF systems have been published for the chlorinated dibenzodioxins, dibenzofurans and dioxin-like polychlorinated biphenyls, and for polycyclic aromatic hydrocarbons.

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

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

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

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

**Transcriptomics:** Techniques available to identify mRNA from actively transcribed genes.

**Transfer RNA (tRNA):** RNA molecules which bond with amino acids and transfer them to ribosome's, where protein synthesis is completed.

**Transfection:** A process by which the genetic material carried by an individual cell is altered by incorporation of exogenous DNA into its genome.

**Transgenic:** Genetically modified to contain genetic material from another species (see also genetically modified organism).

**Transgenic animal models:** Animals which have extra (exogenous) fragments of DNA incorporated into their genomes. This may include reporter genes to assess *in-vivo* effects such as mutagenicity in transgenic mice containing a recoverable bacterial gene (lacZ or *lacI*). Other transgenic animals may have alterations of specific genes believed to be involved in disease processes (e.g. cancer). For example strains of mice have been bred which carry an inactivated copy of the p53 tumour suppressor gene (qv) -, or an activated form of the *ras* oncogene which may enhance their susceptibility of the mice to certain types of carcinogenic chemicals.

**Translation:** In molecular biology, the process during which the information in mRNA molecules is used to construct proteins.

**Tumour** (Synonym – neoplasm): A mass of abnormal, disorganised cells, arising from pre-existing tissue, which are characterised by excessive and uncoordinated proliferation and by abnormal differentiation. **Benign** tumours show a close morphological resemblance to their tissue of origin; grow in a slow expansile fashion; and form circumscribed and (usually) encapsulated masses. They may stop growing and they may regress. Benign tumours do not infiltrate through local tissues and they do not metastasise (qv). They are rarely fatal. **Malignant** tumours (synonym – cancer) resemble their parent tissues less closely and are composed of increasingly abnormal cells in terms of their form and function. Well differentiated examples still retain recognisable features of their tissue of origin but these characteristics are progressively lost in moderately and poorly differentiated malignancies: undifferentiated or anaplastic tumours are composed of cells which resemble no known normal tissue. Most malignant tumours grow rapidly, spread progressively through adjacent tissues and metastasise to distant sites. Tumours are conventionally classified according to the anatomical site of the primary tumour and its microscopical appearance, rather than by cause. Some common examples of nomenclature are as follows:

Tumours arising from epithelia (qv): benign – adenomas, papillomas; malignant – adenocarcinomas, papillary carcinomas.

- Tumours arising from connective tissues such as fat, cartilage or bone: benign – lipomas, chondromas, osteomas; malignant – fibrosarcomas, liposarcomas, chondrosarcomas, osteosarcomas.

- Tumours arising from lymphoid tissues are malignant and are called lymphomas (qv); they are often multifocal. Malignant proliferations of bone marrow cells are called leukaemias.

Benign tumours may evolve to the corresponding malignant tumours; examples involve the adenoma → carcinoma sequence in the large bowel in humans, and the papilloma → carcinoma sequence in mouse skin.

**Tumour initiation:** A term originally used to describe and explain observations made in laboratory models of multistage carcinogenesis, principally involving repeated applications of chemicals to the skin of mice. Initiation, in such contexts, was the first step whereby small numbers of cells were irreversibly changed, or initiated. Subsequent, separate events (see tumour promotion) resulted in the development of tumours. It is now recognised that these early, irreversible heritable changes in initiated cells were due to genotoxic damage, usually in the form of somatic mutations and the initiators used in these experimental models can be regarded as genotoxic carcinogens (qv).

**Tumour promotion:** An increasingly confusing term, originally used, like 'tumour initiation' to describe events in multistage carcinogenesis in experimental animals. In that context, promotion is regarded as the protracted process whereby initiated cells undergo clonal expansion to form overt tumours. The mechanisms of clonal expansion are diverse, but include direct stimulation of cell proliferation, repeated cycles of cell damage and cell regeneration and release of cells from normal growth-controlling mechanisms. Initiating and promoting agents were originally regarded as separate categories, but the distinction between them is becoming increasingly hard to sustain. The various modes of promotion are non-genotoxic, but it is incorrect to conclude that 'non-genotoxic carcinogen' (qv) and 'promoter' are synonymous.

**Uncertainty factor:** Value used in extrapolation from experimental animals to man (assuming that man may be more sensitive) or from selected individuals to the general population: for example, a value applied to the NOAEL to derive an ADI or TDI. The value depends on the size and type of population to be protected and the quality of the toxicological information available.

**Unscheduled DNA Synthesis (UDS):** DNA synthesis that occurs at some stage in the cell cycle other than the S period (the normal or 'scheduled' DNA synthesis period), in response to DNA damage. It is usually associated with DNA repair.

**Volume of distribution:** Apparent volume of fluid required to contain the total amount of a substance in the body at the same concentration as that present in the plasma, assuming equilibrium has been attained.

**Xenobiotic:** A chemical foreign to the biologic system.

**Xenoestrogen:** A 'foreign' compound with estrogenic activity (see estrogen).

## ANNEX 5 – Index to subjects and substances considered in previous annual reports of the Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

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## ANNEX 6 – Previous Publications

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

1991 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321529 0 Price £9.50.

1992 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. HMSO ISBN 0 11 321604-1 Price £11.70.

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

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

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

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

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

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

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

2000 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Department of Health.\*

2001 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Food Standards Agency/Department of Health, FSA/0681/0802.\*\*

2002 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Food Standards Agency/Department of Health, FSA/0838/0803.\*\*

2003 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Food Standards Agency/Department of Health, FSA/0900/0504.\*\*

2004 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Food Standards Agency/Department of Health, FSA/0992/0804.\*\*

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

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

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

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

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

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

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

Guidance on a Strategy for testing of chemicals for Mutagenicity. Department of Health (2000)\*

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment: Risk Assessment of Mixtures of Pesticides and Similar Substances, Food Standards Agency, FSA/0691/0902 (2002).\*\*

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment: Phytoestrogens and Health, Food Standards Agency, FSA/0826/0503 (2002).\*\*

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

Guidance on a Strategy for the Risk Assessment of Chemical Carcinogens. Department of Health (2004)\*

\*Available on the COM website at <http://advisorybodies.doh.gov.uk/com/publications.htm>

\*\*Available on the COT archive at [http://archive.food.gov.uk/dept\\_health/archive/cot.htm](http://archive.food.gov.uk/dept_health/archive/cot.htm)

+Available on the COC website at <http://www.advisorybodies.doh.gov.uk/coc/publications.htm>

\*\*<http://www.food.gov.uk/science/ouradvisors/toxicity/reports/>



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