

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 2004

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Consumer Products
and the Environment

Annual Report
2004

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About the Committees

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

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

The year 2004 has seen a number of changes in the membership of the committees and these are shown in the membership lists at the end of each committee's report.

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

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

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

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

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

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

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

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

Preface



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 2004 the Committee has provided advice on a number of chemicals including phosphate, tryptophan, the flame retardant tetrabromobisphenol A, the enzyme newlase and the bulk sweetener erythritol. It also reviewed evidence for an association between chlorinated drinking water and reproductive outcomes, and possible chemical causes of adverse reactions to acid sweets, of adverse trends in the development of the male reproductive system and of the atypical response in the lipophilic shellfish toxin mouse bioassay. Urgent advice was provided on arsenic in seaweed, bromate in bottled water and the results of chemical analyses on breast milk.

Generic issues discussed included exposure assessment, approaches to risk assessment for mixtures of chemicals in food, toxicogenomics and nanotechnology.

In spring of 2005, I complete the first three-year term of my appointment as COT chairman. I am pleased to accept the opportunity to serve another term leading this important Committee.

Finally, I would like to add my sincere thanks and appreciation of the work of the administrative and scientific secretariats without out 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

Adverse reactions to acid sweets

- 1.1 In 2003 the Food Standards Agency (FSA) received a number of reports of adverse reactions in children eating sour acid sweets designed to be kept in prolonged contact with the tongue.
- 1.2 At present there are no regulatory limits for acidity in confectionery products and no restriction on their sale in the UK. The COT was asked to consider the available data on acid sweets and, in particular, whether it was the composition, pH or acidity, the method of use, or a combination of these factors, that was likely to be responsible for the adverse reactions.
- 1.3 The COT considered that the acute effects of acid sweets in the mouth appeared to be due to a combination of the acid content, method of application and duration of contact. While these effects were reversible, acid sweets could have the potential to contribute to long term irreversible damage to dental enamel.
- 1.4 The COT statement is included at the end of this report.

Adverse trends in the development of the male reproductive system – potential chemical causes

- 1.5 In February 2003 the COT was asked to consider whether it would be appropriate to review the available evidence for adverse trends in development of the male reproductive system and whether chemical exposure might be a possible contributory factor to such trends.
- 1.6 The Committee noted that extensive reviews had been carried internationally. To date these have not provided convincing evidence that exposure to reportedly endocrine disrupting chemicals has led to adverse effects on the male reproductive system. However, it is considered that there is sufficient evidence for effects on wildlife. The committee suggested that new approaches were needed for identifying possible causes of adverse trends in reproductive health. The possible causes to be considered would include lifestyle factors and other toxic effects not necessarily just those mediated by steroid hormone receptors. It was agreed that the area should be reviewed, but as much of this work was outside the COT's remit, it would best be considered as part of a scientific meeting held by a relevant society.
- 1.7 A draft statement was prepared during 2003, but not published because of the rapidly emerging new data. In 2004, further information was considered by the Committee including:
 - the possible role of the Y chromosome;
 - parallels between effects in wildlife and possible effect in humans, especially in relation to testicular dysgenesis syndrome, where similar effects can be reproduced by certain phthalates in animals;
 - the need for better exposure data especially in relation to the exposure of the fetal testes;
 - the prevalence of each of the reproductive disorders.

- 1.8 The final statement reiterates the need to review the evidence for adverse trends in human male reproductive health before considering possible causes and notes that much of this work is outside the COT's remit. However, it notes that the conclusions may need to be reviewed in the light of new data.
- 1.9 The COT statement is included at the end of this report.

Atypical results in the lipophilic shellfish toxin mouse bioassay

- 1.10 In the UK, the mouse bioassay (MBA) is used for statutory monitoring of shellfish for lipophilic toxins of algal origin. The MBA involves intraperitoneal injection of a shellfish extract and the assay is considered positive for the presence of lipophilic toxins if severe symptoms or death are observed in the animals.
- 1.11 The COT previously considered diarrhoetic shellfish toxins (DSP) in 1994 when it reviewed a report by the MAFF Working Party on Naturally Occurring Toxicants in Food. At that time, the COT recommended that the surveillance programme initiated by MAFF for detecting shellfish contaminated with DSP toxins be continued and that the MBA be used but that efforts be made to develop a more quantitative assay. The Agency, and before that, MAFF funded research into alternative testing methods.
- 1.12 Since June 2001, an atypical response has been observed in some MBAs after testing shellfish extracts, primarily cockle extracts, in the monitoring programmes in England, Wales and Northern Ireland. The Agency commissioned a toxicology study to investigate the nature of the atypical response and to provide information for an assessment of the possible public health implications of ingestion of cockles that have shown the atypical response in the MBA.
- 1.13 The preliminary findings, together with a plan for a more extensive acute toxicology study, were presented to COT on 2 Feb 2004. The Committee was asked to comment on its design and asked whether the proposed study would provide sufficient information to allow a risk assessment, whilst noting that the cockle extract was in very short supply.
- 1.14 COT indicated that, on its own, the proposed study would not provide sufficient information to allow a risk assessment of the possible public health implications of the atypical response in the MBA. This was because the causative agent(s) in the shellfish extracts was undefined and the validity of basing an assessment on an acute study would need to be justified, possibly by a short-term repeat dosing study. However, it was recognised that these issues could not be addressed at the present time due to the limited amount of cockle extract available.

Chlorinated drinking water and reproductive outcomes

- 1.15 The COT originally considered the issue of chlorinated drinking water and adverse reproductive outcomes in 1998 and produced a statement which was published in 1999. In May 2001 the COT was asked to consider the findings of a draft report of the first phase of a Government-funded epidemiological study conducted in England by the Small Area Health Statistics Unit (SAHSU), along

with additional relevant studies published since 1998. An updated statement was produced to be released on publication of the SAHSU study. However, in response to peer-reviewers comments, the study was not published but was reanalysed following remodelling of the exposure data, inclusion of additional years and revision of the links between postcodes and boundaries of water supply zones.

- 1.16 In 2004 the COT considered the results of the reanalyses together with additional relevant studies and reviews published since 1998. The second phase of the SAHSU study, which will include data on congenital abnormalities will be completed in 2005 and will be considered by the COT in due course. The current COT consideration therefore focused on adverse reproductive outcomes other than congenital abnormalities.
- 1.17 The SAHSU study showed small associations between total trihalomethane exposure and mean birth weight, prevalence of low and very low birth weight and prevalence of stillbirths in one water company area; however there was evidence of confounding by social deprivation, adjustment for which may not have been entirely successful in the analysis. The effect of social deprivation appeared to be much greater than the effect observed for trihalomethane exposure. In two other water company areas no associations were shown, other than a decreased prevalence of very low birthweight with increasing trihalomethane exposure in one area. Taking the results of the SAHSU study and all other studies together, the COT concluded that the data evaluated did not show a causal relationship between chlorinated drinking water and the adverse pregnancy outcomes considered, i.e. low or very low birthweight, stillbirth, spontaneous abortion, perinatal death, infant death, low Apgar score, infant's head circumference at birth, infant's body length, pre-term delivery, length of gestation, neonatal jaundice and neonatal hypothyroidism. However, the COT recommended further research, particularly prospective studies, to reduce uncertainties in the interpretation of reported associations between patterns of drinking water intake and the incidence of adverse reproductive outcomes.
- 1.18 The COT statement is included at the end of this report.

Erythritol

- 1.19 Erythritol is a polyol that has potential uses as a sweetener and as a bulking agent, thickener, binding agent, sequestrant, flavour enhancer and freezing point depressant in a variety of foods. Erythritol was considered by the Committee in 2003, as the manufacturers were seeking a Temporary National Authorisation in the UK to permit the use of erythritol in beverages at a level of 3.5%, as well as in foods. Other polyols are not authorised for use in beverages as excessive consumption causes laxative effects and intakes from beverages would potentially be much higher than from other food sources.
- 1.20 The COT concluded that the local concentration of erythritol in the gut was the critical factor regarding laxative effects rather than the intake on a per kg bodyweight basis. However, as the small and large intestine continue to grow until the age of around 10 years, younger children would be expected to be more susceptible to laxation from a given amount of erythritol. In order to determine a NOEL for erythritol, information on the sensitivity of young children to polyols would be required. It was suggested that it may be possible to obtain this information from post-market surveillance of foods and beverages (where authorised) containing erythritol or other polyols.

- 1.21 In 2004, the manufacturers submitted a protocol for a study of erythritol in young children. Whilst it would not be appropriate for the COT to approve a specific protocol, it was considered useful to discuss some of the generic issues raised. The COT concluded that the proposed study would not clarify whether erythritol would have a greater or lesser effect than other polyols and a comparative study would be needed. It was noted that if a study was conducted in a small group of healthy children concerns would remain that some children could be more susceptible. Furthermore, if the dose tested was the proposed use level it would not be possible to apply an uncertainty factor to allow for inter-individual variation.
- 1.22 However, it was considered that studies in young children would only gain ethical approval if any potential distress was minimal and if there was a potential benefit to children. It was agreed that the general issue of how to assess safety of substances in young children should be referred to the working group on Variability and Uncertainty in Toxicology (VUT). The COT reiterated its previous advice that information on the relative sensitivity of young children might be obtained from post-market surveillance of erythritol in countries where it is currently authorised.

Enzyme submission – Newlase analytical method to detect rhizoxin

- 1.23 Newlase is an enzyme preparation used for the inter-esterification of fats and as a digestive aid. Newlase has been reviewed by the COT on a number of occasions since 1994. In 2000 and 2002, the COT considered a revised analytical method to confirm the absence of the mycotoxin rhizoxin. The COT considered that the analytical method was suitably sensitive to detect rhizoxin, but had some remaining concerns, and the manufacturers were asked to provide data from additional analyses of batches of newlase, including the use of spiked samples to assess recovery.
- 1.24 In response to this request, the manufacturers of Newlase submitted two years of analytical data on different batches of Newlase that had been analysed for the presence of rhizoxin.
- 1.25 Variation in retention times were noted by the COT as were additional minor peaks, however, these were not in the same retention time region as rhizoxin and were not increased by spiking with rhizoxin and therefore were not likely to be rhizoxin. Since the peak had not been chemically identified, it was not possible to exclude the possibility of it being a breakdown product of rhizoxin but the COT considered this unlikely given that no rhizoxin had been detected in any of the batches.
- 1.26 The COT agreed that the samples had been analysed as they had previously recommended and the data provided indicated that the samples were assessed at the correct retention time and that the clean-up column had been eluted properly, addressing their previous concerns.
- 1.27 Members recommended that the company should continue to test samples from one in every four batches of Newlase and that if any rhizoxin was detected the affected batch should be discarded. Subject to this recommendation, Newlase was granted full clearance.

PAVA (Nonivamide) use as an incapacitant spray: consideration of an updated statement in the light of new data

- 1.28 PAVA (pelargonyl vanillylamide, also known as Novinamide) is the synthetic equivalent of capsaicin, the active ingredient of pepper. It is used as an incapacitant spray by police forces in other European countries and in the United States. In 2001 the Home Office asked for COT advice on the health effects of PAVA. At the time the Sussex Police Force was using a 0.3% PAVA containing spray as an alternative to CS spray.
- 1.29 In its 2002 statement the COT noted that limited data were available and highlighted certain gaps in the toxicological information. These included the need for a second *in-vivo* test for mutagenicity as recommended by the COM, data on possible skin sensitisation and studies to assess possible developmental or reproductive toxicity. In addition the COT recommended further monitoring of experience in use with special consideration being given to eye irritancy among contact lens wearers and effects on asthma sufferers and pregnant women.
- 1.30 Sussex Police commissioned additional studies to meet these requirements, and additional data were provided on experience-in-use of PAVA as a medicine for topical application.
- 1.31 The COT concluded that the available information, both from the toxicity data in experimental studies, and experience in use, indicated that the low exposures arising from the use of PAVA incapacitant spray would not be expected to be associated with any significant adverse health effects. However, the committee recommended that monitoring of experience in use should be continued.
- 1.32 The COT statement, incorporating the COM opinion of the new mutagenicity data, is included at the end of this report.

Phosphate and the calcium-parathyroid hormone axis

- 1.33 Phosphorus was one of the nutrients considered in the 2003 report of the Expert Group on Vitamins and Minerals (EVM). The EVM concluded that there were insufficient data to establish a Safe Upper Level but gave guidance on a level of supplemental phosphorus that would not be expected to result in any adverse effects. This guidance was based on both the occurrence of osmotic diarrhoea in human volunteer studies and the possible adverse effects of phosphorus on the parathyroid hormone (PTH)-calcium axis in subjects with hypovitaminosis D who would be vulnerable to the effects of hyperparathyroidism.
- 1.34 The COT was asked to consider data on phosphate, PTH, calcium balance and bone health in detail, in order to allow the Food Standards Agency to formulate appropriate consumer advice. This review included new data on epidemiology and on the regulation of serum phosphate levels, which were not available to the EVM.

- 1.35 The COT considered that the elevated PTH levels associated with supplemental phosphorus intakes reflected a short term adjustment to serum calcium levels and did not necessarily represent an adverse effect of phosphate on bone health. The long term effects of elevated PTH levels arising from high phosphate intakes were unknown. Low calcium and vitamin D intakes are known to have adverse effects on bone health; these effects may be exacerbated by high phosphate intakes but this was uncertain.
- 1.36 The COT statement is included at the end of this report.

Tetrabromobisphenol A

- 1.37 In 2003 the COT was asked to give an opinion on possible health risks from exposure to brominated fire retardants found in brown trout and eels from the Skerne-Tees river (see 2003 Annual Report). At the time the COT noted that it was important to consider other brominated fire retardants that might find their way into the food chain.
- 1.38 Tetrabromobisphenol A (TBBPA) can be used as an additive flame retardant in the manufacture of acrylonitrile-butadiene styrene resins, high impact polystyrene and phenolic resins. When used for this purpose TBBPA does not react with the other components of the polymer matrix and can leach out. TBBPA residues have been found in marine fish and shellfish and in the eggs of wild birds. TBBPA is one of the compounds included in the Food Standards Agency's programme of surveys. The COT was asked to review its toxicology and provide an assessment of a tolerable daily intake in order to be in a position to advise on the survey results when completed.
- 1.39 Published data on the toxicology of TBBPA are limited and it has not been evaluated by the EU Scientific Committee on Food (SCF) or the Joint FAO/WHO Expert Committee on Food Additives (JECFA). However, a draft risk assessment prepared for its evaluation under the EU Existing Substances Regulations Council Regulation (Council Regulation EC/793/93) provided much of the information on which the COT assessment was based.
- 1.40 The committee reviewed the data for TBBPA and concluded that there were no specific toxicological concerns. On the basis of results from a 90 day repeat dose study and a two-generation reproductive toxicology study the committee recommended a TDI of 1mg/kg bw/day.
- 1.41 The COT statement is included at the end of this report.

Tryptophan and the eosinophilia-myalgia syndrome

- 1.42 In 1990 the COT endorsed a ban on the addition of the isolated amino acid tryptophan to foods, including dietary supplements. This followed reports of a new epidemic illness in the USA, known as the eosinophilia-myalgia syndrome (EMS), which was associated with the consumption of L-tryptophan 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.

- 1.43 In 2003, the Food Standards Agency received a submission claiming that the cause of EMS was now known to be a contaminant in the tryptophan produced by one manufacturer and that there was no need for the continuing ban on the addition of tryptophan to foods. The COT was therefore invited to reconsider the issue.
- 1.44 On the basis of the available data, the COT concluded that the likely cause of the EMS outbreak was one or more contaminants in several batches of tryptophan produced by one manufacturer rather than tryptophan *per se*. However, some uncertainties remained, including whether EMS could also occur sporadically. It was also not possible to determine specific contaminants causal of EMS.
- 1.45 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 medicinal dose taken of 2228 mg/day and concluded that taking 220 mg/day tryptophan, produced to European Pharmacopoeia standards, as a dietary supplement would be unlikely to present a risk to health.
- 1.46 The COT statement is included at the end of this report.

Urgent advice provided by COT

Arsenic in seaweed

- 1.47 In 2004 the Food Standards Agency received the results of a survey of levels of total and inorganic arsenic in seaweed. One variety, hijiki, contained high levels of inorganic arsenic. Other varieties tested did not contain detectable levels of inorganic arsenic.
- 1.48 The COT has previously concluded that there are currently no relevant tolerable intake guidelines or reference doses for inorganic arsenic and recommended that exposure should be As Low As Reasonably Practicable (ALARP). An intake assessment by the FSA indicated that an 18 g portion of hijiki seaweed, providing 2.9 micrograms/kg bodyweight of inorganic arsenic, would increase the calculated average adult intake of inorganic arsenic by at least 20 times. The FSA consulted the Chairman of the COT, who agreed that consumption of hijiki had the potential to represent a significant increase in the intake of inorganic arsenic, and that applying the ALARP approach indicated a need for risk management to reduce this source of exposure. The FSA subsequently issued advice to avoid consumption of hijiki seaweed.
- 1.49 The COT advice is included at the end of this report.

Bromate in bottled water

- 1.50 The Food Standards Agency was informed by Coca Cola that levels of bromate up to 25 µg/L (with typical levels of 10-20 µg/L) had been found in batches of Dasani bottled water. This infringed the UK legal limit of 10 µg/L. The source of the excess bromate was identified as bromide present as an impurity in calcium chloride added to the water. Bromide is converted to bromate during ozonation of water.
- 1.51 Bromate is considered to be a genotoxic carcinogen and is categorised as “possibly carcinogenic to humans” by the International Agency for Research on Cancer (IARC). The FSA consulted with the Chairman of the COM, who confirmed that bromate was considered to be a genotoxic carcinogen and that as such exposure should be As Low As Reasonably Practicable (ALARP). He endorsed the FSA view that the addition of the contaminated calcium chloride had resulted in an unnecessary risk to consumers, and that the product should be removed from the food supply.

Toxicological evaluation of chemical analyses carried out as part of a pilot study for a breast milk archive

- 1.52 The Food Standards Agency received the results of pilot studies exploring alternative methods for the recruitment, collection, storage and management of an archive of breast milk samples. The study involved investigation of methods for recruitment, collection and storage of breast milk samples, and included analyses of a range of contaminants to test sample integrity and effects of storage. The report included analytical data on a range of environmental contaminants (dioxins, polychlorinated biphenyls, organochlorine pesticides, phthalates, heavy metals and other elements). The Committee was invited to advise on whether these data raised toxicological concerns.
- 1.53 The Committee reviewed estimates of potential intake of the contaminants by breast-fed infants, taking into account previous COT discussions on contaminants in infant foods, where data were available.
- 1.54 The COT statement is included at the end of this report.

Committee procedures and working groups

Horizon scanning

- 1.55 The Committee was informed of future agenda items and asked for suggestions for specific issues to be discussed, whether as routine agenda items, topics for one-day open meetings, or generic issues requiring the setting up of a new working group.

1.56 Issues suggested included:

- interactions between diet and pharmaceutical agents,
- effects of diet regimes,
- the relation of plant sterols and stanols to vascular disease,
- potential risks associated with the increasing use of nanomaterials,
- safety of disinfection by-products that may be left as residues on food.

1.57 It was noted that some of these topics would require cross-committee views in order to deal adequately with the relevant issues. 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

1.58 The working group held an open symposium in February 2004 in Cardiff. The programme included presentations from working group members and invited experts, and a panel discussion.

1.59 Two other working group meetings were held during 2004. A draft report will be published for consultation in summer 2005.

Lowermoor subgroup

1.60 The Lowermoor subgroup held six further meetings in 2004. A draft report will be published for consultation in January 2005.

Risk assessment strategies

Mixtures of food contaminants and additives

1.61 The COT's 2002 report on Risk Assessment of Mixtures of Pesticides and Similar Substances considered the risk assessment of multiple residues of pesticides and veterinary medicines in food, and of multiple sources of exposure to these substances. This was at the request of the Food Standards Agency in response to consumer concerns about mixtures of pesticides in food. Similar concerns had been noted regarding mixtures of chemical contaminants and/or additives in food.

- 1.62 Several approaches are currently used by expert committees, including the COT, in performing risk assessments for mixtures of food additives or contaminants. Toxic equivalency factors (TEFs) and toxic equivalents (TEQs) are used for the dioxins and dioxin-like PCBs, which have common structural, toxicological and biochemical properties. Toxicity of mixtures may be assessed on the basis of the most toxic component of the mixture or of a marker compound. Group ADIs may be set for additives with similar structure or toxicity or common metabolites.
- 1.63 The different types of possible combined actions of component mixtures (e.g. those with and those without a similar mechanism of action) were based on the definitions of Cassee *et al.* In: General and Applied Toxicology edited by Ballantyne B, Marrs TC, Syversen T. London, Macmillan Reference Limited, 1999).

Concept	Term used in report	Synonym(s)	Effects observed
Non-interaction	Simple similar action	Simple joint action	Concentration/dose addition
	Simple dissimilar action	Simple independent action, independent joint action	Effect/response addition
Interaction	Potentialiation	Synergy, supra-additivity	Greater than additive effect
	Antagonism	Sub-additivity	Less than additive effect

- 1.64 The COT was asked if some conclusions of the 2002 report were also applicable to mixtures of food contaminants and additives, and on the adequacy of the current approaches used.
- 1.65 It was noted that the 2002 report concentrated on two groups of chemicals that were toxic at relatively low doses. Additives and other food chemicals may be of relatively low toxicity and levels of intake could potentially be much higher. This introduced the possibility that toxicokinetic interactions may occur and that high intakes may saturate metabolic enzymes, receptors or transport mechanisms leading to unpredicted effects.
- 1.66 It was noted that allocation of TEFs and assumption of additivity for dioxin-like compounds involves a pragmatic approach because of the limitations in the scientific basis. A Member noted that synergistic effects had been documented at low, non-toxic, concentrations, for example for furafylline and naringenin in combination with other chemicals. The 2002 report found little or no evidence of interactions at levels below those at which individual components produced effects. However, the implications of toxicokinetic interactions, such as blocking of metabolic pathways, had not been extensively investigated at low levels of exposure.
- 1.67 Where groups of chemicals with similar mechanisms of action had been studied, additivity (or less than additivity) had been demonstrated rather than synergy. Examples of such groups were endocrine disrupters that acted by agonism of oestrogen receptors, dioxin-like compounds and organophosphates. It was noted that many compounds bind to the aryl hydrocarbon (Ah) receptor but not all are agonists or antagonists. However, if partial agonism were of concern then making the assumption that the effect was additive would make the risk assessment over-protective rather than under-protective.

- 1.68 A number of interactions are known for nutrients. For example, calcium and magnesium compete for absorption. Antioxidants had been demonstrated to induce metabolic enzymes at intakes below the NOAEL.
- 1.69 Members agreed the conclusions from the 2002 report on Risk Assessment of Mixtures of Pesticides and Similar Substances, with some modification to allow for the possibility that exposure to some food additives and ingredients of very low toxicity may be much higher than exposure to pesticides and veterinary medicines from food. The following conclusions are generally applicable:
- i. Because of the complexity and variability of chemical mixtures that may occur in the environment, risk assessment of any toxic effects of chemical mixtures is extremely difficult. Most experimental work has been directed at toxic effects due to combined actions on biological systems at relatively high levels of exposure in laboratory experiments in laboratory animals or using *in vitro* systems.
 - ii. Direct chemical reactions can occur between the components of a mixture: there are relatively few studies of these substances that have investigated such reactions.
 - iii. Several studies claim to have identified synergistic interactions of some mixtures. However, for the most part, these studies have been inadequately designed and based on an incomplete understanding of the concepts involved, but a few well-designed studies have demonstrated the occurrence of both synergistic and antagonistic interactions, as well as additive effects in mixtures. These effects have usually been demonstrated at high concentrations or high experimental exposure levels, which are probably unrepresentative of exposure doses to chemicals present at very low levels in food.
 - iv. Some interactions may not be easy to predict, such as those that may occur at the transcriptional level of the genome or second messenger signalling pathways.
 - v. The type of combined action or interaction found at clearly toxic effect levels may not predict what will happen at non-toxic levels, including levels only slightly lower than the lowest observed adverse effect levels (LOAELs).
 - vi. In relation to most examples of possible human exposure to multiple residues, it will be important critically to evaluate whether any effects are likely to occur at low levels of exposure, such as those that will occur through food and water.
 - vii. Studies *in vivo* with chemicals that exhibit the same mode of action in the same target organ have shown that the effects of mixtures of similarly acting toxicants show additivity (dose addition), which results from simple similar action. This is the case, over the whole dose range.

- viii. It is essential to know what happens at non-toxic levels, including exposure levels just below the LOAEL, in order to assess the health risk for humans exposed to mixtures of pesticides, veterinary drugs and similar substances. Generally, when exposure levels of the chemicals within a mixture are in the range of the NOAELs, and the components of the mixture have different modes of toxic action, no additivity and no potentiating interactions are found, indicating the applicability of the basic concept of “simple dissimilar action,” which suggests that adverse reactions would be unlikely.
- ix. Some studies (acute and subacute toxicity, genetic toxicity, carcinogenicity) have addressed the combined effect of mixtures of pesticides and in a few studies clear cases of potentiation were observed in animals exposed to levels of toxic substances showing adverse effects of individual compounds. However, direct extrapolation of these findings to much lower dose levels is not valid. Thus the probability of any health hazard due to additivity or potentiating interaction of mixtures of pesticides at (low) non-toxic doses of the individual chemicals is likely to be small, since the dose of pesticides to which humans are exposed is generally much lower than the NOAEL, at least through food.
- x. Some endpoints that have been studied in animals or in *in vitro* systems are relevant to groups in the population believed to be at higher risk than the general population. Such endpoints include developmental toxicity studies, endocrine and neurotoxic effects and genotoxicity studies. On the basis of limited information it seems likely that the default assumptions in relation to mixtures in children and pregnant and nursing mothers, would be the same as for the rest of the population.

- 1.70 It was noted that prioritisation of chemical mixtures for exposure evaluation and COT consideration could be based upon consideration of whether chemicals acted via common mechanisms or on common target organs.
- 1.71 Members noted the limited information on which to draw conclusions about the effects of mixtures in potentially vulnerable populations such as children and pregnant and nursing mothers. Deficiency conditions may predispose some vulnerable subgroups to adverse effects. The absence of suitable information on vulnerable groups such as children and the elderly or pregnant women was considered an area that might warrant further work in the form of observational studies. However, it was stressed that there was a need for a relevant experimental model for such studies.

RCEP study on pesticides and bystander exposure

- 1.72 In August 2004, the Royal Commission on Environmental Pollution (RCEP) announced a new study on pesticides and bystander exposure. The COT was invited to comment on the intended scope and questions to be addressed. The study would examine the scientific evidence on which DEFRA had based its decision on bystander exposure and its policy on access to information on crop spraying. It had been requested by the Rt Hon Alun Michael MP, Minister of Rural Affairs, in response to claims by campaigners that spray drift from pesticides has affected the health of people living in rural communities.

- 1.73 The COT considered that the scope of the study provided was very brief, and it was not clear whether it would be considering new data or re-examining and considering the reliability of the data that had been used to form DEFRA's decision. Additional information may be available via GP-based systems, but the health outcomes of interest were not clear. As the study would consider wider aspects of risk, uncertainty and communication, it was noted that the COT working group on uncertainty and variability in toxicology would be relevant to some of these aspects.
- 1.74 There was a need for consideration of perception of risk to include consideration of perception of exposure. People may smell solvents or non-active components of pesticide formulations and believe they are being exposed to the active pesticide with risk of adverse health consequences.
- 1.75 Members were surprised that the study was only focusing on bystander exposure from field spraying and not including other types of bystander exposure such as the spraying of pavements, golf courses and verges, which could involve a much larger sector of the population. It was considered that spraying by local authorities may be less able to take account of weather conditions than spraying on farms. Members recommended that the study should be set in the context of other sources of pesticide exposure to the public and to workers.
- 1.76 The RCEP was informed of the COT comments, and that the draft COT report on variability and uncertainty in toxicology will be issued in the summer of 2005.

Reassessment of the toxicological testing of tobacco

- 1.77 The Department of Health asked the COT, COC and COM for advice in preparing a statement to submit to the European Commission on the application of Directive 2001/37/EC of the European Parliament and of the Council of June 5 2001 on the approximation of the laws, regulations and administrative provisions of the Member States concerning the manufacture, presentation and sale of tobacco products. This will contribute to the Commission's first biennial report to the Council and Economic and Social Committee of the European Parliament.
- 1.78 The request focused on strategies that have been used to assess tobacco products and in particular tobacco-based Potentially Reduced Exposure Products (PREPs). This is one of the requirements listed in the Directive ("evaluation of tobacco products which may have the potential to reduce harm"). Specifically expert committee advice was requested on:
- Methods for more realistically assessing and regulating toxic exposure and harm.
 - Toxicological data to be required from manufacturers on ingredients and the manner in which they should be tested in order to allow public health authorities to assess their use.
- 1.79 The COT was asked to consider:
- the application of *in-vitro* cytotoxicity assays;

- the assessment of markers for chronic diseases other than cancer associated with tobacco use such as chronic obstructive pulmonary disease;
- generic aspects of testing tobacco products using inhalation studies in experimental animals;
- behavioural aspects of tobacco use as assessed by biomonitoring studies;
- smoke chemistry as a predictor of toxicological effects.

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

Royal Society study on nanoscience and nanotechnology

1.81 During the February 2004 COT discussion on horizon scanning nanotechnology had been identified as an emerging issue for which there was a potential for future discussion.

1.82 The COT was subsequently invited to comment on the Royal Society report, “Nanoscience and Nanotechnologies: Opportunities and Uncertainties.” The report highlighted the need for research to address uncertainties about the health and environmental effects of nanoparticles, one small area of nanotechnologies. It also made recommendations about regulation to control exposure to nanoparticles.

1.83 Members noted that nanoscience and nanotechnology is a new and rapidly emerging technology and as such there were divergent views on the potential for human health risks. Whilst most reports focussed on risks associated with inhalation exposure, theoretical concerns had also been raised about possible risks associated with oral or dermal exposure. The COT agreed that there is a need for critical evaluation of the basis for these concerns and development of evidence to support conclusions. There are many diverse applications of nanotechnology, and it should not be assumed that any risks would be generic to all of them.

1.84 The report raised theoretical concerns that nanotechnology was capable of changing the toxicity of chemicals although almost no studies had been identified in the report providing conclusive evidence for this. However, COT noted that recent research had demonstrated altered toxicity in specific situations, and also shown the potential for transplacental transfer of nanoparticles.

1.85 The COT agreed that future discussions of specific applications of nanotechnology would be welcomed.

1.86 The report recommended that all relevant data related to safety assessments should be placed in the public domain. Members considered that procedures for handling commercially sensitive material might be necessary to ensure that such information is not withheld from discussion, as this would inhibit comprehensive safety evaluation.

- 1.87 Members welcomed the Royal Society proposal for an interdisciplinary research centre and noted they would be willing to advise on the types of research needed as the centre develops. Members asked to be kept informed of the research centre and also other initiatives, such as those of the Health and Environment Sciences Institute of the International Life Sciences Institute and in the USA.

Uncertainty in chemical exposure assessment

- 1.88 The COT commented on recent work by the Food Standards Agency on reducing the uncertainty in exposure assessment, and which identified further areas that may need to be researched. The COT had previously commented on a draft FSA best practice guide for food chemical exposure assessment and on draft guidelines for exposure assessment practice for human health effects of chemicals produced by the Interdepartmental Group on Health Risks from Chemicals (IGHRC).
- 1.89 The COT welcomed the FSA consideration of uncertainty in exposure assessment and noted that the more complex techniques, such as probabilistic modelling, were being incorporated. This would involve a tiered approach, starting with a basic exposure assessment and moving towards more refined and complex methodology as appropriate.
- 1.90 Members noted that the comparison of energy expenditure with energy intake undertaken as part of feasibility work for the most recent National Diet and Nutrition Survey (NDNS) of adults, indicated that energy intakes were underestimated by around 25% on average. However, it was not possible to distinguish between under-reporting of actual consumption and people changing their dietary habits when they are surveyed, nor to assess under-reporting of specific foods or nutrients. It has not proved possible to address this problem with correction factors but members suggested the use of nutritional biomarkers could be appropriate. The COT questioned the assumption that under-reporting of food intakes is one of the most important sources of uncertainty in chemical exposure assessments and suggested that major sources of uncertainty could be identified by sensitivity analysis.
- 1.91 The COT agreed that using the intake at the 97.5th percentile to assess high level intake would cover most consumers where intakes were normally distributed; however, there were often specific subgroups of the population with higher intakes than most of the population, meaning the exposure distribution was skewed. Surveys could be designed to over-sample sub-populations with higher exposures to ensure that sufficient numbers of high levels consumers were surveyed.
- 1.92 The COT agreed that the FSA continued to make progress in ensuring realistic estimation of dietary exposure to chemicals. Current deterministic approaches to exposure assessment were more likely to over-estimate than under-estimate chemical exposures. However, improved communication of issues and specifying confidence intervals in future deterministic assessments would be helpful in this respect.
- 1.93 Members noted there was little quantitative consumption data for those below the age of 1½. Some dietary data for younger children has been provided by the Avon Longitudinal Study of Parents and Children (ALSPAC), but the FSA may need to perform its own survey of infants and younger children.

- 1.94 The COT considered that the FSA should assess whether the NDNS could be tailored towards reducing uncertainty in chemical exposure assessment, for example by ensuring that sample sizes for at-risk sub-groups were sufficient.

Use of toxicogenomics in toxicology (update on statement published in 2002)

- 1.95 In 2001 the COT/COC/COM held a joint symposium on the use of genomics and proteomics in toxicology. The committees issued a joint statement which considered that although these techniques might in some cases serve as a useful adjunct in toxicological risk assessment, further validation was needed before they could be used in regulatory risk assessments. The statement is available at <http://www.food.gov.uk/science/ouradvisors/toxicity/statements/cotstatements2002/jointstatement>.
- 1.96 Following on from this meeting the committees have been provided with annual updates of literature on this topic. In February 2004, the committees agreed that considerable progress had been made, and it would therefore be appropriate to update the joint statement. The COT noted the importance of considering genomics, proteomics and metabonomics as a single package rather than as individual packages. It also agreed that toxicogenomics might be used as part of a screening process for toxicological mechanisms and their investigation. However, members still considered it premature for such data to be used in risk assessment.
- 1.97 Data generated using these techniques usually require sophisticated statistical interpretation and the committee requested more information on the statistical methods used in the analysis and interpretation of toxicogenomic studies. This was provided by Dr David Lovell of the University of Surrey, who attended the September COT meeting to provide an overview of the use of statistics and bioinformatics in toxicogenomics. In addition, members were provided with an update on toxicogenomic and related literature produced since the meeting in February 2004. Dr Lovell noted that the use of statistical and bioinformatic techniques not only related to data analysis but also to the way in which studies were carried out. He highlighted some of the pitfalls and problems of study design and data analysis and their interpretation. Study design has a major impact on the predicted reliability of the data produced. The need to be able to assess the difference between background changes and possible adverse effects was often not well defined and further work was needed to provide validation of changes in individual genes.
- 1.98 An updated statement was produced incorporating the new data and information on bioinformatics, an update of information reviewed by the COM and COC, and minor amendments to the previous conclusions.

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

Ongoing work

Joint meeting with the Committee on Safety of Medicines on food-drug interactions

- 1.100 Food-drug interactions was one of the issues raised during the horizon scanning discussion in February 2004. The potential for interactions of dietary components and medicines has been known for some years but is increasing in prominence following the observation of interactions such as cranberry and warfarin and grapefruit and CYP 3A4 enzymes which are involved in the metabolism of a wide range of pharmaceuticals including statins, cyclosporin and oestrogens.
- 1.101 It was agreed that, in order to fully consider all aspects of this subject, it would be appropriate to consider the issue of diet-drug interaction at an open meeting to be held jointly with the Committee on the Safety of Medicines in February 2005.

Statements of the COT

Adverse reactions to acid sweets

Adverse trends in the development of the male reproductive system – potential chemical causes

Chlorinated drinking water and reproductive outcomes

PAVA (Nonivamide) use as an incapacitant spray

Phosphate and the calcium-parathyroid hormone axis

Tetrabromobisphenol A – review of toxicological data

Tryptophan and the eosinophilia-myalgia syndrome

Toxicological evaluation of chemical analyses carried out as part of a pilot study for a breast milk archive

Joint COT/COM/COC statements

Reassessment of the toxicological testing of tobacco

Use of toxicogenomics in toxicology (update on statement published in 2002)

Adverse reactions to acid sweets

Introduction

1. Since April 2003 the Food Standards Agency (FSA) has received a number of reports of adverse reactions in children eating sour acid sweets designed to be kept in prolonged contact with the tongue. Some reactions have been sufficiently severe for the children to attend hospital. At present there are no regulatory limits for acidity of confectionery products and no restriction on their sale in the UK. The COT was asked to consider the available data on acid sweets and whether it was the composition, pH or acidity, the method of use, or a combination of these factors, that was likely to be responsible for the adverse reactions.

Background

Acid Sweets

2. An increasing number of acid sweet products are available. These sweets are being manufactured by different countries, with some produced by UK manufacturers. Those reported to cause adverse reactions include both liquid and solid sweets. King Regal “Roll on King” (liquid sour candy), is part toy, part sweet and consists of syrup which is contained in a “roll on” bottle (50 ml) similar to deodorant container. These sweets are designed to be rolled on to the tongue, thus dispensing the sour candy directly onto the tongue. Public analyst reports have indicated that the pH of King Regal “Roll on King” was 2.14-2.26 and titratable acidity* expressed as citric acid was 6.3-6.7%. Brain Licker is a similar “roll on” product (60 ml). The pH of these products was reported to be 1.8-2.0 and titratable acidity as citric acid was 1.98-5%.
3. “Toxic Waste” is a solid sweet available in novelty drum containers. These sweets have an extremely sour soft centre with a hard outer coating. According to public analyst reports, some of the Toxic Waste sweets had a coating containing high levels of titratable acidity (39.8-64.2% as citric acid). When dissolved in water, the pH of the sweets was 2.2-2.7 and titratable acidity as citric acid 9.1-20.3%, calculated as a proportion of the whole sweet. There is a warning that Toxic Waste may cause temporary irritation to sensitive mouths, but consumers are encouraged to keep the sweets in their mouths for as long as possible to “show how tough they are.”
4. Complete compositional data are not available for acid sweets. According to the label, they contain sugars, acids, colours and preservatives. Of these the acids are most likely to be a contributing factor in the reported adverse effects. All of the products concerned contain citric, malic or lactic acid. Citric acid is the most commonly used acid.

* Total dissociated plus undissociated acid measured by titration. When referring to a mixture of acids it is expressed as the acid assumed to be predominant.

Acids in foods

5. Acids are commonly used in the food industry to control pH, preserve food, chelate metal ions and enhance flavour. Organic acids are used in sugar confectionery to offset the general sweetness of sugar and to add flavour. There is a trend for use of a combination of two or more acids in formulated food products¹.
6. Citric acid occurs naturally in plants and animals as an intermediary substance in oxidative metabolism, being a component of the tricarboxylic acid cycle. Malic acid is also one of the steps in the tricarboxylic acid cycle and plays a role in cellular energy production. Lactic acid is a principal metabolic intermediate in glycogen breakdown and other metabolic processes in most living organisms and occurs naturally in small quantities in the body. Citric acid is found in the greatest amounts in citrus fruits. Malic acid is the principal acid contained in apples, apricots, peaches, plums and many other fruits and vegetables. Lactic acid is used in food and food applications as an acidifier, antimicrobial agent, curing agent, pH control agent and flavour enhancer and is present in many foods, both naturally or as product of microbial fermentation.
7. Citric acid anhydrous and monohydrate (E330), malic acid (E296) and lactic acid (E270) and their salts are permitted food additives in the European Union. Conditions of use are “*quantum satis*” which means that no maximum levels are set, but the additive must be used in the food concerned in accordance with good manufacturing practice and must not be used at a level higher than is necessary to achieve intended purpose. These acids are also listed as GRAS (Generally Recognised as Safe) in the USA, where maximum concentrations are specified for malic acid in foods as served: hard candy 6.9%; processed fruits and fruit juices 3.5%; non-alcoholic beverages 3.4%; soft candy 3.0%; jams and jellies 2.6%; 0.7% in all other foods when used in accordance with good manufacturing practice.

Toxicological considerations

Acidity

8. Organic acids are weak acids, in which the hydrogen ions are only partially dissociated from the base. In the mouth, the fully dissociated hydrogen ions of a strong acid would be diluted by saliva, thereby raising the pH. In contrast, dilution of weak acids is likely to progressively release hydrogen ions, prolonging the low pH.

Irritation and sensitisation

9. Citric acid appears to be the most potent irritant of the three acids, although there are no direct comparative data. Eye irritation has been reported in the rat at 0.5% and 2% citric acid². Neat malic acid caused severe ocular irritation in rabbits and was moderately irritating to rabbit skin³. Eye irritation has been reported in the rat at 10 – 20% lactic acid^{2, 4}. Skin irritation has also been reported with application of neat lactic acid and slight skin irritation with occluded application of a 20% solution⁴

10. Skin irritation has been reported in humans at 3% citric acid⁵. Some studies have reported individual differences in the perception of a stinging sensation when 10% lactic acid solution was applied to the skin⁶.
11. In a single report, citric and malic acid have been reported to cause skin sensitisation, as demonstrated by a reaction in 34 patients with atopic dermatitis who were patch tested with a 10% aqueous solution of the acids for 48 hours. Ten patients reacted to citric acid, six reacted to malic acid and 18 reacted to both. Rashes that were either generalised or on the extremities were observed 48 hours after challenge⁷. The test subjects appeared to be a highly selected group with respect to the severity and persistence of their symptoms. None of the controls, whether atopic or non-atopic reacted to citric or malic acid, suggesting that a sensitive subgroup exists.

Effects on dental enamel

12. Data from case studies suggest that ingestion of citric acid frequently or in large doses may cause erosion of teeth and local irritation, probably because of the low pH⁸. Tooth erosion is common in the UK: 50% of 5 year olds had evidence of tooth erosion in 1993¹⁰. However there are no data available on the extent to which citric acid may contribute to this prevalence.
13. Data from *in-vitro* studies indicate that the rate of enamel erosion associated with consumption of soft drinks, increases with decreasing pH for all acids⁹. Enamel erosion in the presence of citric, malic and lactic acids has been reported and factors such as exposure time, acid concentration, pH and presence of calcium have been shown to affect rate of dental erosion^{11, 12, 13}.

Systemic effects

14. Toxicity of citric, malic and lactic acid has only been demonstrated at very high doses. Signs of acute toxicity in rats following ingestion of 11.7 g/kg body weight citric acid include motor ataxia, mydriasis (abnormal pupil dilation) and decreased rate of respiration and heart beat¹⁴.
15. Mice and rats dosed with a single administration of a 25% malic acid solution showed signs of toxicity including ataxia, prostration and convulsions¹⁵. The World Health Organisation (WHO) stated that the oral lethal dose of L-malic acid for rabbits was 5 g/kg and for sodium malate in dogs was 1g/kg. The signs of acute poisoning in rats and mice were weakness, retraction of the abdomen, respiratory distress and cyanosis¹⁶.
16. In rats given doses of lactic acid up to 1.3 g/kg body weight by gastric gavage, signs of toxicity included difficulty breathing, runny nose and abdominal inflation immediately after dosing¹⁷.
17. The Joint FAO/WHO Expert Committee of Food Additives (JECFA) evaluated the safety of citric, malic and lactic acid and their calcium, potassium, sodium and ammonium salts¹⁸. Taking into account the well established metabolic pathways, JECFA concluded that there was no significant toxicological

hazard posed by these compounds, hence no limits were set for their acceptable daily intake (ADI). However, a conditional ADI of 0-100 mg/kg body weight was recommended for D(-) and DL-malic acid and for D(-) and DL-lactic acid.

COT Evaluation

18. A number of factors may contribute to the reported acute effects of acid sweets on the oral mucosa. These include pH, total acid content, method of delivery direct onto the tongue and duration of contact. Conventional sweets such as acid drops are of low pH but stimulate salivary flow which reduces viscosity and raises the pH in the mouth. The liquid acid sweets are more viscous and are expected to inhibit salivary flow, which would reduce the buffering capacity of the saliva, thus further lowering the pH in the mouth.
19. It is possible that there is a subgroup with sensitivity to citric and malic acids. The available information indicates that allergic responses are rare and required challenge with very high concentrations of the acid. The reported allergic responses were systemic, producing reactions at sites distant from the site of patch-testing. In contrast, the reported adverse reactions to acid sweets were local effects, and are therefore more likely to have been irritant rather than allergic responses. However, the mode of application of the acid sweets and the irritancy of the acids could potentially act as an adjuvant, i.e. enhancing the sensitising effects of other ingredients in the sweets.
20. Damage to the oral mucosa was unpleasant but reversible, whereas damage to teeth would be permanent. As yet there was no evidence of a link between dental erosion and consumption of acid sweets. However there is evidence that high consumption of products such as cola beverages with a pH of 2.8 could be associated with dental erosion. Since the new acid sweets had a much lower pH it was likely that they could also lead to dental erosion. Concern was also expressed about possible long term health implications of repeated exposure.
21. Based on the case reports, a pH of 2.5 might be considered as a level below which acute effects would be expected. However, the variability of the product in terms of manufacturing was unknown, the reported pH of 2.5 could not be taken as representative. There could be difficulties in measuring low pH accurately, which could result in significant inter-laboratory variability making interpretation of results difficult and inconsistent. Therefore the reliability of this value was uncertain.
22. Because the effects were related to a combination of factors it would be difficult to set a level at which an individual might adversely react based on pH alone. It might be possible to investigate key characteristics in determining a response in a human volunteer study, which would need to be conducted in adults for ethical reasons. The mode of application would be difficult to replicate in animals. Some *in-vitro* tests for skin corrosion, such as those used for industrial chemicals, might provide a benchmark against materials already classified as corrosive. However, the issue was complex, with several variables being involved, i.e. acidity, pH and buffering capacity; even exposure to pH 4 could be a problem if individuals were exposed to it for a prolonged period.

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23. Safe product design is the responsibility of the manufacturer. If products are shown to be harmful, the relevant regulatory authorities could take action under the appropriate legislation.

Conclusions

24. We *conclude* that the acute effects of acid sweets in the mouth appear to be attributable to a combination of the acid content, the method of application and duration of contact.
25. We *consider* that whilst the acute effects are reversible, acid sweets may also have the potential to contribute to long term irreversible damage to dental enamel. There is also uncertainty with respect to the long term implications of repeated exposure on the oral mucosa.
26. We *recommend* that the manufacturers and distributors should be required to provide evidence of the safety of acid sweets to the relevant regulatory authorities.

COT statement 2004/05

August 2004

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Adverse trends in development of the male reproductive system – potential chemical causes

Introduction

1. In February 2003, the COT considered a number of possible discussion topics as part of its annual horizon scanning exercise. One of the topics considered was whether the Committee should contribute to ongoing debate of reported adverse trends in development of the male reproductive system and the possibility of chemicals being responsible for these reported effects. This statement summarises information reviewed by the Committee over a number of subsequent discussions.
2. Over the past 10-12 years an increasing body of literature has suggested there may have been a deterioration in human male reproductive health^{1,2,3}. Examples of reported changes include a decline in sperm counts and other aspects of semen quality such as sperm morphology and motility, in some places but not others,^{4,5,6,7,8,9,10} a widespread and marked increased incidence of testicular cancer¹¹, and possible increases in hypospadias^{12,13} and cryptorchidism¹⁴. Cryptorchidism is the most common developmental abnormality occurring in 2.2- 3.8% boys at birth¹⁵ whereas the prevalence of hypospadias is between 1 in 125 live births and 1 in 250 new borns (0.4-0.8%)¹⁶, and testicular cancer ranges from 0.0025% – 0.0085%¹⁷. Geographical and ethnic differences have also been reported in these conditions^{3,11,18,19,20,21}. One consistent finding is that the reproductive health of Finnish men tends to be healthier than that of Danish or British³. For instance using time to pregnancy as an indicator, Britain is reported to have lower fertility than Finland²². It is now recognised that some of the most important disorders of human male reproductive health may be caused or influenced by events in perinatal life. In addition over the last few decades, a decline in male to female sex ratios has been reported in Denmark, the Netherlands, England, Wales, the USA and Canada^{23,24,25}.
3. Potential chemical causes of cancer fall within the remit of the Committee on Carcinogenicity (COC). However, the existence of parallel findings for the risk of testicular cancer with that of impaired semen quality, hypospadias and cryptorchidism, e.g. in Finland compared with Denmark, suggest linkage between these endpoints³, and therefore testicular cancer was included in the COT consideration.
4. One hypothesis that has attracted much attention in recent years is that these observations on male reproductive health could be due to the effects of natural and synthetic endocrine disrupting chemicals (EDCs). The literature has been discussed extensively at meetings worldwide and in reports, including those of the Danish Environmental Protection Agency²⁶, the EU Weybridge workshop²⁷, the Royal Society²⁸, an expert panel of the International Programme on Chemical Safety (IPCS)²⁹, and an International Symposium convened by IUPAC/SCOPE³⁰.
5. Most initial reports of possible adverse effects of EDCs focused on effects in wildlife. The IPCS²⁹ report states that there is “strong evidence that there are effects observed in wildlife that can be attributed to substances that function as EDCs”. Some examples are briefly highlighted below (paragraphs 9-12).

6. While a range of chemicals (both natural and man-made) have been shown to possess the potential to interfere with endocrine systems in experimental systems, it is now recognised that in most cases their potency and level of exposure is too low to have had any effect in humans, especially in the case of “environmental oestrogens” such as alkylphenolpolyethoxylates and bisphenol A^{31,32}. The potent oestrogen diethylstilboestrol (DES) has been recognised as an endocrine disrupting chemical with established effects on male reproductive development in humans, and this occurred only when it was given at pharmacological doses of more than 2000 µg/kg/day during early pregnancy; however no effect was reported at lower doses³².
7. Endocrine disruptors other than oestrogens could affect the male reproductive system, in particular those that inhibit the synthesis or the action of testosterone, such as phthalates or DDE (the stable breakdown product of DDT), respectively³¹. In addition they may act as anti-androgens to affect the androgen-oestrogen balance; studies in male rats have suggested the induction of reproductive tract abnormalities from the alteration of the androgen-oestrogen balance rather than from the absolute level of exposure to androgens or oestrogens³¹. Compounds may also interfere with the biosynthesis of oestrogen by suppressing the activity of aromatase, required for the conversion of testosterone to oestrogen. This may result in an excess of testosterone and hence could disturb the androgen-oestrogen balance. Such alterations to the production or action of oestrogen may lead to abnormal development of Sertoli and germ cells in the fetal testis, as these are sites containing oestrogen receptors³¹.
8. However, the beginning of the rise in testicular cancer incidence predates the introduction of such chemicals, and the widespread use of DDT in malaria control programmes has not resulted in an epidemic of this disease³. Another possibility requiring further evaluation is whether exposure to dioxins, and/or to other substances that have a similar activity, could have adversely affected the male reproductive system³³. However, as dietary exposure to dioxins has been decreasing for over 20 years³⁴ it is not likely to be responsible for the postulated increase in congenital abnormalities such as cryptorchidism and hypospadias.

Wildlife

9. It has been suggested that the abnormally small gonads and significantly depressed testosterone concentrations observed in juvenile male alligators from Lake Apopka, Florida could have been caused by contamination of the lake with various chemicals including the pesticide DDT and its metabolite DDE^{34,35}. This effect may explain the decline in the number of juvenile alligators in this area, whereas alligator populations elsewhere were increasing or stable at the same time.

10. Begeron *et al.*^{37,38} demonstrated that a number of polychlorinated biphenyl (PCB) metabolites are capable of acting as synthetic estrogens. Some reptiles such as crocodilians and turtles exhibit environmental sex determination so that the temperature at which the egg is incubated determines the sex of the offspring³⁹. Turtle eggs incubated at 26°C produce 100% males. However, if eggs incubated at the male producing temperature were “painted” with either one of the PCB metabolites (2,4,6-trichloro-4-biphenylol or 2,3,4,5-tetrachloro-4-biphenylol) sex reversal occurred as if the eggs had been treated with the natural estrogen, estradiol-17 β ³⁷.
11. The observation of an increased prevalence of hermaphroditism in fish from sewage treatment water (STW) lagoons in England and Wales initiated a series of studies examining the effects of environmental estrogens in STW-exposed rainbow trout. This was done using an assay measuring vitellogenin production. Vitellogenin produced in the liver of female fish stimulates the growth of ova and is under estrogenic control. In contrast, males do not produce vitellogenin unless exposed to supra-physiological levels of estrogens⁴⁰, which can therefore be used as a biomarker for environmental estrogenic activity. STW-exposed caged rainbow trout showed an induction of 500-100,000-fold increase in plasma vitellogenin concentration and males were shown to achieve levels almost as high as females, indicating the contamination of water by estrogenic compounds⁴¹. These estrogenic compounds will include hormones excreted by women taking the contraceptive pill. A variety of adverse environmental conditions, including sub-optimal temperatures, restricted food supply, low pH, environmental pollutants and/or parasites, may also induce intersex effects in fish⁴².
12. To date, the tributyltin (TBT)-induced masculinisation (imposex/intersex) in female molluscs, particularly prosobranch snails, is the best example of endocrine disruption in invertebrates that is causally linked to an environmental pollutant⁴³. TBT is the active ingredient in antifouling paint applied below the waterline on ship hulls to prevent marine creatures from sticking to them. This application explains the wide-spread contamination of this compound in both fresh and seawater. TBT boosts the production of testosterone in female molluscs resulting in imposex, which is the growth of a penis and occlusion of the oviduct due to the development of a superficial vas deferens. The oviduct is blocked by the penis leading to sterility as reproduction is prevented. This is a key example of population-level impact resulting in a decline in population. TBT-containing antifouling paints have been banned from use on vessels under 25 metres in length in the late 1980s, and recently the International Maritime Organisation called for a ban on any new application of TBT, with a total ban on the use of TBT by January 2008.

Experimental work

13. A large body of experimental work on the adverse effects of endocrine disrupting chemicals (EDCs) on male reproductive toxicity has been carried out both *in vitro*⁴⁴ and *in vivo*^{45,46,47,48}. However, the relevance of the data to the observed trends in human reproduction remain unclear.

14. The Organisation for Economic Co-operation and Development (OECD) began the development of new revised guidelines for the screening and testing of potential endocrine disruptors in 1998. One of these activities is validation of the rodent uterotrophic bioassay, an *in vivo* screen to identify suspected estrogen agonists or antagonists of estrogen. The phase two validation studies are now complete. The OECD validation studies demonstrated that all four uterotrophic bioassay protocols were robust and reliable for identifying estrogen agonists and antagonists, and are transferable across laboratories⁴⁹. The results from the validation studies have been submitted for independent peer-review to provide support for the validation of the uterotrophic bioassay, the data will be used to develop the draft OECD test guidelines for the uterotrophic bioassay.
15. The second assay under OECD validation is the *in vivo* Hershberger assay, which is based on the principle that the sex accessory tissues are under the control of androgens to stimulate and maintain growth. In the castrated male rat, sex accessory tissue weights can be restored by androgens, and similarly anti-androgens can block such effects. Phase 2 validation of this assay has also been completed^{50,51,52}. All five accessory sex organs and tissues tested, consistently responded with statistically significant changes in weight, supporting the conclusion that the Hershberger assay is a reliable and reproducible assay for the detection of androgen agonistic and antagonistic effects. Phase 3 validation of the Hershberger assay will now follow.
16. Tinwell and Ashby have demonstrated that a variety of different estrogen receptor (ER) agonists, present individually at doses which are too low for an effect to be detected, can act simultaneously to evoke a ER-regulated response in the immature rat uterotrophic assay⁵³. However simple addition of the activities overestimated the actual effect.

Recent and current activities on observations in humans

17. A current UK government research programme investigating the trends in male reproductive health and the possible effects of chemicals is in progress. This programme includes the following projects;
 - An assessment and analysis of existing data on hypospadias in UK and Europe, publication of some of the results can be accessed at http://www.sickkids.on.ca/frontiersinfetalhealth/FFH_SeptOctNov2000.pdf
 - Environmental risk factors for hypospadias: a population-based case control study in three health regions

- Historically prospective cohort study of Scottish male reproductive health, additional information about this project is available on <http://www.med.ed.ac.uk/hew/repro>
- UK multi-centre study of occupational and environmental exposure to chemicals and male fertility.

18. A number of similar projects are in progress in the European Union, including:

- Inuendo – Biopersistent organochlorines in diet and human fertility. Epidemiological studies of time to pregnancy and semen quality in Inuit and European populations – further information is available at <http://www.inuendo.dk>
- Envir.Reprod.Health – Increasing incidence of human male reproductive health disorders in relation to environmental effects on growth and sex steroid-induced alterations in programmed development
- EDEN – Endocrine disrupters: Exploring Novel Endpoints, Exposure, Low-Dose and Mixture-Effects in Humans, Aquatic Wildlife and Laboratory Animals – further information is available at <http://www.credocluster.info/eden.html>
- COMPRENDO – Comparative Research on Endocrine Disrupters, Phylogenetic Approach and Common Principles focussing on Androgenic/Antiandrogenic Compounds – further information is available at <http://www.credocluster.info/comprendo.html>
- Eurisked – Multi-organic risk assessment of selected endocrine disrupters – further information is available at <http://www.credocluster.info/eurisked.html>
- FIRE – Risk assessment of brominated flame retardants as suspected endocrine disrupters for human and wildlife health – further information is available at <http://www.credocluster.info/fire.html>
- CASCADE – Chemicals as contaminants in the food chain – further information is available at http://europa.eu.int/comm/research/endocrine/index_en.html

19. The potential adverse effects of phytoestrogens on the development of the male reproductive system were addressed by the COT Working Group on phytoestrogens; the report was published in May 2003⁵⁴. Studies on the effects of phytoestrogens on human reproductive development are limited in number, and there are no published human studies specifically investigating the potential effects of *in utero* exposure to phytoestrogens.

20. There are constraints on the ability to identify possible causes of adverse effects in the development of the human male reproductive system. This is due to the limitations that are common to epidemiological studies, as well as some specific problems such as uncertain ascertainment of hypospadias and cryptorchidism²¹, and variations in population recruitment and in laboratory procedures in the case of semen quality. Existing studies on trends in semen quality have been retrospective and have reached different conclusions, and the issue remains controversial.

21. The IPCS Global Assessment²⁹ concluded that analysis of the human data, while generating concerns, has so far failed to provide firm evidence of direct causal associations between low level (general population) exposure to EDCs and adverse health outcomes. In part, this was due to limitations in the available data and the IPCS report highlighted a number of points, including:
- *“A number of studies report a decline in human sperm quality in several countries. The issue remains controversial. Even if there has been deterioration in semen quality, this would not necessarily be due to endocrine disruption.”*
 - *“Available human and experimental animal studies demonstrate that high-level exposure to certain environmental chemicals can impair fertility and increase the risk of spontaneous abortion, but the relationship to endocrine disruption remains speculative.”*
 - *“Declining sex ratios (fewer males) have been recorded in a number of regions and countries and there is evidence that unidentified external influences are associated with such changes, but the mechanism(s) is unknown;”*
 - *“Temporal increases in the frequency of development abnormalities of the male reproductive tract, particularly cryptorchidism and hypospadias, have been reported, but the role of exposure to EDCs is unclear. Experimental data have shown that a number of chemicals can disrupt development of the male reproductive tract via endocrine mechanisms.”*

Other possible risk factors

Possible causes of cryptorchidism

22. Causes of cryptorchidism are multiple and the precise etiological factors are still unknown; a recent review⁵⁵ has outlined a few of the risk factors. Some biomarkers, including a reported decrease in serum inhibin B levels and sperm concentration, have been associated with cryptorchidism⁵⁶.
23. Recently the role of insulin-like factor-3 (INSL3), which acts to retain the gonad in the inguinal region, has been highlighted in mice⁵⁷ INSL3 expression in fetal testis is inhibited by maternal exposure to estrogens. Although to date no mutations have been found in the human INSL3 gene responsible for cryptorchidism, one associated mutation in the INSL3 receptor has been reported⁵⁸.

Lifestyle

24. An increasing number of publications are citing individuals' lifestyle as possible risk factors for some of the adverse trends observed in the development of the male reproductive system^{59,60,61}. These include advanced maternal age at birth of the first child, and low parity (number of siblings at the time of birth)^{59,62}.

25. An association between in utero exposure of the male fetus to maternal smoking, and reduction in semen quality⁶³ and testis size, has been reported. Diet and physical activity in adolescence have also been reported to be potential risk factors for the incidence of testicular cancer^{64,65}.

Body size at birth and in adulthood

26. Positive associations have been reported for high birth weight⁶⁶ low birth weight (possibly due to intrauterine retardation)⁵⁹ height in young adulthood⁶⁷, and risk of testicular cancer, cryptorchidism and hypospadias. However, there is inconsistency between the studies.

Genetic factors

27. An increasing number of papers have focused on the potential role of genetic factors and DNA damage, in the reported adverse trends in the development of the male reproductive system^{68,69}. Large DNA deletions on the Y chromosome are associated with infertility, such deletions remove genes crucial for the progress of normal spermatogenesis^{70,71}. It had been considered that the Y chromosome was particularly susceptible to genetic damage that will affect male fertility. However, there is now evidence, which suggests that the Y chromosome has developed a novel mechanism for dealing with its unique situation, in which mutations in single copy genes might arise. This involves a process termed gene conversion whereby multiple palindromic copies of genes exist within the Y chromosome, between which recombination may occur⁷².
28. A recent study identified an association between male subfertility, and the chromosomal region 11p15⁶⁹. Mutations in the Zinc Finger genes, *ZNF214* and *ZNF215*, which are localised on chromosome 11p15, were found in patients (50% of whom reported a history of cryptorchidism) but not in the controls. These genes are predominantly expressed in the testis.

COT discussion

29. Although there is good evidence that the incidence of testicular cancer has increased, evidence for changes in sperm density, motility and morphology is less clear. Data on the quality of human sperm are subject to a number of sources of uncertainty, including analytical and methodological differences in assessment.
30. The Committee noted that extensive reviews had been conducted, including those of the IPCS²⁹, various other official bodies and academics. These have not provided convincing evidence that exposure to endocrine disrupting chemicals has adversely affected the human male reproductive system (in contrast to wildlife).
31. Efforts are being made to collect exposure data on various chemicals with endocrine disrupting potential. So far these have shown little correlation with reproductive effects. Furthermore the majority of these chemicals are of low potency compared to mammalian hormones. Although many of these compounds are persistent in the environment, exposure is generally very low. While some bioaccumulate in man, this is not true of all of them (e.g. phthalates).

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32. The Committee agreed that there is a need for new approaches to consider possible causes of adverse trends in reproductive health, reported in some countries. In addition to endocrine disrupting chemicals, there is a need to consider whether chemicals without a direct effect on the endocrine system might be involved in the deterioration of male reproductive health, by some other toxic or genotoxic mechanism not mediated via classical hormone signalling mechanisms. Other possibly relevant factors include lifestyle changes that may have occurred in the last few decades, such as an increase in sedentary occupations and maternal age at first pregnancy, as well as decreased numbers of older siblings at birth (parity).
 33. It was considered that although current animal studies demonstrate effects of EDCs on certain endpoints, such as maldescended testis, these data often cannot be extrapolated to humans and therefore may not be useful. For instance the poor quality of human sperm compared to that of animals may have implications when comparing human and animal data.
 34. It was agreed that it would not be useful for the Committee to repeat the work of existing and ongoing international reviews and activity relating to endocrine disruption and adverse trends in the male reproduction system. There was a need for a new approach investigating the mechanisms involved in the formation of developmental abnormalities. The first step of the process would be to review the evidence for adverse trends in human male reproductive health, and then to consider possible causes including lifestyle factors and the role of chemicals in general. Since much of this would be outside of the Committee's terms of reference, this might best be explored by experts from appropriate medical and scientific disciplines at a scientific meeting organised by one or more learned societies.

Conclusions

35. We *note* that although the evidence of endocrine disruption in wildlife is more convincing, the extensive international reviews currently do not provide direct evidence that exposure to endocrine disrupting chemicals has adversely affected the human male reproductive system. This may be because of the uncertainties involved in undertaking prospective long-term studies in humans.
36. We *consider* that the evidence of adverse trends in human male reproductive health should be reviewed first before considering possible causes. These could include the role of chemicals in general, not merely those affecting the endocrine system, as well as lifestyle factors.
37. Since much of this work is currently outside the Committee's remit, we *recommend* that a scientific meeting be held to review the evidence of adverse trends in male reproductive health. This will need to involve experts from relevant medical and scientific disciplines.
38. These conclusions may need to be reviewed as new data emerge.

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Chlorinated drinking water and reproductive outcomes

Introduction

1. The Committee considered the issue of chlorinated drinking water and adverse reproductive outcomes in 1998 and produced a statement which was published in 1999¹. In May 2001 we were asked to consider the findings in a draft report of the first phase of a Government-funded epidemiological study⁵ conducted in England by the Small Area Health Statistics Unit (SAHSU), along with additional relevant studies published since 1998^{8,9,12,13,20}. Our conclusions were unchanged. An updated Committee statement was drafted, for release on publication of the English study. In response to peer-reviewers' comments, however, the study was not published but was reanalysed following remodelling of the exposure data, inclusion of additional years, and revision of the links between postcodes and the boundaries of water supply zones. In April 2004, we were asked to consider the results of the reanalyses, together with the additional relevant studies and reviews published since 1998^{7-14,16-33}. The SAHSU study, incorporating additional results, has now been published¹⁵. We were asked to restrict our evaluation to pregnancy outcomes. We were also advised that a second phase of the SAHSU study, in progress and expected to report in 2005, would include data on congenital anomalies (which were not considered in the first phase). We were informed that we would be asked to advise on the results of the second phase in due course, and that therefore it was appropriate to defer reassessment of the scientific literature on chlorinated drinking-water and congenital anomalies until then.

1998 Evaluation

2. In 1998, at the request of the Drinking Water Inspectorate, we were asked to consider the evidence linking the consumption of chlorinated tapwater and adverse reproductive outcomes. We had not previously considered the health effects of chlorinated water or the by-products of chlorination. However, the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) had reviewed cancer epidemiology data in 1992. At that time the COC concluded that the 1986 opinion of CASW, the Department of Health Committee on the Medical Aspects of Air, Soil and Water, (that there was no sound reason to conclude that the consumption of by-products of chlorination in drinking water increased the risk of cancer in humans) was adequately founded and that more recent studies did not alter that conclusion². COC also in 1999 reviewed additional relevant epidemiological studies of cancer published since the 1992 evaluation, and issued a revised statement³. COC did not find "persuasive evidence of a consistent relationship between chlorinated drinking-water and cancer" but noted that "it remains possible that there may be an association" and therefore advised that "efforts to minimise exposure to chlorination by-products remain appropriate, providing that they do not compromise the efficiency of disinfection of drinking-water".
3. The 1998 request to us followed the publication of two prospective epidemiological studies conducted in three geographic regions of California, USA. One study reported a weak to moderate association between high consumption of tapwater and the incidence of spontaneous abortion, albeit in only one of the three regions⁴. The second study, taking data from all three regions together, reported a weak to moderate association between high exposure to certain chlorination by-products in tapwater and spontaneous abortion⁶.

4. As most of the drinking water in the United Kingdom is chlorinated and similar levels of certain chlorination by-products could occur in UK tapwaters, we were asked to comment on the relevance of these data for public health in the UK.

Consideration of the epidemiological and toxicological data

5. In 1998 we considered available epidemiological information on the association of chlorination by-products in drinking water and a range of adverse reproductive outcomes. Of the seventeen reviewed studies concerned with consumption of drinking water, eight had examined a potential association with chlorinated water or chlorination by-products in the tapwater. Of these, particular attention was focused upon the study from California⁶, which the Committee considered to be particularly well-designed and well-conducted.
6. This study, taking data from all three regions together, reported a weak to moderate association (adjusted odds ratio 3.0, 95% confidence interval 1.4-6.6) between high exposure to certain chlorination by-products in tapwater and spontaneous abortion. Nevertheless, the study did not exclude unidentified biases and other confounding factors and the findings, along with information from earlier studies, did not provide persuasive evidence of a causal association between exposure to chlorination by-products in drinking water and adverse reproductive outcomes.
7. Exposure to chlorination by-products could occur not only through drinking but also through showering, bathing and so forth, especially for the more volatile compounds; also these would tend to be removed by boiling. In addition, the available reproductive toxicity studies with some of the individual chlorination by-products indicated that the levels of exposure to these substances in drinking water are about four orders of magnitude (ie 10,000 times) lower than levels at which adverse effects may occur in animals.

1998 Conclusions

8. In 1998 we concluded as follows:
 - We *consider* that there is insufficient evidence to conclude that the presence of chlorination by-products in tapwater increases the risk of adverse reproductive outcomes.
 - We *recommend*, however, that the claimed associations between patterns of drinking-water intake and the incidence of adverse reproductive outcomes be investigated further, since any causal association would be of significant public health concern.
 - We therefore *consider* that efforts to minimise exposure to chlorination by-products by individuals and water authorities remain appropriate, providing that they do not compromise the efficiency of disinfection of drinking water.

The SAHSU study (first phase) and 2004 evaluation

9. Following our 1998 recommendation for further investigations, the Small Area Health Statistics Unit (SAHSU), in collaboration with, and supported by, UK water supply companies, undertook a major study using small area statistical methodology. The first phase of the study utilised routinely collected trihalomethane (THM) measurements in drinking water (as an index of exposure to chlorination by-products) and available health statistics on stillbirths and birthweight, to examine the possible effects¹⁵.
10. Modelled estimates of quarterly THM concentrations in water zones from 3 water companies in England (Northumbrian Water, Severn Trent Water and United Utilities [formerly North West Water]) were linked to about 1 million routine birthweight and stillbirth records based on location of maternal residence at the time of birth. THM estimates corresponding to the final three months (93 days) of pregnancy were used. Three total THM exposure categories were defined: low (below 30 mg/l), medium (30-60 µg/l) and high (above 60µg/l).
11. The findings, for low birthweight (less than 2500g), very low birthweight (less than 1500g), mean birthweight and stillbirth, were different in the three water supply areas.
12. In the North West (United Utilities), where there was increasing social deprivation as exposure increased, there was a graded, inverse association between level of exposure and mean birth weight, and a direct association with stillbirth rate and the prevalence of low and very low birthweight. After adjustment for maternal age, deprivation, year of study (for birthweight data) and sex of baby (for low birthweight data), the risk of very low birthweight (in the high versus the low exposure categories) was elevated (adjusted odds ratio = 1.20, 95% confidence interval 1.07-1.34). Similarly, the risk of low birthweight (in the high versus the low exposure categories) was elevated (adjusted odds ratio = 1.19, 95% confidence interval 1.14-1.24) as was the risk of stillbirth (adjusted odds ratio = 1.21, 95% confidence interval 1.03-1.42). In this area, but not in the other two areas, adjustment for deprivation reduced odds ratios by up to about one-half, suggesting the possibility of residual confounding.
13. In contrast, in the Severn Trent region, the risk of very low birthweight was decreased in the high versus the low exposure categories (adjusted odds ratio = 0.90, 95% confidence interval 0.82-0.99). There was no association between level of exposure and deprivation, stillbirth rate, or prevalence of low birthweight. No statistically significant associations were found in the Northumbrian region, but the number of births included in the study was much smaller than in the other regions and the confidence intervals were therefore wide.
14. In a random-effects model to obtain overall summary estimates, allowing for heterogeneity in THM exposure effects across the three regions, a statistically significant elevated risk was found in the high compared to low exposure areas for stillbirths (adjusted odds ratio 1.11, 95% confidence interval 1.00-1.23). For low birthweight (adjusted odds ratio 1.09, 95% confidence interval 0.93-1.27) and very low birthweight (adjusted odds ratio 1.05, 95% confidence interval 0.82-1.34), the risks were elevated but not

statistically significant. The authors concluded that their findings overall suggest a significant association of stillbirths with maternal residence in high total THM exposure areas. Nonetheless, they could not exclude residual confounding by socio-economic deprivation. They noted that adjusted excess risks in areas of high deprivation relative to areas of low deprivation were, on average, 15 to 20 times those found in areas of high relative to low total THM exposure. Further work was recommended, to help differentiate between alternative (non-causal) explanations for the association with THMs and those that may be due to the water supply.

Evaluation

15. In the North West total THM exposure showed an inverse association with mean birth weight, a direct association with prevalence of low and very low birthweight, and a direct association with the prevalence of stillbirths. However, there was evidence of confounding by social deprivation, adjustment for which may not have been completely successful in this analysis. In the Severn Trent region, in contrast, the prevalence of very low birthweight decreased with increasing total THM exposure, and there was no association for prevalence of low birthweight or stillbirth rate. In the Northumbrian region, there was no evidence of associations between total THM levels and any of the pregnancy outcomes, but the number of births included in the study was relatively small.
16. We note that the SAHSU study was confined to THM concentrations and that data on other chlorination by-products in water were not routinely available. We note also that data on other sources of exposure to THMs, such as swimming pools, were not available.
17. We have reviewed data from thirteen other epidemiological studies^{7-14,16-20} which were reported after our evaluation in 1998, and which also investigated associations between chlorinated drinking water and pregnancy outcomes (other than congenital malformations, on which we have not yet reassessed the data). The pregnancy outcomes considered were: low and very low birthweight, stillbirth, spontaneous abortion, perinatal death, infant death, Apgar score, infant's head circumference at birth, infant's body length, pre-term delivery, length of gestation, neonatal jaundice and neonatal hypothyroidism. Results from these studies were inconsistent and inconclusive and we consider that further research is needed on this issue. In particular, prospective studies with appropriate assessment of exposure, with a better assessment of confounding factors and allowance for seasonal variations in chlorination by-product concentrations, should be considered.
18. We have also noted the findings in recent papers on exposure to and uptake of chlorination byproducts²¹⁻²³, recent studies in laboratory animals²⁴⁻³⁰, and recent reviews³¹⁻³³.
19. We conclude that the data which we have evaluated do not show a causal relationship between chlorinated drinking-water and adverse pregnancy outcomes. This conclusion excludes congenital malformations, the data on which we have not yet reassessed.

20. The Committee statement published in 1999 advised that “...efforts to minimise exposure to chlorination by-products by individuals...remain appropriate...”. We were asked for clarification. We recalled that bathing and showering may result in greater exposure to some volatile chlorination byproducts in drinking-water than the use of the water for drinking and cooking. Other sources of exposure, such as chlorinated swimming-pools, may also be important. Effective avoidance of chlorination byproducts could therefore require major changes in behaviour. We agreed that the evidence does not justify any such changes, and that advice to pregnant women to minimise exposure to chlorination byproducts is not warranted. We consider, however, that it remains prudent for water companies to minimise consumers’ exposure by restricting the concentrations of chlorination byproducts in tapwater, provided always that this is consistent with effective disinfection to protect public health.

Overall conclusions

21. We *conclude* that the data which we have evaluated do not show a causal relationship between chlorinated drinking-water and pregnancy outcomes, namely: low and very low birthweight, stillbirth, spontaneous abortion, perinatal death, infant death, low Apgar score, infant’s head circumference at birth, infant’s body length, pre-term delivery, length of gestation, neonatal jaundice and neonatal hypothyroidism. We have not yet reassessed the data on congenital malformations.
22. We *recommend*, however, further research to reduce uncertainties in the interpretation of the reported associations between patterns of drinking-water intake and the incidence of adverse reproductive outcomes. In particular, we *recommend* prospective study designs which include more precise assessment of individual exposures, allowance for seasonal variations in chlorination byproduct concentrations, and more comprehensive analyses of the influence of other potential causative agents and confounding factors.
23. We *consider* that, while research to determine the effects of chlorinated drinking water continues, efforts by water companies to minimise consumers’ exposure to chlorination by-products remain appropriate, providing that such measures do not compromise the efficiency of disinfection of drinking water.

COT Statement 2004/08

October 2004

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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 related 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 NAOEL of 750 mg/day (Brixen *et al.*, 1992) to allow for inter-individual variability, resulting in a guidance level of 250 mg/day phosphorus.
2. In the interests of maintaining choice and promoting consumer understanding, the Food Standards Agency is working with manufacturers to develop a labelling initiative, which would allow higher amounts of phosphorus (as phosphate) to be present in food supplements than those recommended by the EVM provided that appropriate advisory statements were also included.
3. The COT was asked to consider data on phosphate, PTH, calcium balance and bone health in detail, in order to allow the FSA to formulate the appropriate consumer advice. This review included new data which were not available to the EVM.

Background

4. Phosphorus is a non-metallic, group V element. It is most commonly found in its pentavalent form in combination with oxygen as phosphate (PO_4^{3-}).
5. Phosphorus is present in a wide variety of foods largely as phosphate. Particularly rich sources are red meats, dairy products, fish, poultry, bread and other cereal products. Food additives are also a significant source of phosphate (Calvo and Park, 1996). Phosphorus intakes in the UK in 2000 were estimated to be 1494 (mean) and 2381 (high level) mg/day in men and 1112 (mean) and 1763 (high level) mg/day in women (Henderson *et al.*, 2003). These intakes had increased since the previous survey in 1986/6 (Gregory *et al.*, 1990).
6. Food supplements provide doses of up to 1100 mg/day phosphorus (EVM, 2003), though 150 mg or less is more common (OTC, 2003). Tablet forms of supplement products may also contain calcium phosphates as fillers or diluents. It has been suggested that these could represent a dose of up to 250

mg phosphorus per day (personal communication, Proprietary Association of Great Britain). The phosphate may not be declared on the label as an active ingredient.

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

Phosphate: Absorption, distribution, metabolism and excretion

8. Phosphate is readily absorbed throughout the small intestine (Koo and Tsang, 1997). The majority of phosphate absorption occurs via passive diffusion but active transport, dependent on potassium and calcium ions, sodium-potassium ATPase and vitamin D, also occurs. Active transport of phosphate is regulated by PTH which promotes calcium absorption via increased synthesis of calcitriol. However, even at low levels of calcitriol, substantial phosphate absorption occurs, such that dietary phosphate content is the most important determinant of phosphate absorption. To some extent, the degree of phosphate absorption varies with need, being 90% in infants fed human milk with low phosphate levels and 60-70% in adults consuming a normal diet, but it is essentially linear over the normal physiological range (Lemann, 1996).
9. Soluble phosphates from meat or milk are almost completely absorbed, whereas the phosphate present as phytate in certain vegetable fibres may not be (Koo and Tsang, 1997). Phosphate absorption is affected by calcium, with an insoluble calcium phosphate precipitate being formed in the intestine (Calvo and Heath, 1988; Calvo and Park, 1996). However it is likely that excess calcium affects phosphate absorption rather than phosphate affecting calcium absorption (IOM, 1996).
10. Phosphorous 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 eg 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.

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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.

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Tetrakisphenol A – review of toxicological data

Introduction

1. In 2003, the COT considered data on some brominated flame retardants (BFRs) in fish from the Skerne-Tees river system. In the course of the discussions, Members noted a need to consider other BFRs, such as tetrabromobisphenol A (TBBPA). As the Food Standards Agency is planning to conduct a survey of TBBPA in fish and shellfish during 2004, the Committee was invited to consider the toxicological data in advance of receiving the results of the survey.
2. Decabromodiphenyl ether (decaBDE) and tetrabromobisphenol-A (TBBPA) have been found commonly in marine fish and shellfish but studies have shown that decaBDE and TBBPA are present in the eggs of wild birds^{1,2} a finding that was unexpected. There appear to be no data on BFRs in hen eggs; they are being included in the survey to assess whether the unknown source of the BFRs in the wild bird eggs is also affecting free range hen eggs.
3. TBBPA has not been evaluated by the Scientific Committee on Food (SCF) or the Joint FAO/WHO Expert Committee on Food Additives (JECFA). However it is currently under evaluation under the Existing Substances Regulations. A draft risk assessment is available³. Many of the studies referred to are unpublished and not freely available.
4. The COT evaluation of the toxicology of TBBPA was based primarily on the EU risk assessment. The data described in this statement are summarised from the EU risk assessment unless otherwise indicated.

Background

5. BFRs are structurally diverse chemicals used in plastics, textiles and other materials to enhance their flame-retardant properties. TBBPA may be present as an additive or may be reacted into the polymer matrix. Reactive flame retardants are covalently bonded to the plastic itself whilst additives are mixed into the material. This means that reactive materials are less likely to leach or volatilise whereas additives are more easily released. Where TBBPA is used as an additive flame retardant it is generally used with antimony trioxide for maximum performance. Antimony trioxide is generally not used in conjunction with TBBPA in reactive flame retardant applications.
6. TBBPA is produced by the bromination of bisphenol A (BPA) in the presence of either a hydrocarbon solvent (with or without water), 50% hydrobromic acid or aqueous alkyl monoethers. TBBPA is not produced in the EU however 13,800 tonnes were used in the EU during 1999 (620 tonnes in the UK in 2001).
7. The primary use of TBBPA (approximately 90% of use) is as a reactive intermediate in the manufacture of epoxy and polycarbonate resins. When used as a reactive intermediate it becomes covalently bound in the polymer and is effectively lost. The only potential for exposure is from unreacted TBBPA which may exist where excess has been added during the production process. It is used as a reactive flame retardant in polycarbonate and unsaturated polyester resins. It may also be used as an additive flame

retardant in the manufacture of acrylonitrile-butadiene styrene resins, high impact polystyrene and phenolic resins. As an additive flame retardant TBBPA does not react chemically with the other components of the polymer and therefore may leach out of the polymer matrix. Additive use accounts for approximately 10% of the TBBPA used.

Toxicological profile as described in the EU risk assessment

Toxicokinetics

8. Toxicokinetic data are available for the rat but not for other experimental animals. TBBPA is well absorbed from the gastrointestinal tract. Systemic distribution of TBBPA is low with most distributed directly to the liver and eliminated in the faeces via the bile. There is some evidence to suggest that the compound undergoes enterohepatic recirculation. TBBPA undergoes metabolism via glucuronidation and/or sulphation. The half-life in blood is about 20 hours and 95% of administered dose was reported to be eliminated in the faeces within 72 hours, with only a small percentage eliminated in the urine. Repeat dose studies have shown no potential for bioaccumulation in fat. A toxicokinetic study in pregnant animals indicated no significant transfer of TBBPA or its metabolites to the fetus.
9. TBBPA has been detected in samples of human serum or plasma. In Sweden the concentrations ranged from <0.5 to 3.8 µg/kg lipid, and one study estimated the half-life of TBBPA in serum to be 2.2 days. In Norway, the range was <0.4 ng/kg lipid to 1.8 µg/kg lipid. A further study in Norway analysed the level of TBBPA in pooled serum samples at six timepoints during 1977 to 1999. TBBPA was not detected in samples from 1977-1981 but was found to increase from 0.44 µg/kg lipid in 1986 to 0.65 µg/kg lipid in 1999. The range of TBBPA in pooled samples from 8 groups of individuals collected in 1998 was 0.34 – 0.71 µg/kg lipid. In Japanese volunteers, the maximum blood concentration detected was 12 µg/kg lipid. There is no information on blood concentrations of TBBPA in the UK population.
10. Limited data demonstrate that TBBPA has also be detected in human breast milk. Concentrations of 0.29 – 0.94 µg/kg lipid were detected in two of four samples collected from West Berlin during 1998/99; 11 µg/kg lipid was detected in a single sample from the Faroe Islands, but no information was available on the possible reason for this high value. Analysis of pooled samples of breast milk collected from Norway during 2001 found TBBPA was present at 0.010 – 0.1 µg/kg lipid.

Acute and sub-acute toxicity

11. TBBPA is of low acute toxicity via oral administration. The LD₅₀ in the rat is >50,000 mg/kg body weight (bw) and in the mouse is >10,000 mg/kg bw. There is no information available on the acute toxicity of TBBPA in humans.
12. In a recent study conducted to OECD guidelines, by MPI research⁴, rats were administered 0, 100, 300 or 1000 mg/kg/day TBBPA in corn oil by gavage for 13 weeks, followed by a 6-week recovery period. There were no treatment-related deaths, no effects on body weight gain, and no abnormal ophthalmic observations. A functional observational battery (FOB) and assessment of motor activity at week 12 revealed no evidence of neurobehavioural effects. Platelet counts were significantly reduced (by 17%)

in the high dose males at 13 weeks, but not at the end of the recovery period. There were no other haematological changes. The values were comparable to control at the end of the recovery period, other markers of liver toxicity were normal and there were no abnormal histopathological findings in the liver.

13. There was a reduction in T4 levels in all treated males and females on day 33 and in males at day 90, with no clear dose-response relationship. There were no reported differences in TSH and T3 at any timepoint, and the T4 levels were comparable to control following the recovery period.
14. Overall, the EU risk assessment concluded there were no clear toxicologically significant effects up to the highest nominal dose of 1000 mg/kg bw/day. However, it was noted that due to the relative insolubility of TBBPA, it was only partially dissolved at the highest level. In view of this the EU risk assessment concluded that only 50% of the TBBPA would be available for absorption and suggested that the nominal dose of 1000 mg/kg bw/day was equivalent to an actual dose of 500 mg/kg bw/day.
15. Two dietary studies in rats reported no treatment-related effects at TBBPA doses up to 100 mg/kg bw/day for 90 days and up to 75 mg/kg bw/day for 28 days. Two short term (3 – 28 days) studies designed to determine the effect of TBBPA on the liver indicated no clear evidence of hepatotoxicity up to doses of 2250 mg/kg. Some significant changes were apparent in one study, including reduced liver glutathione content, following 7 days of TBBPA administration at 750 and 1125 mg/kg bw/day, and indicators of porphyrogenic action at 50 and 250 mg/kg bw/day⁵. The relationship to dose and treatment time was not consistent, and there were no accompanying histological changes. A further study investigated the effect of daily gavage dosing, up to 28 days, of TBBPA (10-250 mg/kg bw/day) on the kidneys in rats. No dose related or toxicologically significant effects were reported.
16. Repeated dermal exposure to 50% of the maximum non-irritating dose of TBBPA did not induce bromacne (analogous to chloracne) in the skin.
17. There is no information on the effect of repeated exposure to TBBPA in humans.

Mutagenicity and carcinogenicity

18. TBBPA was not mutagenic in reverse mutation assays with *Salmonella typhimurium* or *Saccharomyces cerevisiae* in the presence or absence of metabolic activation and up to cytotoxic concentrations. A chromosomal aberration test in human peripheral lymphocytes in the presence and absence of S9 also gave negative results. TBBPA has also been tested for its ability to induce intragenic recombinations in mammalian cells. The study did not show an increase in the number of revertant colonies. TBBPA has not been tested for mutagenicity *in vivo*. Overall, in view of the negative data and absence of structural alerts for genotoxic potential, there are no concerns for genotoxicity. There are no carcinogenicity data for TBBPA.

Reproductive toxicity

19. A comprehensive developmental study by Hass et al.⁶ was recently conducted according to the proposed OECD guideline TG426⁷. TBBPA was administered to rats by gavage at 0, 50 and 250 mg/kg bw/day from gestational day 7 to postnatal day 17. Observations included endpoints associated with endocrine effects (anogenital distance at birth, time of nipple development and sexual maturation) and a range of neurobehavioural tests at timepoints up to the age of 7 months. The authors concluded the study demonstrated that TBBPA caused developmental neurotoxicity at 250 mg/kg bw/day, expressed as decrease of habituation capability and impairment of learning and memory in both sexes. However the draft EU risk assessment questioned the relevance of the observations and considered that the study showed no significant treatment-related toxicity.
20. Data from a recent 2-generation reproductive toxicity study in rats⁸, conducted to OECD guidelines, indicate that TBBPA has no toxicologically significant effects on fertility or neurodevelopment at doses of up to 1000 mg/kg. Observations on neurodevelopment were conducted in the F₂ generation and included neurobehavioural studies of motor activity, learning and memory, and neuropathological evaluation and morphometric measurements. Significant reductions in the levels of T4 were observed in F₀ males at 100 and 1000 mg/kg and in F₀ females at 1000 mg/kg. Levels of T4 were reduced in F₁ males and females at 100 and 1000 mg/kg. Levels of T3 were reduced in F₀ males from 1000 mg/kg. There was no effect on TSH. The authors suggested that these findings indicated that TBBPA can induce UDP-glucuronosyltransferase, the enzyme involved in metabolism of T4.
21. In a study of developmental neurotoxicity, TBBPA was administered as a single oral dose of 0.75 or 11.5 mg/kg bw to 10-day old male mice. No differences in performance were observed in behavioural tests conducted on the mice at the age of 2 and 4 months³. This study was conducted by Eriksson and colleagues, who reported that some polybrominated diphenyl ethers produced significant effects when tested using this protocol, as discussed in the COT statement on BFRs in fish from the Skerne-Tees river system⁹.
22. There are no human data available on the reproductive toxicity of TBBPA.

Other studies

In vivo

23. Following oral exposure of pregnant rats to TBBPA (5 mg/kg/bw) on days 10-16 of gestation there were no effects on maternal bodyweight, total litter size, or number of resorptions¹⁰. There was no effect on total and free T4 plasma levels in dams or offspring and no effect on T3 in the dams. T3 was not detected in the plasma of offspring. An increase in TSH levels was reported in the dams and offspring compared to controls, but this was only significant in the offspring.

In vitro

24. TBBPA has shown evidence of weak oestrogenic activity in some, but not all, *in vitro* assays using cell lines and recombinant yeast models¹¹⁻¹⁵.

COT evaluation

25. The reported effects of TBBPA on thyroid hormones differed between studies. As TBBPA was reported to inhibit the binding of ¹²⁵I-T3 (1×10^{-10} M) to the thyroid hormone receptor in the range 1×10^{-6} to 1×10^{-4} M¹⁵, TBBPA would be expected to have thyroid hormone agonist effects and to inhibit thyroid stimulating hormone (TSH). However, this did not appear to be the case. Given the lack of consistency in the data, it was considered difficult to ascertain whether TBBPA had significant thyroid effects. A reduction in T4 levels in one study had been reported but levels had returned to baseline following a recovery period, which indicated that any effect was reversible. The relevance of data suggesting that TBBPA may displace T4 from transthyretin (TTR), a major T4 binding protein in the rat, was unclear in view of the limited understanding of the role of TTR. It was possible that induction of UDP-glucuronosyltransferase could account for the findings, but that needed to be demonstrated.
26. No long-term carcinogenicity study was available. However, the results of mutagenicity studies were negative and the subchronic studies provided no indication of a mechanism to suggest that TBBPA could lead to carcinogenesis of relevance to humans following life-time exposure.
27. TBBPA had been shown to have weak estrogenic activity in some *in vitro* assays. The lack of effects in a recent 2-generation reproductive toxicity study in rats suggests that TBBPA is not an endocrine disruptor *in vivo* at doses relevant to human exposure.
28. Discussions focussed on results of neurotoxicity studies in rats^{4,6,8}. Hass *et al.*⁶ reported a significant reduction in habituation at a dose of 250 mg/kg bw/day. There was no consistent pattern of effects across the dose groups, which raised doubts about the biological plausibility of the effect. In contrast, reduced habituation was not observed in the MPI study⁸, at 100 and 1000 mg/kg bw/day. A decrease in parietal cortical thickness was observed at 1000 mg/kg bw/day at day 11. Additional data, not described in the EU risk assessment, showed that this effect was not present at day 60¹⁶, and hence the MPI study was not considered to provide evidence of neurotoxicity. Detailed consideration was therefore given to the comparison of these studies.
29. The two studies used slightly different end points at slightly different ages (postnatal days 21, 27 and 84 in the Hass study and postnatal days 17, 21 and 60 in the MPI study). Both studies showed the expected increase in spontaneous activity after weaning, and both showed significant habituation activity in the controls over the test period. Absolute activity values are not comparable between the two studies due to methodological differences, but the percentage changes over time should be comparable.

30. The MPI study showed less habituation in the controls than the Hass *et al.* study, which would have reduced its ability to detect a toxicant-induced fall in habituation, particularly at postnatal day 21. However the MPI study did have sufficient power to detect as marked a fall in habituation as was seen in the Hass *et al.* study at post-natal day 21 – yet failed to do so at 100 and 1000 mg/kg. The rather more modest decline in habituation, seen in the Hass *et al.* study at post-natal day 84 was not reproduced in the MPI study at post-natal day 60. Overall, the lack of effect in the MPI study, and the lack of internal consistency in the results of Hass *et al.* indicates that the findings of a reduced habituation at 250mg/kg by Hass are likely to have been due to chance.
31. The EU draft risk assessment report concluded that its risk characterisation for oral exposure should be based on a repeat dose study in which rats were administered TBBPA in corn oil. No significant toxicological effects were reported in this study at the highest dose of 1000 mg/kg bodyweight/day. Based on the limited solubility of TBBPA in the dosing vehicle (corn oil) the EU report assumed that only 50% of the TBBPA was bioavailable. However the Committee considered that assumptions on absorption should not be based on solubility and therefore the highest dose of 1000 mg/kg bw/day could be used as the basis for deriving a Tolerable Daily Intake (TDI).
32. A total uncertainty factor of 1000 would be required. This comprises the default uncertainty factor of 100 to allow for inter- and intra-species variation and an additional factor of 10 for the absence of chronic toxicity studies.

Conclusions

33. The available data on TBBPA do not raise specific toxicological concerns. There is a lack of long term or carcinogenicity studies, but this is not considered essential to the evaluation in view of the absence of relevant effects in the available studies.
34. No clear adverse effects were observed at doses up to 1000 mg/kg bw/day, the highest dose tested in a 90-day study and in a two-generation reproductive toxicity study. This dose level may be used as the basis for deriving a Tolerable Daily Intake (TDI).
35. An uncertainty factor of 100 is applied to allow for inter- and intra-species variation. An additional factor of 10 is required because of the absence of chronic toxicity studies. The Committee therefore recommended a TDI of 1 mg/kg bw/day.

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Toxicological evaluation of chemical analyses carried out as part of a pilot study for a breast milk archive

Introduction

1. The Food Standards Agency has received the results of the SUREmilk pilot studies, which explored alternative methods for the recruitment, collection, storage and management of an archive of breast milk samples¹. The report includes analytical data on a range of environmental contaminants (dioxins, polychlorinated biphenyls, organochlorine pesticides, phthalates and heavy metals). The Committee was invited to advise on whether these data raised toxicological concerns for the breast-fed infant, which would require urgent action or indicate a need for further research. The data were collected primarily to explore the viability of sample collection methods, they do not constitute a rigorous survey.

Study design

2. Primiparous pregnant women and mothers were recruited within six NHS Trusts in Yorkshire during 2001-2002. The age range was 16 to 42 (mean 28) years. Samples of breast milk were collected on one or more occasions at 1 to 16 weeks. The research was designed as pilot studies of issues associated with recruitment and storage, and not to investigate the range of contaminant concentrations present at different lactation times, or in different ages of mother, and cannot be assumed to be representative of the UK population.
3. There were three cohorts of women, defined by criteria related to recruitment and not to potential exposure to contaminants.
 - Cohort 1 – the 322 women in this group expecting/having healthy full-term babies were asked to provide two samples – one at a fixed time point between 1 and 16 weeks, the other at a random point. This would give a cross-section for each time point (1, 2, 4, 8, 12-16 weeks).
 - Cohort 2 – 54 self-selected volunteers, also expecting/having healthy full-term babies and especially committed to breast feeding, were asked to provide samples at 1, 2, 4, 8 and 12 weeks to create a longitudinal series.
 - Cohort 3 – an opportunistic approach. Eighteen mothers of babies admitted to a Special Care Baby Unit were asked if they would be prepared for any unused expressed milk stored in the unit to be taken for the study once they had been discharged and there was confirmation that they did not require it. Cohort 3 represented only 5% of the recruits.
4. Concentrations of dioxins, polychlorinated biphenyls, organochlorine pesticides, phthalates and heavy metals were analysed to explore the range of variation in breast milk and to demonstrate stability on storage. For dioxins, the analysis required a sample size of approximately 300mL, therefore samples had to be pooled and do not provide information on the range of variation. A stability study indicated that data on the phthalates were unreliable and these were not considered further. Based on the results of the stability study and prior experience, the data on other contaminants were considered to be of an acceptable quality.

Intake estimations

5. There is no universally accepted methodology for assessing consumption of breast milk in different ages of infant, allowing for different nutritional needs and progressive weaning. Some researchers assume consumption of a fixed amount (e.g. 600ml or 800ml per day) regardless of age. The method employed in this evaluation is based on the results of a study which tracked the consumption of breast milk and growth of 48 infants in Cambridge². Breast milk consumption was highest for infants under 2 months of age, with an average daily intake of 160g/kg bodyweight (bw). These data were previously used in estimating intake of dioxins and polychlorinated biphenyls (PCBs) by breastfed infants in the UK^{3,4}. The estimates of intake from breast milk, and from infant food where available, have been compared with previous COT opinions and available tolerable intakes recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), the EU Scientific Committee on Food (SCF) or the Expert Group on Vitamins and Minerals (EVM).

PCBs and organochlorine pesticide residues in milk

PCBs

6. Seven ortho-PCBs were analysed in individual milk samples. One of these (PCB118) is considered to have weak dioxin-like activity, the others are non-dioxin-like. There is currently no accepted method of risk assessment for the non-dioxin-like PCBs, and there are no established tolerable intakes. These substances are currently being evaluated by a co-operation between the European Food Safety Authority (EFSA) and the World Health Organisation (WHO), which is expected to report at the end of 2004.

Pesticides

7. Fifteen organochlorine pesticides were analysed in 92 individual milk samples from 48 donors. Alpha-HCH, beta-HCH, heptachlor, heptachlor epoxide (cis), heptachlor epoxide (trans), oxychlordane, chlordane (trans), chlordane (cis) and p,p'-DDE were not detected. Hexachlorobenzene (HCB) and p,p'-DDE were detected in almost all samples (47 of 48 donors), p,p'-DDT in 26% (15 donors) and dieldrin in 16% (11 donors). o,p'-DDT was detected in the milk of one woman who also had a relatively high concentration of p,p'-DDE.
8. HCB concentrations ranged from 0.1 to 3.03 µg/kg (mean 0.66 µg/kg). Consumption of breast milk at the highest detected concentration of HCB would result in infants of 7 months and younger exceeding the TDI of 0.16 µg/kg bw/day⁵, with a maximal 3-fold exceedance for infants below 2 months of age. A mean concentration of 1 µg/kg (range <1-5 µg/kg) was previously reported based on 193 samples taken between 1989 and 1991 in the UK⁶.

9. HCB has been shown to induce liver tumours in rats, but from an overview of the predominantly negative mutagenicity data, it is considered not to be genotoxic. A WHO taskforce recommended a health-based guidance value of 0.16 $\mu\text{g/kg bw/day}$, which is used as the TDI for HCB⁵. This was based on the lowest reported no observed effect level (NOEL) of 0.05 mg HCB/kg bw/day, for primarily hepatic effects observed at higher doses in studies on pigs and rats exposed by the oral route, incorporating an uncertainty factor of 300 (x 10 for interspecies variation, x 10 for intraspecies variation, and x 3 for severity of effect). The COT considered that the toxicity profile of HCB did not justify the use of the 3-fold factor for severity of effect, and therefore the TDI was likely to be over-precautionary.
10. Studies involving lactational exposure in mink, rats and cats have shown effects on the offspring at maternal doses of 0.16 to 4 mg/kg bw/day. A NOEL of approximately 1 mg/kg bw/day was derived from a four-generation reproductive toxicity study in Sprague-Dawley rats. The lowest observed effect levels (LOELs) and NOELs represent maternal doses that are higher than the NOEL that provided the basis for the TDI. However, the dose to the pups is not known.
11. WHO (1997) noted that mean intakes of HCB by nursing infants have been estimated to range from <0.018 to 5.1 $\mu\text{g/kg bw/day}$ in various countries⁵. Thus, the maximal intake of HCB estimated from the SUREmilk project (0.485 $\mu\text{g/kg bw/day}$) is at the lower end of the WHO range. The results of most studies on the levels of HCB in foods and human tissues over time indicate that exposure of the general population to HCB declined from the 1970s to the mid-1990s in many locations.
12. Based on representative levels of HCB in air, water and food, WHO (1997) estimated the total intake of HCB by adults in the general population to be between 0.0004 and 0.003 $\mu\text{g/kg bw/day}$, predominantly from the diet⁵.
13. Overall, the exceedances above the TDI are undesirable, but the significance of this should be considered in the context of the use of maximum levels in the intake calculations, the precautionary derivation of the TDI and that the period of exceeding the TDI from breast-feeding is relatively short. The SUREmilk data do not highlight a new problem with respect to HCB levels in breast milk.

Dieldrin

14. Consumption of breast milk containing dieldrin at the highest detected concentration of 0.54 $\mu\text{g/kg}$, would result in an intake of 0.087 $\mu\text{g/kg bw/day}$ in an infant of less than 2 months, which is below the PTDI of 0.1 $\mu\text{g/kg bw/day}$.
15. In 1977 JMPR recommended an ADI of 0.1 $\mu\text{g/kg bw}$ (combined total for dieldrin and the closely related substance aldrin)⁷. Aldrin is readily metabolised to dieldrin in plants and animals. Only rarely are aldrin residues present in food or in the great majority of animals, and then only in very small amounts. Therefore, national and international regulatory bodies have considered aldrin and dieldrin together. The ADI was based on NOAELs of 1 mg/kg of diet in the dog and 0.5 mg/kg of diet in the rat, which are equivalent to 0.025 mg/kg bw/day in both species. JMPR applied an uncertainty factor of 250 based on concern about carcinogenicity observed in mice.

16. This ADI was reaffirmed in 1994 by JMPR⁸, and was converted into a Provisional Tolerable Daily Intake (PTDI) because aldrin and dieldrin were no longer used as pesticides and are therefore viewed as environmental contaminants. Although levels of aldrin and dieldrin in food have been decreasing, dieldrin is highly persistent and bioaccumulates.
17. WHO reported that dieldrin has been detected in breast milk at a mean concentration of 0.5–11 µg/kg of milk in Europe and the USA. Breast-fed babies receive doses of approximately 1 µg/kg bw/day when mothers' milk contains 6 µg of dieldrin per litre. Although concentrations in breast milk decreased from an average of 1.33 µg/kg of milk in 1982 to 0.85 µg/kg of milk in 1986, higher concentrations (mean 13 µg/litre) have been found in breast milk from women whose houses were treated annually with aldrin⁹.

DDT and DDE

18. Consumption of breast milk containing the highest detected concentrations of p,p'-DDE (38.7 µg/kg), o,p'-DDT (0.43 µg/kg) and p,p'-DDT (1.72 µg/kg), would result in intakes of 6.19, 0.069 and 0.275 µg/kg bw/day, respectively in an infant of less than 2 months. Thus total intakes of DDT (both isomers) and DDE would be below the PTDI of 10 µg/kg bw/day set by JMPR for any combination of DDT and its metabolites DDD and DDE¹⁰.

Metals

19. Thirteen elements were analysed in 91-114 individual milk samples. In most cases, the reporting does not allow identification of numbers of donors that these samples represent. Worst-case intake from breast milk has been calculated, using the maximum detected concentration. Total intakes of these metals were estimated from the maximum intakes from breast milk at different ages together with maximum estimated intakes from non-soya infant foods recently reviewed by the Committee¹¹. The non-soya data are used because it is assumed that breastfed infants will not be fed on soya formula.
20. Cadmium, chromium, antimony and mercury concentrations were below the limit of quantitation in all samples, with very few above the limit of detection. The data are therefore considered insufficiently accurate for further analysis but are not of toxicological concern.
21. Aluminium was above the limit of quantitation in 24% of 91 samples, with a maximum concentration of 186 µg/kg. Estimated intakes from breast milk ranged from 30 µg/kg bw/day below 2 months of age to 7 µg/kg bw/day at 8-10 months. The total estimated intakes of aluminium ranged from 44 to 184 µg/kg bw/day and are considerably below the JECFA PTWI¹², which is equivalent to 1000 µg/kg bw/day.
22. Cobalt was above the limit of quantitation in 7% of 91 samples, with a maximum concentration of 0.31 µg/kg. Estimated intakes from breast milk ranged from 0.05 µg/kg bw/day below 2 months of age to 0.01 µg/kg bw/day at 8-10 months. These are considerably below the EVM guidance level, which is equivalent to 23 µg/kg bw/day¹³. No estimates are available of intake from infant foods.

23. Nickel was above the limit of quantitation in 57% of 91 samples (6 donors), with a maximum concentration of 39 $\mu\text{g/kg}$. Estimated intakes from breast milk ranged from 6.2 $\mu\text{g/kg bw/day}$ below 2 months of age to 1.4 $\mu\text{g/kg bw/day}$ at 8-10 months. The total estimated intakes of nickel ranged from 6.2 to 9.0 $\mu\text{g/kg bw/day}$. This is up to 80% above the TDI of 5 $\mu\text{g/kg bw/day}$ set by WHO¹⁴. The COT previously concluded that the exposure estimates from infant food were likely to be over-estimates due to use of upper bound concentrations and worst case scenario consumption, and therefore the exceedance of the TDI was unlikely to be of significance. Furthermore, the COT noted that ingestion of nickel may exacerbate eczema in pre-sensitised individuals, and infants are less likely than adults to be sensitised to nickel¹¹.
24. Arsenic was above the limit of quantitation in 7% of 91 samples, with a maximum concentration of 4.0 $\mu\text{g/kg}$. Estimated intakes from breast milk ranged from 0.64 $\mu\text{g/kg bw/day}$ below 2 months of age to 0.15 $\mu\text{g/kg bw/day}$ at 8-10 months. The total estimated intakes ranged from 0.65 to 2.05 $\mu\text{g/kg bw/day}$, which are all within the JECFA PTWI¹⁵, equivalent to 2.14 $\mu\text{g/kg bw/day}$. The COT has previously concluded that there are no appropriate safety guidelines for inorganic or organic arsenic, and that exposure to inorganic arsenic should be As Low As Reasonably Practicable¹⁶. No data are available relating to the species of arsenic, or the sources of maternal exposure leading to the levels detected in breast milk. The majority of dietary exposure is organic arsenic from fish, and the small number of samples in which arsenic was quantifiable may be consistent with a small proportion of the women eating larger amounts of fish. However, no information is available on the form of arsenic present in the breast milk.
25. Tin was above the limit of quantitation in only two of 91 samples, with a maximum concentration of 6 $\mu\text{g/kg}$. The estimated intakes from breast milk ranged from 0.96 $\mu\text{g/kg bw/day}$ below 2 months of age to 0.22 $\mu\text{g/kg bw/day}$ at 8-10 months. The total estimated intakes ranged from 1.5 to 19 $\mu\text{g/kg bw/day}$. All estimated intakes are considerably below the EVM guidance value¹³, which is equivalent to 220 $\mu\text{g/kg bw/day}$.
26. Lead was above the limit of quantitation in 7% of 114 samples, with a maximum concentration of 2.6 $\mu\text{g/kg}$. The estimated intakes from breast milk ranged from 0.42 $\mu\text{g/kg bw/day}$ below 2 months of age to 0.1 $\mu\text{g/kg bw/day}$ at 8-10 months. The total estimated intakes ranged from 0.44 to 0.69 $\mu\text{g/kg bw/day}$. All estimated intakes are below the JECFA PTWI¹⁷, which is equivalent to 3.6 $\mu\text{g/kg bw/day}$.
27. Copper was above the limit of quantitation in all 114 samples, with a maximum concentration of 896 $\mu\text{g/kg}$. The estimated intakes from breast milk ranged from 143 $\mu\text{g/kg bw/day}$ below 2 months of age to 33 $\mu\text{g/kg bw/day}$ at 8-10 months. The total estimated intakes ranged from 109 to 184 $\mu\text{g/kg bw/day}$. All estimated intakes are below the JECFA PTWI¹⁸, which is equivalent to 500 $\mu\text{g/kg bw/day}$, but total estimated intakes for the younger age groups marginally exceeded the EVM Safe Upper Level¹³, which is equivalent to 160 $\mu\text{g/kg bw/day}$. The dietary exposure estimates are likely to overestimate actual intakes, and since copper is an essential element, extrapolation to infants on a bodyweight basis may not be appropriate.

28. Zinc was above the limit of quantitation in all 114 samples, with a maximum concentration of 9424 $\mu\text{g/kg}$. The estimated intakes from breast milk ranged from 1508 $\mu\text{g/kg}$ bw/day below 2 months of age to 349 $\mu\text{g/kg}$ bw/day at 8-10 months. The total estimated intakes ranged from 1411 to 2264 $\mu\text{g/kg}$ bw/day. All estimated intakes exceed the JECFA PTWI¹⁹, which is equivalent to 1000 $\mu\text{g/kg}$ bw/day, by 40-140%. Zinc is an essential element, and extrapolation to infants on a bodyweight basis may not be appropriate.
29. Selenium was above the limit of quantitation in all samples, with a maximum concentration of 28 $\mu\text{g/kg}$. The estimated intakes from breast milk ranged from 4.5 $\mu\text{g/kg}$ bw/day below 2 months of age to 1.0 $\mu\text{g/kg}$ bw/day at 8-10 months. The total estimated intakes ranged from 3.1 to 5.8 $\mu\text{g/kg}$ bw/day. All estimated intakes are below the EVM Safe Upper Level¹³, which is equivalent to 7.5 $\mu\text{g/kg}$ bw/day. Selenium is an essential element and extrapolation to infants on a bodyweight basis may not be appropriate.

Dioxin and dioxin like-PCBs

30. Based on analysis of 15 pooled samples of breast milk, the total TEQ content, including the dioxin-like PCBs was estimated to range from 12 to 28 pg WHO-TEQ/g fat, corresponding to 0.26 to 0.78 WHO-TEQ/g whole weight.
31. Table 1 compares the estimated dioxin and dioxin-like PCB intakes derived from the SUREmilk data, with intakes from milk collected from women in Birmingham, Glasgow and Cambridge in 1993/4^{3,20}, and from infant foods²¹. The data indicate that intakes by breastfed infants have decreased by over 50% since 1993/4. It should be noted that the accuracy of these estimates at different ages is unclear, since all data are derived from pooled milk and do not allow for the decreasing concentrations of dioxins and PCBs with duration of lactation.

Table 1 Dietary intakes of dioxins and dioxin-like PCBs by breast-fed infants

Age (months)	Mean consumption of breast milk ² (g/kg bw/d)	Estimated upper bound dietary intakes of dioxins + PCBs (pg WHO-TEQ/kg bodyweight/day)		
		Human milk	Baby foods	
<2	160	Suremilk Pools A-Z	1993/94 ^{3,20}	2003 ²¹
2-3	140	41-109 (mean 72)	163	
3-4	124	36-97 (mean 64)	144	
4-5	103	30-80 (mean 53)	120	
5-6	79	23-62 (mean 41)	92	0.3-0.4
6-7	63	18-49 (mean 33)	73	
7-8	42	12-33 (mean 22)	49	
8-10	37	11-29 (mean 19)	43	0.4

32. The majority of the TEQ derives from 1,2,3,7,8-pentachlorodibenzodioxin (24%), 2,3,4,7,8-pentachlorodibenzo-furan (20.6%), PCB126 (15%), PCB156 (8.4%), 1,2,3,6,7,8-hexachlorodibenzodioxin (8.0%) and 2,3,7,8-tetrachlorodibenzodioxin (7.5%). The half-lives of the dioxin and furan congeners in humans have been estimated to be 5.3-16, 5.0-19.6, 3.5-14 and 4-11 years, respectively, and the estimated half-life for PCB156 is 4.2 years²². Half-life data are not available for PCB 126 and there is a need for generation of these data to support the risk assessment.

Previous COT evaluations

33. COT last discussed concentrations of dioxins and PCBs in human milk in 1997, when the TDI for dioxins and dioxin-like PCBs was 10 pg TEQ/kg bw/day⁴. At that time the mean total intake of breast-fed infants was reported to range from 170 pg Int-TEQ/kg bw/day at 2 months of age to 39 pg TEQ/kg bw/day at 10 months of age. Since the toxicity equivalency factors have been revised in the intervening period, these data have been converted to the WHO-TEQ in Table 1. The Committee noted a number of factors, including:
- infants may be more susceptible to these compounds
 - a high level of exposure during a critical period of early postnatal development may have adverse effects, whereas exposure to the same level in later life would not
 - the period of breast-feeding is short compared with the time needed to accumulate these compounds in the body
 - there are known benefits to breast-feeding.
34. Overall, the Committee concluded that, although intakes of dioxins and PCBs by breast-fed babies were higher than is desirable, breast-feeding should continue to be encouraged on the basis of convincing evidence of the benefits of human milk to the overall health and development of the infant. The Committee recommended that the levels of dioxins and PCBs in human milk should be surveyed at regular intervals and, if levels did not continue to fall, that a review should be instigated to investigate whether inputs of these pollutants to the environment can be reduced further, so as to reduce human exposure. The COT also noted that it would be desirable to investigate the potential for accumulation of the dioxin and PCB congeners which occur in breast milk, that is their disposition in and elimination from the body, especially in early life.
35. In 2001, the COT revised the Tolerable Daily Intake (TDI) for dioxins and dioxin-like PCBs²³. The Committee noted the advice of the COC, that TCDD should be regarded as a “probable human carcinogen”, but that the negative genotoxicity data and evidence from mechanistic studies suggested that a threshold approach to risk assessment was appropriate. The COT considered that, because of the long half-life of TCDD, the risk assessment should be based on the body burden. A TDI of 2 pg

WHO-TEQ/kg bw/day was established, based upon effects on the developing male reproductive system mediated via maternal body burden. The body burden of TCDD at steady state is about 2000-fold higher than the average daily intake, and therefore occasional exceedance of the TDI would not be expected to result in harmful effects, provided that intake averaged over a prolonged period is within the TDI.

36. In 2003, the FSA published the results of the 2001 Total Diet Study of dioxins and dioxin-like PCBs²⁴. The key observations were:
- Estimated total dietary intakes of dioxins and dioxin-like PCBs by all age groups fell by around 50% between 1997 and 2001, continuing the reduction in intakes seen from the previous surveys.
 - The percentage of school-aged children estimated to exceed the TDI fell from 62% in 1997 to 10% in 2001.
 - The percentage of toddlers (aged 1.5 – 4.5 years) estimated to exceed the TDI fell from 97% in 1997 to 37% in 2001.

Comments on new data

37. Data on dioxins and PCBs in breast milk may not be directly comparable because of the factors that influence the levels. Dioxin content of breast milk varies with maternal age, and previous breast-feeding history. The method of milk collection will influence the concentrations detected because the fat content varies during the course of a single feed, and dioxin levels tend to decrease with duration of lactation. However, the SUREmilk data indicate that concentrations have decreased, which would be consistent with the decreasing dietary exposure to dioxins and PCBs.
38. In infants under 2 months of age, the estimated intakes of dioxins and dioxin-like PCBs from breast milk could result in daily intakes of 20-60 times the TDI. The TDI is set at an intake that would prevent accumulation of dioxins in a woman's body to a body burden that could be harmful to the fetus. The half-life of TCDD is considered to be in the region of 7.5 years, and the other predominant congeners also have half-lives of several years. It could therefore take in the region of 30 years or more for the body burden to reach steady-state.
39. The infant's body burden is lower than that of its mother because of its low body fat. Weisglas-Kuperus *et al.* (2000) reported on concentrations of marker PCBs in blood of children aged 42 months²⁵. The data indicated that the average children's PCB body burdens were 15-20% of the average maternal body burden, and the PCB body burden of breast-fed children was about 3-4 times that of formula-fed children. Similar differences might be expected for the TEQ body burden, but data are not available because of the large volume of blood needed for the analysis.
40. Pollitt (1999) calculated accumulated TEQ body burden, assuming a half-life of 9 years for all congeners, 100% absorption and a zero body burden at birth, using the previous UK data for dioxins

and dioxin-like PCBs in breast-milk²⁶. Breast-feeding for 6 months was predicted to result in increased body burden in early life, but the steady state level, reached at about 40-50 years of age, was not increased. The most recent JECFA monograph on dioxins and dioxin-like PCBs used a physiologically-based pharmacokinetic (PBPK) model to predict the effects of transplacental exposure and 6 months of breast-feeding on the body burden of the infant²². This model uses a number of different assumptions on the relative partition coefficients of milk fat and adipose tissue. It is unclear which of these are most appropriate, but the worst case predicts a higher body burden in young children than in adults.

41. The COT previously commented on studies of development of children in Rotterdam and Groningen, with cohorts sub-divided into groups that were breast-fed for at least 6 weeks or formula fed²³. The researchers reported that dioxin exposure was associated with impaired cognitive development and changes in the children. The COT concluded that concerns over the known and potential confounders made it impossible to reach firm conclusions. However, if the effects were real, they were most likely to be due to pre-natal exposure, and breast-feeding ameliorated the effects. In these studies, the mean concentrations of dioxins and dioxin-like PCBs in breast milk were in the region of 60-70 pg WHO-TEQ/g fat, i.e 2-5 times higher than those reported in the SUREmilk study.
42. The most sensitive effect of dioxins and dioxin-like PCBs are considered to be impairment of sperm production and morphology in the male offspring resulting from exposure *in utero*. It is unclear whether effects on sperm quality or other reproductive effects would be expected to result from post-natal exposure. There are no available studies of sperm quality in adult men who were breast-fed as infants compared with those who were formula-fed. However, compared with the previous COT evaluation on dioxins and PCBs in breast-milk (paragraph 33), there is less concern that infants could be more susceptible to these compounds. Any possible risks need to be considered in the context of the revised basis for the TDI, the decreasing concentrations of dioxins and dioxin-like PCBs in food and in breast milk, and the well-established benefits of breast-feeding²⁷.

Conclusions

43. We *note* that the SUREmilk samples were collected primarily to explore the viability of breast milk collection methods and do not constitute a rigorous survey. Nevertheless, it is possible to draw some conclusions on the toxicological implications of the data.
44. We *conclude* that the estimated intakes of metals and other elements associated with the highest detected concentrations in the breast milk samples do not raise toxicological concerns.
45. We *consider* that the data on non-dioxin-like PCBs should be reviewed after completion of the international risk assessment of non-dioxin-like PCBs.
46. We *consider* that although intakes of HCB by some breast-fed babies may be higher than is desirable, this relatively short period of exceeding the TDI is unlikely to result in adverse effects. We *consider* that the intakes of the other organochlorine pesticides measured in this study were within relevant tolerable intakes and do not raise toxicological concerns.

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47. The intakes of dioxins and dioxin-like PCBs derived from the SUREmilk study appear to be less than half of those from breast milk sampled in 1993/4. This indicates that concentrations of dioxins and dioxin-like PCBs in breast milk have decreased; however we caution that the data may not be directly comparable to those reported previously or representative of the UK population.
 48. Dietary exposure to dioxins and dioxin-like PCBs has decreased considerably over the past 20 years, and these substances are eliminated slowly from the body. The concentrations of these substances in breast milk are therefore expected to continue to fall.
 49. The TDI is set to protect against the most sensitive effects of dioxins and dioxin-like PCBs, which occur in the male fetus as a result of the mother's accumulated body burden. There is uncertainty with respect to whether similar effects would arise from post-natal exposure, but there is currently no basis for assuming that the young infant is at increased risk.
 50. Taking into account that the TDI is now set to protect against reproductive effects and the evidence that concentrations of dioxins and dioxin-like PCBs in breast milk are declining, the new data do not suggest any reason to alter Government advice that breast-feeding should continue to be encouraged on the basis of convincing evidence of the benefits of human milk to the overall health and development of the infant.
 51. We *recommend* that monitoring of contaminants in breast milk should continue, particularly for HCB and the dioxins and PCBs, incorporating appropriate studies of stability and recovery. Data on other contaminants of potential concern, such as the brominated flame retardants, are also required.

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July 2004

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Tryptophan and the eosinophilia-myalgia syndrome

Introduction

1. In the autumn of 1989, a previously unrecognised epidemic illness was reported in the United States. Its principal features included an elevated eosinophil count and severe myalgia, often accompanied by arthralgia, oedema, dyspnoea, fever and rash, and it was termed the eosinophilia-myalgia syndrome (EMS). The syndrome was quickly associated with the consumption of L-tryptophan-containing dietary supplements and these were withdrawn from the US market. In total, over 1500 cases of EMS were reported in the US, including at least 37 deaths.
2. Several cases of EMS were also reported in the UK, Germany, Canada, Belgium, France, Israel and Japan. In 1990 the COT endorsed a ban on the addition of tryptophan to foods (including supplements) in the UK. Since that time, a number of epidemiological, analytical and toxicological studies have been published, which have investigated possible causes of the EMS epidemic. It has been claimed that the causes of EMS are now known and that there is no need for the continuing ban of added tryptophan in food¹. The Committee was therefore asked to consider the available data on tryptophan and EMS and to advise on the risks to health of tryptophan in food supplements.

Background

3. L-tryptophan is one of 8 essential amino acids that cannot be made by the body and therefore must be included in the diet daily to maintain nitrogen balance. It is important for protein synthesis and is the precursor of a number of biologically important compounds including serotonin, melatonin, tryptamine, quinolinic acid, kynurenic acid. It is also the precursor of NAD and NADP and can replace niacin².
4. Good food sources of L-tryptophan are foods high in protein such as meat (230 mg/100g in beef), cheese (325 mg/100g in Edam cheese), fish, milk and eggs (165 mg/100g)². In 1991, the Committee on Medical Aspects of Food and Nutrition Policy (COMA) concluded that the protein quality of the UK diet was sufficient not to set recommendations for individual amino acids. It noted that studies of the amino acid pattern of the British diet indicated that the average household consumes a diet which supplies adequate amounts of essential amino acids³. There have been no recent estimates of dietary L-tryptophan intakes but the National Food Survey of 1974 indicated the average intake to be 890 mg/day (equivalent to 14.8 mg/kg bw/day in a 60 kg adult)⁴. Tryptophan requirements in adults have been estimated to be 6 mg/kg bw/day⁵.
5. Although the addition of isolated tryptophan to foods in the UK is generally banned, the addition of tryptophan is permitted in certain foods such as infant formulas, follow on formulas and processed cereal based foods and baby foods, which are regulated by EU legislation. Tryptophan was permitted in such foods to ensure protein quality and it was understood that natural protein sources of tryptophan, rather than isolated tryptophan, were used. EU legislation further permits the addition of tryptophan to foods for particular nutritional uses (PARNUTS). Manufacturers of certain categories of PARNUTS food such as those intended for sportsmen, dieting or diabetics are obliged to notify the FSA prior to marketing.

6. Prior to the 1990 ban, L-tryptophan-containing dietary supplements were typically used for insomnia, depression and premenstrual syndrome, and had been used by bodybuilders. In the UK, one to two 500 mg tablets were typically taken per day, usually at night. In the US, at the time of the EMS outbreak it was reported that people were taking up to 8000 mg/day supplemental L-tryptophan.

Eosinophilia-myalgia syndrome

7. The case definition of EMS by the US Centers for Disease Control (CDC) was: (1) a total eosinophil count greater than $1 \times 10^9/L$, (2) generalised myalgias at some point during the course of the illness of sufficient severity to limit the ability to pursue normal activities, and (3) exclusion of other neoplastic or infectious conditions that could account for the syndrome⁶. Case reports also described arthralgia, swelling of the extremities, rash, fever, cough, interstitial lung disease, arrhythmias, ascending polyneuropathy and sclerodermiform skin thickening in EMS patients. After ceasing to take L-tryptophan supplements, improvement was slow, with symptoms continuing to progress in some patients⁷. While most symptoms were reported to have improved 2 years later, a study of 205 patients reported that cognitive changes (impaired memory, impaired concentration or mood change, reported in 28% of patients) and neuropathy had not⁸.
8. The features of EMS closely resemble the 1981 Spanish toxic oil syndrome epidemic. The toxic oil syndrome was associated with the consumption of adulterated rapeseed oil, affected approximately 20,000 people and resulted in more than 300 deaths; however, the causes were never established. The features of toxic oil syndrome included fever, rash, pneumonitis, myalgias, eosinophilia, neuromuscular abnormalities, pulmonary hypertension and scleroderma-like skin changes. The differences between the two syndromes are few, the incidence and intensity of pulmonary effects such as coughs, infiltrates, dyspnoea and plural effusions being fewer and less severe in EMS patients than toxic oil syndrome patients⁷.
9. There are also some similarities with eosinophilic fasciitis, a rare disorder first described in 1975, which is characterised by eosinophilia of both the peripheral blood and connective tissue, fascial inflammation and thickening, and pain and swelling of the extremities^{9,10}. The pathophysiology and aetiology of eosinophilic fasciitis remain poorly understood.

Possible causes of EMS

Link to L-tryptophan produced by one manufacturer

10. L-tryptophan used in dietary supplements on sale in the US was manufactured by 6 bulk manufacturers. In epidemiological studies, 97-100% of EMS cases meeting the CDC criteria were traced to L-tryptophan manufactured by one company, Showa Denko KK of Japan^{11,12,13}. Furthermore, EMS symptoms were reported to resolve in patients who switched from Showa Denko L-tryptophan to non-implicated L-tryptophan supplements^{14,15}. The authors of one of the epidemiological studies noted that all the EMS cases that could be traced back to Showa Denko in their study had consumed L-tryptophan produced between October 1988 and June 1989, possibly suggesting that manufacturing variables were important.

11. National surveillance in the US indicated that on average 10% of consumers of Showa Denko EMS-implicated L-tryptophan were diagnosed with EMS and there was no dose-risk relationship⁷. EMS patients who had taken L-tryptophan supplements had taken them for between 0 and 3,668 days before onset of illness (0 indicating onset of illness on the same day as first taking L-tryptophan supplements), with a median of 127 days and a mean of 275 days⁶.

Identification of contaminants in EMS-implicated L-tryptophan

12. Showa Denko L-tryptophan was produced by a fermentation process involving the use of *Bacillus amyloliquefaciens*. On 25 December 1988 the company started using a new strain (Strain V) of *Bacillus amyloliquefaciens* which increased the synthesis of two intermediates in L-tryptophan biosynthesis, serine and 5-phosphoribosyl-1-pyrophosphate. Additionally, the amount of powdered activated carbon used in purification steps was reduced from usually 20 or more kg/batch in 1988 to 10 kg in most batches in 1989. Furthermore, between October 1988 and June 1989 some L-tryptophan batches partially bypassed a filtration step using a reverse osmosis filter to remove chemicals with molecular weights of more than 1000. Statistical analysis showed significant associations between batches of L-tryptophan associated with EMS and the use of Strain V *Bacillus amyloliquefaciens* and the reduction in the amount of activated carbon used¹¹.
13. More than 60 minor contaminants were identified in EMS-associated batches of Showa Denko L-tryptophan. On comparing batches of EMS-associated Showa Denko L-tryptophan, non-EMS associated Showa-Denko L-tryptophan and control L-tryptophan from another manufacturer, six of these contaminants were associated with EMS¹⁷. Three of the contaminants were identified as 1,1'-ethylidenebis[tryptophan] (EBT), 3-(phenylamino)-L-alanine and 2(3-indolylmethyl)-L-tryptophan. Two others were later identified as 3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo-[2,3-b]-indole-2-carboxylic acid and 2-(2-hydroxyindoline)-tryptophan¹⁷. One of the contaminants remains unidentified.

Toxicological studies of tryptophan and its implicated contaminants

14. L-tryptophan has low oral toxicity². The lowest reported LD₅₀ is 1.6 g/kg bw in rats, where death was thought to have resulted from an accumulation of metabolites such as ammonia and urea. Equivocal changes in the liver, including fatty change and fibrosis, were seen in a study in rats dosed with 250 mg/kg bw/day for 3 days¹⁸. In a rat carcinogenicity bioassay conducted by the US National Cancer Institute no evidence of carcinogenicity was found².
15. Treatment of rats with adrenal dysfunction with 100 or 1000 mg/kg bw/day L-tryptophan (Japanese pharmacopoeia grade, not manufactured by fermentation) by gavage for 7 days resulted in a statistically significant increase in the number of eosinophils in the peripheral blood¹⁹. Guinea pigs supplemented orally via gelatine capsules with 400 mg/kg bw/day L-tryptophan (purchased from a laboratory chemical supplier several years after Showa-Denko had ceased production of L-tryptophan) showed a statistically significant decrease in the number of circulating eosinophils and increase in the number of eosinophils in the bronchial alveolar lavage fluid compared to controls²⁰. The significance of these results is not clear.

16. An investigation in female Lewis rats, dosed by gavage for 38 days with 1600 mg/kg bw/day EMS-implicated L-tryptophan, showed fasciitis and perimyositis, whereas those dosed with 1600 mg/kg bw/day control L-tryptophan did not²¹. A study was conducted to determine whether EBT was responsible for these effects. All animals treated with EMS-associated L-tryptophan (2000 mg/kg bw/day), EBT (40 mg/kg bw/day) or EBT plus control L-tryptophan by gavage, 6 days/week for 6 weeks showed significant myofascial thickening compared to animals receiving control L-tryptophan or vehicle-only controls²². However, even animals treated only with control L-tryptophan showed a mild but statistically significant increase in the thickness of the myofascia compared to vehicle-only controls. This thickening of the myofascia could be related to prior resolved inflammation rather than a direct effect of the tryptophan. Immune effects, including increased frequency of CD8, Ia and IL-2 receptor-positive cells in the peripheral blood, were observed in EMS-associated L-tryptophan-treated animals only. Control L-tryptophan in both studies was US pharmacopoeia-grade L-tryptophan which had not been associated with cases of EMS and contained no measurable EBT.
17. In a study in mice, administration of EBT (40 µg/kg bw/day, i.p. for up to 6 weeks) resulted in an inflammatory reaction in the dermis and subcutis, with hyperplasia of the epidermis in 20% of animals²³. Focal inflammation was also seen in saline and L-tryptophan (non-EMS implicated, 30 mg/kg bw/day)-treated animals but was not as severe as in EBT-treated animals. Cellular infiltration was accompanied by fibrosis and destruction of the adipose layers and panniculus carnosus muscle. These changes were more severe in EBT-treated animals than in other groups.
18. Three female Lewis rats were treated with 40 mg/kg bw/day EBT and four with 40 mg/kg bw/day non-EMS-implicated L-tryptophan, i.p., for up to 132 days, with tail vein blood drawn at days 26 and 72 for haematological analysis²⁴. No abnormalities were seen in the four L-tryptophan-treated rats. Some necrotic muscle fibres were observed in all three animals treated with EBT and in two there was inflammation of the fascia and perimysium. No abnormalities were observed in the four L-tryptophan-treated rats.
19. *In vitro* studies have shown that EBT stimulates human fibroblast proliferation and increases collagen synthesis and type I collagen mRNA levels in human fibroblasts^{25,26}.
20. 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA), the breakdown product of EBT, has been shown to affect the survival of 1 month old spinal cord cultures derived from fetal mice. This effect only occurred with the S-isomer, and not the R-isomer of the compound. Co-treatment of cultures with antisera to interleukin-1α or the murine IL-1 receptor abolished the effect²⁷.
21. Mononuclear cell supernatants from hypereosinophilic syndrome patients (an idiopathic disease in which eosinophil levels in the peripheral blood are raised to > 1500/µL for 6 months or more, leading to multiple organ system effects), when incubated with IL-2 and either EBT or MTCA, supported the formation of colonies with neutrophils, macrophages and 'a small number' of eosinophils²⁸. Two to three-fold increased levels of IL-6 were found in the supernatant of mononuclear cells from a hypereosinophilic syndrome patient and two normal volunteers when stimulated with IL-2 and either EBT or MTCA. IL-5 has been reported to be induced by stimulating human splenic T cells *in vitro* with EBT²⁹.

22. 3-(Phenylamino)-L-alanine is closely related to aniline contaminants identified in the oil consumed by patients of the toxic oil syndrome. One of the toxic oil syndrome contaminants, 3-(phenylamino)-1,2-propanediol, is metabolised to 3-(phenylamino)-L-alanine by rat hepatocytes and human liver tissue³⁰. However, in a subchronic toxicity study of 3-(phenylamino)-L-alanine in rats, no EMS-like or other abnormalities were found³¹. One study showed that 3-(phenylamino)-L-alanine affects the binding of L-tryptophan to rat hepatic nuclear membranes (which contain a tryptophan receptor) *in vitro*³² but the significance of this is not clear.
23. None of the other four contaminants appear to have been studied toxicologically. Two were identified only relatively recently¹⁷ one remains unidentified.

Susceptible subgroups

24. One study showed that people with EMS were more likely to have a genotype associated with poor CYP2D6-dependent metabolism³³. However, the majority of EMS cases had extensive CYP2D6-dependent metabolism.

EMS not linked to implicated L-tryptophan

25. A small number of EMS cases were not traced to Showa Denko L-tryptophan. In two of the epidemiological studies, all but one EMS case in each study was conclusively linked with Showa Denko L-tryptophan^{13,14}. In one of the studies, the authors analysed the L-tryptophan consumed by the non-Showa Denko associated case and other L-tryptophan supplements. They observed that the L-tryptophan consumed by the non-Showa Denko associated case contained EBT and that the HPLC 'signature' exactly matched that of other Showa-Denko L-tryptophan samples and did not match that of L-tryptophan samples from the manufacturer it was apparently from. The authors suggested that incorrect labelling at some stage of the tableting or distribution process could have lead to erroneous information on the source of the L-tryptophan¹³.
26. One of the epidemiological studies also looked at 'possible' EMS cases, which did not meet the CDC criteria for EMS but in which there was either raised eosinophil count in the absence of other symptoms or 2 or more other features of EMS without raised eosinophil count¹³. Thirty-two possible EMS cases were identified from users of non-Showa Denko L-tryptophan.
27. Twelve case of EMS were reported to have been diagnosed in Canada between July 1992 and June 1993, more than 2 years after the EMS epidemic³⁴. The cases were not linked to L-tryptophan consumption. The authors also identified 14 'possible' EMS cases, which did not fully meet the CDC criteria for EMS but noted that there were other possible diagnoses for these cases, including eosinophilic fasciitis, hypereosinophilic syndrome, Churg-Strauss syndrome (severe asthma associated with raised eosinophil counts), small vessel vasculitis, fibromyalgia, atypical arthritis, drug reactions and asthma.

28. Three percent of 1056 cases of EMS in the USA denied having taken L-tryptophan supplements⁶. In addition, of 1055 cases for whom the information was available, one had first reported symptoms in 1954, one in 1980, one in 1981, four in 1983, two in 1985, four in 1986, 13 in 1987, 31 in 1988 and 31 in 1989 before May, i.e. prior to the EMS epidemic. It is not clear whether these cases had consumed L-tryptophan supplements.
29. Two case reports are available of cases of EMS in people who denied having taken L-tryptophan supplements^{35,36}.
30. Several reports have described cases of EMS and EMS like symptoms linked to consumption of 5-hydroxy-L-tryptophan, a metabolite of L-tryptophan^{37,38,39}. 5-Hydroxy-L-tryptophan is not manufactured in the same way as L-tryptophan, but is either synthesised from 5-benzyloxyindole or extracted from the seeds of the African tree *Griffonia simplicifolia*. A contaminant, 1,2,3,4,4a,9a-hexahydro- β -carboline-3-carboxylic acid, which has a similar chemical structure to MTCA, has been found in 5-hydroxy-L-tryptophan produced by both methods^{39,40}.

Current availability of tryptophan medicines and supplements

31. Due to its role as a precursor to serotonin, L-tryptophan is available in the UK as a prescription drug for the treatment of depression. Its use is limited to severe and disabling depression which has continued for more than 2 years, following the trial of other antidepressant drugs and only together with the use of other antidepressant drugs. The dose taken is 1 to 2 grams, three times a day (maximum dose 6 grams), to be reduced if the patient is elderly or taking monoamine oxidase (MAO) inhibitors. Prescribing GPs are advised to regularly survey and monitor eosinophil count, haematological changes and muscle symptomatology.
32. Only one product is currently available in the UK, the Merck Pharmaceuticals product Optimax. At the time of the EMS epidemic four possible cases of EMS were linked to Optimax. One of the cases was confirmed as matching all the CDC criteria for EMS⁴¹. It has been suggested that Optimax may not have been the only source of L-tryptophan this case had been exposed to⁴². Because of the concern over EMS, a unit was established by the manufacturers in 1994 to facilitate the monitoring of patients prescribed Optimax. Prescribers and patients must be registered with the unit in order to receive Optimax. Prescribers are sent an initial questionnaire on registration, followed by questionnaires at 3 and 6 months and thereafter every 6 months. Since monitoring began over 5000 patients have been treated with Optimax, with a mean dose of 2228 mg/day L-tryptophan⁴². The manufacturers stated that up-to-date questionnaires are available for 96% of patients. No patients meeting all the CDC criteria for EMS have been identified.
33. The purity criteria for the L-tryptophan used in Optimax are stated to be similar to the European Pharmacopoeia monograph⁴². In particular there is a limit for EBT of 10 ppm.

34. A small number of dietary supplements containing L-tryptophan are currently available in Belgium and the Netherlands^{1,43} and one product is available in the Czech Republic⁴⁴. Literature searches have not identified any reports of cases of EMS in these countries. The source of the L-tryptophan used in these products and the purity criteria used in their manufacture are not clear. The Health Council of the Netherlands has recommended a maximum supplementary intake of 0.6 g/day tryptophan. This was based on the amount of tryptophan that would be supplied by the protein intake advised for women in the Netherlands. No specific purity criteria were recommended⁴⁵.

COT evaluation

35. On the balance of evidence, it is likely that L-tryptophan *per se* was not causal for EMS, and that EMS was due to one or more contaminants. Changes to the manufacturing and purification process by one particular manufacturer may have increased levels of these contaminants to harmful levels. EBT was found in some samples of non-EMS implicated tryptophan supplied by other manufacturers, although at lower levels.
36. However, there are some uncertainties. These include whether EMS could arise sporadically. There are no reliable data on the prevalence of EMS and as EMS is a difficult condition to define, with a variety of non-specific symptoms, it is possible that mild cases may not be diagnosed and that there could have been underreporting of EMS prior to its recognition in 1989. An epidemiological study indicated that the use of L-tryptophan supplements increased substantially between 1988 and 1989, the start of the EMS epidemic. It therefore cannot entirely be ruled out that the apparent epidemic may have been due to the increased use of L-tryptophan supplements and the recognition of EMS.
37. There is little information on the possible mechanisms that could lead to EMS. The limited available animal data have not reproduced all the features of EMS. Administration to rats of EMS-implicated L-tryptophan and the contaminant EBT resulted in myofascial thickening. A US pharmacopoeia-grade L-tryptophan, which was not associated with cases of EMS and did not contain any EBT, also caused increased myofascial thickening but to a lesser degree. This thickening of the myofascia could be related to prior resolved inflammation rather than a direct effect of the tryptophan. The toxicological significance of this is therefore unclear.
38. It is not possible to determine specific contaminants causal of EMS. EBT has reproduced some of the features of EMS, such as fasciitis, in animals, but not others, such as eosinophilia. The presence of EBT could be a marker for another contaminant which is responsible for EMS or it could be a number of contaminants that are responsible for the features of EMS. Some of the contaminants found in EMS-implicated L-tryptophan have not been studied toxicologically.
39. It has not been possible to identify any particular subgroups with increased susceptibility to developing EMS.

40. L-Tryptophan supplements are on sale in a small number of countries, including the Netherlands, Belgium and the Czech republic. We are not aware of any cases of EMS being reported in these countries; however, it is not clear that symptoms of EMS would be monitored for or necessarily recognised. We have not been informed of any specific purity criteria used in the manufacture of these supplements.
41. L-Tryptophan is also available on prescription as a licensed medicine, the Merck product Optimax. GPs are advised to closely monitor patients receiving Optimax, including regularly monitoring eosinophil count, and are sent questionnaires at 6 monthly intervals to complete and return. Since Optimax returned to the market in 1994 over 5000 patients have been prescribed Optimax, with no confirmed cases of EMS. This provides reassurance that the purity criteria used for Optimax reduces the risk of EMS. The purity criteria for the L-tryptophan used in Optimax are stated to be similar to the European Pharmacopoeia monograph for L-tryptophan, and include a limit for the contaminant EBT of 10 ppm.

Conclusions

42. We conclude that the new data offer reassurance that as a prescription medicine, tryptophan has not resulted in a detectable increase in risk of EMS. Applying an uncertainty factor of 10 to the mean therapeutic dose of 2228 mg tryptophan per day, to allow for uncertainty with respect to the actual cause of EMS, indicates that a dose of 220 mg tryptophan per day as a dietary supplement would not present an appreciable risk to health, providing that it meets the purity criteria specified in the European Pharmacopoeia.

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June 2004

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Use of PAVA (Nonivamide) as an incapacitant spray

Introduction

1. In 2001 the Home Office requested advice from COT on the health effects arising from the use of a chemical incapacitant spray containing pelargonyl vanillylamide (PAVA or Nonivamide). PAVA is the synthetic equivalent of capsaicin the active ingredient of natural pepper. It is a potent sensory stimulant. It is also used both as a food flavour (at up to 10 ppm in the diet) and in human medicine (topical application as a rubifacient). In the USA it has been given GRAS (Generally Regarded as Safe) status by the FDA as a food flavour.
2. The Sussex Police Force have now been using PAVA spray since 2001, following a pilot exercise in 2000. It is now being used by 2 other police forces in the UK, as well as by police forces in other European Countries and in North America.
3. The COT considered this use in 2001 and agreed a statement in April 2002, which incorporated the advice of the COM on the available mutagenicity data. A copy is attached at Appendix 1. This gives details of the structure of PAVA, its use and a summary of the available toxicity data at that time.
4. The following conclusions were reached:
 - i) We consider that it is not possible to make a complete assessment of the likely adverse health effects that could arise from the use of PAVA spray as a chemical incapacitant in view of the limited data available.
 - ii) We recognise that exposures would be low and for a short period. It is impossible to calculate exposure with any accuracy but we note that dermal exposure would be of the order of 30 mg PAVA from a 1 second burst, with about 3 mg being absorbed. Any systemic exposure is likely to be low (of the order of 0.04 mg/kg bw).
 - iii) The animal model data and experience in use do not give rise to any concerns regarding long term harm to the skin or eyes. However consideration needs to be given as to whether those wearing contact lenses might experience increased irritant effects. It is also noted that no data are available on the potential of PAVA to induce skin sensitisation.
 - iv) The *in-vitro* mutagenicity data, and consideration of metabolites, indicate that PAVA has some mutagenic potential; although negative results were obtained in an *in-vivo* study to investigate mutagenic effects in the bone marrow, data from a further study are needed to provide adequate assurance that this activity cannot be expressed *in vivo*. An *in-vivo* study to investigate the induction of unscheduled DNA synthesis (UDS) in the liver would be appropriate in this regard.
 - v) No data are available to assess whether PAVA has any effects on the reproductive system. In particular the lack of any developmental toxicity studies is of concern as it is possible that pregnant women may be exposed to the spray.

- vi) The data from inhalation studies in volunteers, including those with mild asthma, indicate that there are unlikely to be any adverse respiratory reactions in normal individuals. Some respiratory effects may well occur in asthmatics, particularly since effects were observed in asthmatic volunteers at 0.1% PAVA, which is lower than the 0.3% used in the spray, and given the conditions of increased stress likely when the spray is used.
- vii) Further monitoring of experience in use, including the police officers using the spray, is recommended with particular consideration being given to eye irritancy in those wearing contact lenses and to effects in those with asthma or hay fever and in women who may be pregnant.

New data

- 5. In response to the conclusions in the COT statement Sussex Police have commissioned further studies to provide information on the data gaps highlighted. These comprise a further *in-vivo* mutagenicity study (the liver UDS assay)¹, an investigation of skin sensitisation potential using the local lymph node assay², and an investigation of effects on reproduction using a developmental toxicity study^{3,4}. In addition they have provided some information on experience in use since the COT last considered the issue⁵⁻⁸. PAVA is now being used as an incapacitant spray by 3 Police Forces in the UK.
- 6. The COM considered the new *in-vivo* mutagenicity data at their meeting on 5th February 2004. They concluded that the *in-vivo* liver UDS assay was done to the current OECD guideline (No 486) and was adequate. There was no evidence for the induction of DNA repair, as measured by unscheduled DNA synthesis, in the assay. The COM concluded that the information sought by the Committee had now been provided and that it was possible to conclude that PAVA would not be expected to be an *in-vivo* mutagen⁹. No further mutagenicity data were required.
- 7. The COT considered the new data on skin sensitisation, reproductive toxicity and experience in use at their meeting in May 2004.
- 8. The ability of PAVA to induce skin sensitisation has been investigated in mice using the local lymph node assay². The methodology was consistent with that given in the OECD guideline No 429. Dose levels of 0.8, 2.1 and 4.1% PAVA were employed. Negative results were reported. However there was a high level of inter-animal variability, with for example, very low individual scintillation counts in 2/5 animals treated at the highest dose level. In addition it was felt that a concurrent positive control should have been carried out, as the laboratory concerned appeared to be relatively inexperienced in this assay. No conclusions could therefore be drawn from this study.

9. The potential of PAVA to induce adverse effects on development following exposure *in utero* has been investigated in the rat in a study that conformed to OECD test guideline No. 414, with oral dosing (gavage). Dose levels used in the main study were selected following a preliminary developmental toxicity study in which no effects were seen on embryo-fetal development at doses up to 1000 mg/kg (the maximum dose level recommended in the OECD guideline 414)³. In the main study animals were dosed at 100, 500 and 1000 mg/kg on day 5-19 of gestation⁴. They were then killed and their uteri and contents examined in the usual way. The only significant effect seen was a slight but statistically significant, reduction in fetal weight at the top dose level. The no observed adverse effect level (NOAEL) was 500mg/kg. This was not of concern in view of the large margin of safety.
10. Further data provided by Sussex Police did not indicate any significant adverse effects arising from the use of this spray either in the general public or in officers using the spray⁵. Experience in use has not identified any groups that are particularly sensitive to the spray.
11. Regarding the COT conclusions in 2002, the only outstanding data related to skin sensitisation, in view of the inability to draw any conclusions from the local lymph node assay on mice. As an alternative to repeating this study, consideration was given to obtaining information on the experience in use of PAVA as a topical medicine. As noted earlier it is used, at up to 0.4%, in topical medicines, sometimes under occlusion. Information on whether there was any history of skin sensitisation arising from such use was considered by the Committee at their September 2004 meeting. Data provided by industry and also by the Medicines Healthcare products Regulatory Agency (MHRA) were considered⁶⁻⁸. Products containing up to 0.4% PAVA have been used in human medicines for topical application in many countries, including the UK, for over 50 years. They are generally well tolerated with an insignificant number of adverse reactions. In the UK there have been reports of only 2 adverse reactions (both involving a rash) over the last years. It can be concluded that PAVA does not have any significant skin sensitisation potential in practice.

Revised conclusions

12. Following consideration of these new data the COT agreed the following revised conclusions on the health effects of the use of PAVA incapacitant spray
 - i) We recognise that exposures would be low and for a short period. It is impossible to calculate exposure with any accuracy, but we note that dermal exposure would be of the order of 30 mg PAVA from a one second burst, with about 3 mg being absorbed. Any systemic exposure is likely to be low (of the order of 0.04 mg/kg bw).
 - ii) The animal model data and experience in use do not give rise to any concerns regarding long term harm to the skin and eyes, arising from irritant effects. Although no conclusions can be drawn from the one available animal study to investigate skin sensitisation, experience in use, including in human medicines for topical application, indicates that PAVA is not a skin sensitising agent.

- iii) The new *in-vivo* mutagenicity data provided (negative results in an *in-vivo* liver UDS assay conducted to internationally accepted guidelines) in conjunction with previously evaluated studies allow the conclusion to be drawn that PAVA is not an *in-vivo* mutagen.
- iv) The ability of PAVA to induce adverse effects on the developing offspring following *in utero* exposure has been investigated in a prenatal developmental toxicity study in the rat using oral exposure (by gavage). The compound had low toxicity by the oral route, with no significant effects being seen in the maternal animals at doses up to 1000 mg/kg/day. The only effect seen in the developing offspring at this dose level was a small reduction in fetal weight. There was no evidence of any malformations, skeletal anomalies, or any other adverse effects at this dose level. The NOAEL for effects on the offspring was 500 mg/kg/day. This NOAEL is about 4 orders of magnitude above the expected exposure level arising from use of the spray; there are thus no concerns regarding developmental toxicity.
- v) The data from inhalation studies in volunteers, including those with mild asthma, indicate that there are unlikely to be any adverse respiratory effects in healthy individuals. It is possible that some respiratory effects may occur in asthmatics, particularly since effects were observed in asthmatic volunteers at 0.1% PAVA, which is lower than the 0.3% used in the spray, and given the increased stress likely when the spray is used.
- vi) The available information, both from the toxicity data in experimental studies, and experience in use, indicates that the low exposures arising from the use of PAVA incapacitant spray would not be expected to be associated with any significant adverse health effects. However we recommend that monitoring of experience-in-use be continued.

COT statement 2004/06

October 2004

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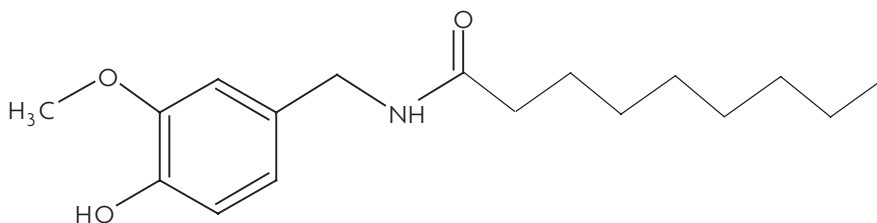
Appendix 1: 2002 COT statement

Use of PAVA (Nonivamide) as an incapacitant spray

Introduction

1. The Home Office has requested advice from the COT on the health effects arising from the use of a chemical incapacitant spray, containing pelargonyl vanillylamide (PAVA or Nonivamide) as the active ingredient. PAVA is the synthetic equivalent of capsaicin the active ingredient of natural pepper. It is a potent sensory stimulant. It is also used both as a food flavour (at up to 10 ppm in the diet) and in human medicine (topical application as a rubefaciant). In the USA it has been given GRAS (Generally Regarded as Safe) status by the Food and Drug Administration as a food flavour.
2. Following a pilot exercise last year the Sussex Police Force is now using PAVA spray as an alternative chemical incapacitant to CS spray. Recently Northamptonshire Police Force also started to use the spray. It is used by police forces in other European countries and in North America.

PAVA has the following chemical structure:



Use of PAVA as incapacitant spray

3. The spray used by Sussex Police consists of a 0.3% solution of PAVA in 50% aqueous ethanol. It is dispensed from hand held canisters (containing nitrogen as propellant) as a coarse liquid stream; the spray pattern is stated to be directional and precise. The canisters contain 50 ml of solution. The instructions are to aim directly at the subject's face, especially the eyes, using a half second burst (still air) or one second burst (moving air), repeating if necessary. The maximum effective range is 8-15 feet and the instructions are not to use at a distance of under 3 feet because of the risk of pressure injury to the eye. The effectiveness of the spray depends on eye contact with small amounts reaching the eyes producing the desired effect. In some cases officers will miss and use more than one burst.
4. Studies on the particle size of the spray indicate that the bulk of the droplets are over 100 μm but a small proportion (1-2%) is in the range 2-10 μm , and there may be traces below 2 μm . Thus it is unlikely that large amounts of PAVA will reach the respiratory system, although the possibility of some reaching the lungs cannot be excluded. It is not possible to estimate the respirable dose.

Toxicity of PAVA

Absorption, Metabolism, Elimination and Excretion

5. Only limited data are available to assess oral absorption of PAVA; the compound shows higher acute toxicity by the parenteral rather than by the oral route suggesting relatively poor oral absorption. More extensive data are available regarding skin absorption, particularly from ointments designed for topical medical use. PAVA has been shown to be well absorbed through the rabbit skin (50-70% in 14 hours) when applied in such ointments (hydrophilic, oil-water emulsions) under an occlusive dressing.¹ More limited skin absorption (12% over 72 hours) was reported in the rat using an aqueous vehicle (phosphate buffered saline) and a non-occlusive dressing³. The only data available on skin absorption from aqueous ethanol are from in-vitro studies using rat skin when the rate of absorption from 50% aqueous ethanol was shown to be considerably faster than that when phosphate buffered saline was used as vehicle³. It should therefore be assumed that there will be some absorption of PAVA following skin or eye contact with the spray.
6. Once absorbed PAVA is distributed throughout the body, extensively metabolised and rapidly excreted (most within 24 hours). The main route of metabolism is hydrolytic cleavage of the amide bond which occurs in liver and other tissue including the skin². There is some evidence for aliphatic hydroxylation, as also occurs with capsaicin³.

Experimental studies in animals

7. Only very limited data are available on PAVA itself; it has a comparable toxicity, as measured by the LD₅₀ value, to capsaicin when given by the intra-peritoneal route to mice (8 mg/kg)^{4,5}. Capsaicin has moderate toxicity by the oral route with LD₅₀ values being reported as 119 and 97 mg/kg in male and female mice and 161 and 148 in male and female rats⁶. Data were provided on an acute inhalation study on PAVA in the rat using exposures of up to 3.6 mg/l for 4 hours, but the report is difficult to follow and no conclusions could be drawn⁷.
8. The skin irritancy of a 3.2% (v/v) solution of PAVA in polyethylene glycol has been investigated in the rabbit using an occlusive dressing and 4 hour exposure⁸. Animals were then observed for up to 3 days post exposure; no signs of irritancy were noted.
9. The ability of the in-use formulation (0.3% in 50% aqueous ethanol) to produce eye irritation has been investigated in the rabbit using the standard OECD test method⁹. Signs of significant irritation were seen from instillation until 3 days post-dose (including some evidence of opacity of the cornea and damage to the iris). However the eyes of all the animals had recovered by 7 days post exposure. These data indicate that the solution should be regarded as irritating to the eyes, but they do not suggest that any long term effects are likely.

10. No data are available on the potential of PAVA to cause skin sensitisation.
11. Few data are available on the effects of repeated exposure to PAVA. These consist solely of the results of a limited 90 day dietary study in the rat¹⁰. Only a single dose level was used, equivalent to about 10 mg/kg body weight/day. This produced no evidence for any toxic effects and can be regarded as a no observable adverse effect level (NOAEL).

Reproductive toxicity

12. No data are available on reproductive toxicity studies on PAVA. Nor are any data available from fertility or developmental toxicity studies using standard methods for either PAVA or capsaicin. There is one report of the treatment of neonatal rats (2 day old) with a relatively high dose level of capsaicin (50 mg/kg using the subcutaneous route) resulting in retardation of sexual development and reduced fertility¹¹. The dose level used resulted in growth retardation throughout adulthood. No conclusions can be drawn from this study regarding any effects of PAVA on the reproductive system. There are no reported investigations of developmental toxicity (teratogenicity), nor of any multigeneration studies to investigate effects on fertility.

Mutagenicity studies

13. The advice of our sister committee, the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) was sought on a package of mutagenicity data on PAVA that had been commissioned by the Sussex Police Force^{12,13,14,15}. Its conclusions are given below:
 - i. The structure of PAVA suggests the possible formation of reactive oxygen species from the phenol moiety, and other possible active metabolites which may be mutagenic.
 - ii. Data are available from 3 in-vitro studies done to current standards. The assay for gene mutation in bacteria gave negative results. Equivocal results were obtained in the mouse lymphoma assay. A clear positive result was however obtained in the assay for chromosome damage in CHO cells in the presence of the exogenous metabolic activation system which was not limited to concentrations producing excessive toxicity. These *in-vitro* data indicate that PAVA has mutagenic potential.
 - iii. Negative results were obtained in a bone marrow micronucleus test, PAVA being given orally at up to dose levels that produced marked toxicity (some lethality).
 - iv. As noted in the COM guidelines, in the case of substances positive in vitro a negative result in a single tissue will not provide sufficient data to conclude that the chemical is inactive in vivo. Thus data from a second in-vivo assay are necessary to provide adequate reassurance that the mutagenic potential identified in the *in-vitro* studies cannot be expressed in vivo. In this regard members felt that data from an *in-vivo* liver UDS assay would be appropriate in this case and that negative results in this assay would provide the necessary reassurance.

Effects in humans

Studies in volunteers

14. A series of studies have been carried out in volunteers to investigate the effect of inhalation of PAVA on the respiratory and cardiovascular system by measuring effects on heart rate, blood pressure, oxygen saturation and airways (FEV₁, Forced Expiratory Volume in 1 second)^{16,17}. These included studies in mild asthmatics. In order to maximise any response an aerosol of respiratory sized particles was produced using a nebulizer, rather than the coarse spray that is used by the police. Subjects (10 'normal' and 10 with mild asthma) were exposed to a range of concentrations, in the case of the normal subjects up to 0.3% PAVA in 50% aqueous alcohol to mirror the in-use condition. In the case of the asthmatics the maximum concentration used was 0.1%. Transient coughing was noted in normal subjects on exposure to the PAVA spray. Minimal effects were seen on FEV₁ heart rate and blood pressure (bp) in the normal subjects at the in-use concentration (1% reduction in mean FEV₁, 15% increase in mean heart rate and 8% increase in mean systolic bp compared to baseline). Similar effects were noted in the asthmatics exposed to 0.1% PAVA (mean reduction in FEV₁ 3%, increase in heart rate 5% and increase in systolic bp 5%), although a transient but clinically significant reduction in FEV₁ (>0.5 l) was noted in 2 subjects; these were judged to have somewhat more severe asthma on the basis of their greater methacholine responsiveness. These data suggest that exposure to the coarse 'in-use' PAVA spray is unlikely to have any significant respiratory effects in normal subjects although some bronchospasm could be induced in asthmatics. It was noted that under operational use the subjects would be likely to be experiencing a high level of stress, and this could lead to clinically significant bronchospasm.

Experience in use

15. Data provided by Sussex Police did not indicate any significant adverse effects arising from the use of this spray. There did not appear to be any persistent harm to skin or eyes in those exposed. The Committee noted that the animal data indicated that it is an eye irritant and there is the possibility that more marked effects could occur in subjects wearing contact lenses.

Quantification of exposure

16. It is extremely difficult to estimate accurately actual exposure levels in use due to the dynamics of the confrontation. The advice given to officers of the Sussex Police Force is to use a one second spray burst, and to repeat only if the first spray does not affect the eyes. However it is theoretically possible to discharge the whole of the container in a 6 second burst and this would be the most extreme (very unlikely) scenario. Assuming that half of this comes into contact with the skin, eye or mouth, this is equivalent to exposure to about 85 mg PAVA ie an external dose of the order of 1 mg/kg for an adult – some of which may be ingested. It was noted that the total exposure is about 150 times less than the estimated oral LD₅₀ in the rat. A more realistic exposure scenario is to assume that all of a 1 second discharge comes into contact with the skin (ie about 28 mg PAVA). Assuming 10% absorption this will

give a systemic dose of the order of 0.04 mg/kg (compared to a NOAEL of 10 mg/kg/day in the sub-chronic rat study referred to in para 11). However it was noted that it was not possible to compare directly the dermal dose to that obtained orally, due to differences in systemic exposure to PAVA arising from the different routes. Any systemic exposure resulting from the use of PAVA spray was however likely to be low.

Conclusions

- (i) We consider that it is not possible to make a complete assessment of the likely adverse health effects that could arise from the use of PAVA spray as a chemical incapacitant in view of the limited data available.
- (ii) We recognise that exposures would be low and for a short period. It is impossible to calculate exposure with any accuracy but we note that dermal exposure would be of the order of 30 mg PAVA from a 1 second burst, with about 3 mg being absorbed. Any systemic exposure is likely to be low (of the order of 0.04 mg/kg bw).
- (iii) The animal model data and experience in use do not give rise to any concerns regarding long term harm to the skin or eyes. However consideration needs to be given as to whether those wearing contact lenses might experience increased irritant effects. It is also noted that no data are available on the potential of PAVA to induce skin sensitisation.
- (iv) The *in-vitro* mutagenicity data, and consideration of metabolites, indicate that PAVA has some mutagenic potential; although negative results were obtained in an *in-vivo* study to investigate mutagenic effects in the bone marrow, data from a further study are needed to provide adequate assurance that this activity cannot be expressed *in vivo*. An *in-vivo* study to investigate the induction of unscheduled DNA synthesis (UDS) in the liver would be appropriate in this regard.
- (v) No data are available to assess whether PAVA has any effects on the reproductive system. In particular the lack of any developmental toxicity studies is of concern as it is possible that pregnant women may be exposed to the spray.
- (vi) The data from inhalation studies in volunteers, including those with mild asthma, indicate that there are unlikely to be any adverse respiratory reactions in normal individuals. Some respiratory effects may well occur in asthmatics, particularly since effects were observed in asthmatic volunteers at 0.1% PAVA, which is lower than the 0.3% used in the spray, and given the conditions of increased stress likely when the spray is used.
- (vii) Further monitoring of experience in use, including the police officers using the spray, is recommended with particular consideration being given to eye irritancy in those wearing contact lenses and to effects in those with asthma or hay fever and in women who may be pregnant.

April 2002

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Joint statement on the re-assessment of the toxicological testing of tobacco products

Introduction

1. Article 11 of the Directive 2001/37/EC of the European Parliament and of the Council¹ sets out a requirement for the European Commission to submit, no later than 31 December 2004 and every two years thereafter, a report on the Directive's application. Such a report will aim to review, and advise on the development of, particular features of the Directive. Two areas of specific concern are;

“methodologies for more realistically assessing and regulating toxic exposure and harm”

and,

“toxicological data to be required from manufacturers on ingredients and the manner in which they should be tested in order to allow public health authorities to assess their use”

2. The Committees (COT/COC/COM) were asked to provide advice on these areas of toxicological assessment with reference to the assessment of Potentially Reduced Exposure Products (PREPs) and in particular tobacco-based PREPs which are smoked. A brief overview of the information reviewed and approach taken by the Committee's is given below. Copies of the discussion papers used by the committees can be obtained from the Committee internet sites.

<http://www.advisorybodies.doh.gov.uk/cot/index.htm>

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

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

3. The Committees agreed that it was important to state that the ideal way forward to reduce risks and hazards of tobacco smoke was to encourage smokers to stop or people not to start in the first place any attempt to reduce toxicity should not be allowed to detract from that. Members acknowledged that the primary remit of the Committees' discussions was to provide advice based on the information provided.

Approach taken and evidence reviewed

4. The Committees considered a covering paper drafted by the secretariat (available on the Committee internet sites) and appended references. These included information from the Symposium proceedings of the 56th meeting of the Tobacco Science Research Conference² and a number of peer-reviewed publications in scientific journals³⁻⁹. In discussing these data members were reminded of the recent considerations (at COT/COC/COM meetings during 2004) on toxicogenomics where a number of studies investigating tobacco smoke had been considered. (See above internet sites for minutes and papers). The Committees were aware that additional studies both in the public domain and possibly held by industry could have been reviewed but noted that they had been asked to provide the best advice possible in the available time based on the information provided to members. The discussion paper and appended references were considered at the COM meeting on 7 October, the COT meeting on 26 October and the COC meeting on 18 November 2004.

Generic consideration of toxicological approaches to evaluation of PREPs

5. The Committees commented that tobacco smoke was a highly complex chemical mixture and that the causative agents for smoke induced diseases (such as cardiovascular disease, cancer, effects on reproduction and on offspring) was unknown. The mechanisms by which tobacco induced adverse effects were not established. The best information related to tobacco smoke -induced lung cancer, but even in this instance a detailed mechanism was not available. The Committees therefore agreed that on the basis of current knowledge it would be very difficult to identify a toxicological testing strategy or a biomonitoring approach for use in volunteer studies with smokers where the end-points determined or biomarkers measured were predictive of the overall burden of tobacco-induced adverse disease.
6. The Committees commented that since it was not possible to define reliable end-points or biomarkers for use in *in-vitro* investigations (with exception of mutagenicity testing see paragraph 10 below), *in-vivo* studies in experimental animals or in volunteer studies using smokers, it would not be possible to compare PREPS using such approaches.
7. The Committees also noted that some weight could be placed on investigations of markers for the tobacco-specific nitrosamine NNK (4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone) and its metabolite NNAL (4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanol) as markers for exposure to a potentially relevant tobacco smoke-derived carcinogen. However, a valid investigation of carcinogenic potency of PREPs would have to examine a wide range of the 50 or so known human carcinogens present in tobacco-smoke. In addition, strategies designed to compare PREPs with regard to one chronic disease associated with tobacco-smoke, such as lung cancer, may have no predictive value for other diseases such as tobacco-smoke induced cardiovascular disease.
8. Overall the Committees agreed that there were considerable difficulties in designing a toxicological testing strategy for the reassessment of tobacco products and that it was not possible to design a valid strategy given current understanding of the diseases associated with smoking tobacco.

Consideration of approaches currently used

9. The Committees commented on the approaches used in the information provided. When tobacco manufacturers wish to assess the influence of any design changes on the overall toxicity of a product, a tiered testing regime has been advocated which currently consists of one or more of the following:
 - 1) A bacterial test for gene mutation (e.g. the Ames test)
 - 2) A test for clastogenicity and for indications of aneugenicity
 - i) *in-vitro* metaphase analysis
 - ii) *In-vitro* micronucleus test

- 3) Mammalian cell mutation assay (preferred choice is the mouse lymphoma assay)
- 4) Cytotoxicity is assessed using the Neutral Red uptake assay.
- 5) *In-vivo* studies in experimental animals to investigate biomarkers of disease.
- 6) Studies in smokers (volunteers) to examine effects on smoking and biomarkers following switching from one tobacco product to a PREP.

Advice from COM on mutagenicity

10. The COM considered the available information and agreed that using suitable protocols it was possible to compare mutagenicity *in-vitro* of different PREPs which could be useful to assess hazard. However, the results of such *in-vitro* tests had no predictive value for risk of *in-vivo* mutagenicity or cancer. No conclusions could be drawn on the approaches using toxicogenomic methods. The available biomonitoring approaches were too limited to draw any conclusions regarding a comparison of PREPs.

Advice from COT on Toxicology testing

11. The COT concluded that *in-vitro* cytotoxicity testing could be used as part of an overall approach for comparing PREPs but the data could not be extrapolated to the *in-vivo* situation and the outcome measured in such tests had no predictive value with regard to tobacco-smoke associated diseases. The COT considered that studies in smokers (volunteers) to investigate tobacco-based PREPs should take account of smoking behaviour in addition to investigation of biomarkers of disease. The COT concluded that there were currently no adequate biomarkers for tobacco-smoke induced diseases and no conclusions could be reached on the available data. The COT concluded that smoke chemistry could not be used to compare PREPs.

Advice from COC on carcinogenicity

12. The COC commented on the complexity of tobacco induced cancer and noted that the mechanism(s) and information on the chemical agents responsible for tobacco induced cancer in humans had not been fully elucidated. In addition members noted the importance of the interaction between chemical carcinogens and susceptibility factors regarding the pathogenesis of tobacco induced cancer. The COC concluded that there is no strategy which could be used to compare PREPs for carcinogenic potency and that the approaches used are not informative on the risk of tobacco induced carcinogenicity. The COC agreed that it was not possible to draw conclusions on the carcinogenic risk of tobacco-based PREPs on the available biomarker studies reviewed⁶⁻⁹. The COC commented on the need to examine a wide range of biomarkers of carcinogenicity and their interaction with susceptibility factors.

Overall conclusions of the Committees

13. The Committees agreed that analysis of tobacco smoke constituents was not useful in comparing tobacco-based PREPs or predicting risks associated with tobacco smoking.
14. The Committees agreed that the current *in-vitro* tests and *in-vivo* approaches used in experimental animals used to evaluate the toxicity of tobacco products and tobacco-based PREPs are not informative on risk of diseases induced by tobacco-smoke.
15. It was noted that comparative assessments between PREPs can be undertaken for data generated in some *in-vitro* mutagenicity tests, but the data cannot be extrapolated to *in-vivo* mutagenicity.
16. The Committees agreed that the available biomonitoring studies in volunteer smokers were too limited to draw any conclusions regarding comparisons of tobacco-based PREPs.
17. The Committees cautioned that biomonitoring studies which focused on one disease (such as cancer) in volunteer smokers had no predictive relevance to other tobacco smoke-induced diseases such as cardiovascular diseases.
18. The Committees advised that future progress on the proposed approach to reduce tobacco smoke-induced disease by modification of tobacco products could only be made when detailed mechanistic information on tobacco-induced diseases were available.

November 2004

COT/04/09; COC/04/S4 & COM/04/02

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Joint COT/COC/COM statement on the use of toxicogenomics in toxicology (update on statement published in 2002)

Introduction

1. The COT/COC/COM held a joint symposium on the use of genomics and proteomics in toxicology in October 2001. The following overall conclusions were subsequently agreed and published in a statement.
 - a) We recognise the future potential of proteomics and genomics in toxicological risk assessment.
 - b) *We note that these techniques may serve as adjuncts to conventional toxicology studies, particularly where proteins under investigation are known to be causally related to the toxicity.*
 - c) *However, we consider that research and validation is required before these techniques can be considered for routine use in regulatory toxicological risk assessment. In particular, there is a need for more research leading to development of genomic/proteomic databases, methods of bioinformatic and statistical analysis of data and pattern recognition and for information on the normal range of gene expression.*
2. The Committees agreed to further consider toxicogenomics as part of the horizon scanning exercise initiated at the February 2004 COT meeting. It was noted that there was a considerable increase in the number of publications using toxicogenomic approaches. A number of discussion papers were subsequently prepared for the Committees which reviewed the available published literature. The data from 50 studies were considered during the review which also included available information from the HESI collaborative scientific program on toxicogenomics[†]. Details of the references consulted during the review can be found in the papers cited at the end of this statement. This current review considered information on use of metabonomics in toxicology for the first time. The COT requested a further paper and presentation on the use of statistics/bioinformatics in toxicogenomics. A presentation was given by Dr David Lovell (University of Surrey) to the COT at its meeting on the 7 September 2004.
3. For the purposes of this statement, the term transcriptomics refers to gene expression as measured through cDNA or oligonucleotide or cRNA microarray based approaches, proteomics refers to determination of protein levels through gel or solid phase approaches and metabonomics refers to measurement of metabolites in tissues, plasma or urine.
4. A summary of the main conclusions reached by each Committee based on the information provided is given below.

[†] HESI = Health and Environmental Sciences Institute (HESI) of the International Life Sciences Institute (ILSI).

Conclusions reached by Committee on Toxicity

5. The COT reached the following conclusions after discussions at its February and September 2004 meetings:
 - a) There had been improvements in the design and reproducibility of studies, and the approaches to the analysis of raw data and statistical approaches to evaluation and identification of toxicologically relevant patterns for gene changes.
 - b) The key areas for future development were development of methods for pattern recognition, the evaluation of functional significance of changes in gene expression and distinction between adverse and adaptive changes.
 - c) There was a need for better toxicogenomic studies on time course for effects and dose-response assessment and the reversal of toxicological effects. At present toxicogenomic data could be considered as part of the overall toxicological data package, but could not be used in the absence of prior knowledge about the toxicity of the chemical from conventional toxicological approaches. There was a potential that toxicogenomic approaches could be designed to screen for specific mechanisms of toxicity but such approaches would require appropriate validation.
 - d) Regarding transcriptomic methods it was agreed that there were a considerable number of sources of variance which might affect the results of studies. The COT confirmed that for the present it was necessary to confirm key gene changes independently such as by quantitative* analysis of mRNA. The design of experiments (e.g pooling of samples), reproducibility of replicate mRNA analyses, the approach to assessment of background changes, use of different fluorometric methods to assess gene expression changes, use of housekeeping genes, variation between laboratories regarding analysis of mRNAs in particular the use of different platforms, and validation of the genes incorporated into microarrays were all examples of the potential sources of variation in transcriptomic analyses.
 - e) There are few comparative data on the use of high density cDNA microarrays (e.g. with thousands of genes) and low density cDNA arrays (with small numbers of genes targeted for a limited number of toxic mechanisms). In general high density arrays are comparatively of greater difficulty and expense to develop and the evaluation and interpretation of data is complex. Low density arrays are cheaper, easier to use and evaluate, but may miss novel mechanisms and have limited coverage of genes.
 - f) Regarding proteomics, it was agreed that there had been considerable important developments since the 2001 meeting particularly in respect of introduction of solid-phase separation methods. Both two dimensional gel methods and solid phase techniques were valuable and should be considered as complimentary techniques.

* polymerase chain reaction

- g) Metabonomics had not been considered in the 2001 joint COT/COC/COM meeting. There had been considerable recent advances in techniques regarding recognition of metabolite pattern changes in tissues and biological fluids. The potential for development of biomarkers was noted. The potential use of trajectomes to visualise onset and recovery for toxicity was noted.
- h) The Committee agreed that transcriptomics/proteomics and metabonomics each need to be considered as part of an integrated approach to toxicological risk assessment. Thus a preliminary study of the acute hepatotoxicity of paracetamol in mice using transcriptomics and metabonomics had provided new insights in the mechanism of hepatotoxicity of this chemical².
- i) It was too early to draw any conclusions as to whether the use of toxicogenomic approaches to toxicology could result in the use of fewer animals in testing.
- j) There were no epidemiology studies retrieved during the review (i.e up to September 2004).

Additional conclusions reached by Committee on Mutagenicity

- 6. The COM reached the following conclusions after discussions held at its February and May 2004 meetings.
 - a) No conclusions can be drawn from the preliminary results of the ILSI/HESI trial of mutagenesis in mouse lymphoma L5178Y tk^{+/−} cells³. Further information on the detailed results from this trial and validation of the findings would be needed before conclusions can be drawn.
 - b) Mutagenicity may be associated with changes in expression of relatively few genes which might be potentially difficult to identify in high density arrays⁴. The COM agreed there were considerable difficulties in developing in-vitro mutagenicity screening assays using toxicogenomic approaches with regard to selection of appropriate microarray platform, confirmation of microarray results using quantitative measures of mRNA levels, identification of appropriate fold change in gene expression, and development of appropriate statistical/bioinformatics approaches for assessment of studies. However it was possible that valid approaches to screening for mutagens might be developed in the future.
 - c) The COM identified the need for more research on time dependent changes in gene expression using mutagens and the application of integrated toxicogenomic approaches to evaluating changes in protein and metabolic pathways in response to exposure to mutagens. No adequate proteomic/metabonomic studies of mutagens had currently been identified.

- d) The COM reviewed a number of published papers which presented data using mouse lymphoma L5178Y tk⁺ cells and agreed that no clearly defined pattern of gene expression changes which could logically be associated with mutagenesis had been identified. The COM reviewed a recent study which had used HepG2 cells and agreed that the authors had been able to distinguish between genotoxic and non-genotoxic carcinogens but only when a number of genotoxic compounds (predominantly methylating agents) were excluded⁴. Overall this latter study provided some useful information but there was a need for considerable additional research involving multiple dose levels and sampling times before conclusions could be reached.
- e) The Committee considered that the limited available in-vivo studies using four hepatocarcinogens did provide some preliminary results which suggested genotoxic responses in gene expression could be identified *in vivo*⁵.
- f) One preliminary investigation provided evidence to suggest that transcriptomics could provide information to aid in the interpretation of conventional *in-vitro* clastogenicity assays to assist in the evaluation of mutagenic or cytotoxic responses in these tests⁶.

Additional conclusions reached by Committee on Carcinogenicity

- 7. The COC reached the following conclusions after discussions held at its June 2004 meeting. The COC reached a number of general conclusions on toxicogenomic studies in experimental animals regarding dose-response evaluation, investigations of reversibility, statistical handling of data and bioinformatic developments which are consistent with those reached by COT. COC members also commented on the need for “pathway mapping” for the identification of toxicologically relevant gene changes. The COC agreed with the COM conclusion that a gene expression pattern had been reported in studies in rodents using genotoxic hepatocarcinogens.
 - a) A number of studies in rodents using model carcinogens had reported on toxicogenomic approaches to investigate the process leading to neoplasia from initiation to tumour formation and growth. However no conclusions could be drawn from these limited studies. It was noted from the preliminary evidence considered by the committee that it was difficult to distinguish between chemical induced changes in gene expression from those occurring as a result of the neoplastic process.
 - b) It was not possible in studies in animals using model non-genotoxic carcinogens to identify common gene expression changes which might be of value in developing an approach to early detection of non-genotoxic carcinogen. The available study identified more distinct than common changes in studies in mice using two model non-genotoxic hepatocarcinogens⁷.
 - c) Potentially valuable information on mechanisms of carcinogenesis could be derived from experiments designed to investigate particular specific mechanisms. Some preliminary information on non-genotoxic liver carcinogenesis in mice was available⁷.

- d) Comparison of gene expression changes in stomach tumours in rodents induced by a model genotoxic carcinogen had shown similarities with gene expression profiles from human stomach cancers⁸. These preliminary data could be used for hypothesis generation regarding the aetiology of stomach cancer. However caution was required in interpreting the studies considered by the committee as the range of toxicological effects in animals given relatively high doses of model carcinogens did not reflect the likely effects in humans exposed to much lower doses in the environment.

Use of statistics and bioinformatics in toxicogenomics.

- 8. The COT heard a presentation from Dr David Lovell on the use of statistics and bioinformatics in toxicogenomics at its 7 September 2004 meeting. The background paper for the presentation is available on the COT internet site⁹.
- 9. The COT reached a number of conclusions based on the main recommendations proposed by Dr Lovell;

Samples and experimental design.

- a) The purpose of a study and experimental design should be clearly specified such as whether it is designed for screening compounds internally within an organization, for providing evidence relating to a mechanism of action or is part of a formal submission to a regulatory body. Sample sizes based upon power calculations depend upon the 'probe' of interest.
- b) Replicates should be biological replicates representing independent experimental units such as subjects, animals or cultures. Technical replicates from repeated sampling of the same experimental unit may also be analysed. The need for biological replication should take precedence over the need for technical replication.
- c) Samples should not be pooled if information on individual experimental units is important and information is required on within group variability in *in-vivo* studies.
- d) Sufficient information on an experiment should be provided to enable independent replication. The strongest evidence to confirm the results of toxicogenomic studies comes through data showing commonality of gene pathways affected in transcriptomic, proteomic and metabonomic experiments. The concept of phenotypic anchorage of data, i.e. linking cause and effect is crucial to the evaluation of toxicogenomic data¹⁰. Confirmatory evidence may also come from similar findings from the analysis of results using different platforms (cDNA and oligonucleotide arrays) and from other techniques such as reverse transcriptase (RT)-PCR. The concordance or otherwise of results from these different systems relates more to the different mechanisms underlying the systems than a formal statistical interpretation and practical issues in terms of amounts of sample may determine how much verification using other techniques will be carried out.

- e) No specific guidelines should be drawn up for the reporting of expression changes between different groups. The relationship between the size of difference such as the fold-change and the significance level is complex and depends upon such factors as the statistical tests used, the sample size, whether multiple comparison methods are used and the relative level of gene expression for the probe. A useful presentation of the data is in the form of a volcano plot which illustrates the relationship between statistical significance and difference between two treatments.
- f) Considerable intra- and inter-laboratory variability has been found in cross-laboratory studies of microarrays.
- g) The extension of the methods from laboratory-based experimental studies to observational epidemiological studies raise a number of issues relating to the collection and preparation of samples to prevent the introduction of biases. This is critical for all biomarker/molecular epidemiology work but is particularly relevant to these sensitive multivariate analyses which are vulnerable to many unintentional biases as a consequence of less experimental control over the material used in the study.

Data Management

- h) Toxicogenomic studies generate large quantities of raw data. Management of the full set of 'raw' data collected from studies is a major challenge¹¹. The approach being developed by MIAME[§] for the data management of data collected in microarray studies has proven to be a useful starting point for data management of toxicogenomics study. The development of draft international guidelines on minimum information about microarray experiments for Toxicogenomics (MIAME/Tox) which builds on the MIAME initiative is to be welcomed. Adoption of a harmonised set of guidance would greatly assist the planning and completion of Toxicogenomic studies. The development of similar standards for proteomic studies (The Proteomics Standards Initiative) is at an early stage but should provide valuable generic guidance in the future¹².

Statistics

- i) No single method can currently be considered the most appropriate way to analyse toxicogenomic data. No attempt should, therefore, be made at present to define specific statistical methods or software that should be used for the analysis of toxicogenomic studies. Toxicogenomics studies may have different objectives and, therefore, the methods used need to be appropriate for the specific aim of a study. Many of the methods currently available within the standard statistical packages and the specialized software associated with genomics are 'mature' methods. Considerable research is in progress in developing new and modified versions of these methods to analyze the multivariate datasets being generated. It is unlikely that this will result in a 'single' agreed method in the short-term.

§ Minimum Information About a Microarray Experiment.

- j) It is critical, though, that the statistical methods used are reported comprehensively. The methods used should be clearly specified and any assumptions or options used in the analysis should be explicitly identified.
- k) Ideally, the data collected should be in a form that it is possible to repeat the analysis using both the specific software initially used and using alternative statistical methods using other software and packages. There is likely to be a need for 'in house' expertise to analyse data using standard significance testing, Principal Component Analysis, cluster analysis and classification packages to get an overview of conclusions being drawn and to try out sensitivity analyses.

Overall discussion and conclusions

10. The Committees agreed that there had been rapid and extensive developments regarding the application of toxicogenomic methods to toxicological hazard characterisation. It was possible that toxicogenomic approaches could potentially be applied in a limited number of situations but could not be applied routinely to toxicological risk assessment. Thus the available evidence supported the view that these techniques could be applied, particularly when used in combination, for the initial screening for a limited number of toxicological mechanisms where appropriate validation was available. The Committees recognised the application of proteomic and metabonomic studies to the examination of chemically-induced responses in whole animal and the possible development of biomarkers of toxicological effect. but could not currently be used for routine screening for target organ toxicity. The available information provided preliminary and limited evidence that toxicogenomic techniques could also provide additional information to aid in the assessment of dose-response relationships but it was important to interpret such data in collaboration with information from existing conventional toxicological methods such as microscopical examination of fixed, sectioned and stained tissues.
11. The main factors which limited the current application of toxicogenomic methods predominantly relate to the limited database of studies available (with regard to limited duration of dosing predominantly to short-term periods, few species and toxicological mechanisms examined, and limited information on interlaboratory reproducibility of results) and to the need for improved methods of data evaluation and interpretation (particularly with regard to the need for interpretation of data in respect of gene expression changes in toxicologically relevant pathways).
12. Members agreed the conclusions and recommendations proposed by Dr Lovell. It was recognised that the statistical methods were undergoing rapid development. It was, however, considered that there was a need for some generic guidance on the most suitable methods for statistical evaluation of different types of toxicogenomic data.
13. The Committees agreed that the overall conclusions reached in 2001 were satisfactory subject to a number of amendments. The following overall conclusions were agreed.

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- a) We *recognise* the rapid development in toxicogenomic methods (transcriptomics, proteomics and metabolomics) in toxicological hazard identification and characterisation since 2001.
 - b) We *confirm* that these techniques may serve as adjuncts to conventional toxicology studies. There is a need to provide appropriate data from studies on gene expression, protein levels and metabolite changes in order to provide sufficient information on toxicologically relevant pathways.
 - c) However we *consider* that further research and validation is required before these techniques can be considered for routine regulatory toxicological risk assessment. At present toxicogenomic approaches can provide valuable supportive data on mechanisms of target organ toxicity which can aid in the risk assessment process.
 - d) There is a need for further refinement and optimisation of methods used, approaches to data interpretation and evaluation using statistical and bioinformatics methods and development of appropriate publicly accessible databases.
 - e) We *note* the need for generic guidance on the most suitable methods for statistical evaluation of different types of toxicogenomic data.

COT 4/10: COM/04/S5 and COC 04/S8

December 2004

COT/COM/COC discussion papers

COT discussion paper and annexes;

<http://www.food.gov.uk/multimedia/pdfs/TOX-2004-02.PDF>

<http://www.food.gov.uk/multimedia/pdfs/TOX-2004-02annexes.pdf>

COM discussion paper and annexes:

<http://www.advisorybodies.doh.gov.uk/pdfs/mut041.pdf>

<http://www.advisorybodies.doh.gov.uk/pdfs/mut042.pdf>

<http://www.advisorybodies.doh.gov.uk/pdfs/Mut0411.pdf>

COC discussion papers and annexes:

<http://www.advisorybodies.doh.gov.uk/pdfs/cc045.pdf>

<http://www.advisorybodies.doh.gov.uk/pdfs/cc046.pdf>

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Urgent advice provided by the COT

Arsenic in seaweed – risk assessment

Inorganic arsenic

1. The Food Standards Agency has received the results of a survey for arsenic in imported edible seaweed. Inorganic arsenic was detected in all of 9 samples of hijiki seaweed, but not in 4 other types of seaweed tested. These UK data are consistent with results of a survey in Canada, showing high levels of inorganic arsenic in 16 of 17 samples of hijiki seaweed. Based upon the average inorganic arsenic content of 11 mg/kg in hijiki seaweed detected in the UK survey, an average portion size of 18g would provide 198 µg of inorganic arsenic, which is equivalent to about 3 µg/kg bodyweight in a 70kg adult. This represents about 150% of the Provisional Tolerable Weekly Intake (PTWI) of 15 µg/kg bodyweight/week (about 2 µg/kg bodyweight/day) set by JECFA in 1989¹, before considering intake from the rest of the diet.
2. In 2002, the International Agency for Cancer Research (IARC) concluded that arsenic in drinking water (primarily inorganic, as arsenate and to a lesser extent arsenite) was carcinogenic to humans on the basis of sufficient evidence for an increased risk for cancer of the urinary bladder, lung and skin².
3. In May 2003, COT noted the additional evidence published since the JECFA evaluation, and the IARC opinion, and concluded that the approach used by JECFA in setting the PTWI would not now be considered appropriate, in view of its genotoxicity and carcinogenicity³. The COT therefore concluded that there are currently no relevant tolerable intake guidelines or reference doses for inorganic arsenic and recommended that dietary exposure should be As Low As Reasonably Practicable (ALARP). The JECFA PTWI is scheduled to be reviewed in February 2005.
4. Based on the results of the survey of total and inorganic arsenic in the 1999 Total Diet Study, dietary exposure of adults at the 97.5th percentile to inorganic arsenic was estimated to be 0.043–0.14 µg/kg bodyweight/day. This range represents lower bound (levels below the limit of quantitation assumed to be zero) to upper bound (levels below the limit of quantitation assumed to be at the limit of quantitation). An 18g portion of hijiki seaweed providing 2.9 µg/kg bodyweight of inorganic arsenic, would increase the intake of inorganic arsenic by at least 20 times. Thus consumption of hijiki has the potential to represent a significant increase in inorganic arsenic. Applying the ALARP approach indicates a need for risk management action to reduce this source of exposure.

Total arsenic

5. The average total arsenic concentrations in the 5 sampled species of seaweed (arame, kombu, wakame, hijiki and nori) ranged from 2.9 to 24.0 mg/kg. At 24 mg/kg, an 18g portion would provide 432 µg of total arsenic, equivalent to 6 µg/kg bodyweight in a 70kg adult. This is a similar order of magnitude to high level intake of total arsenic from the diet (4 to 7 µg/kg bodyweight in different adult groups according to UK surveys). With the exception of hijiki, inorganic arsenic was not detected. Based on the limit of detection, it can be deduced that inorganic arsenic was less than 2% of the total.

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6. In May 2003, the COT also noted that the general assumption that organic arsenic is less toxic than inorganic arsenic is based on an extremely limited database. However, there was no evidence that exposure to organic arsenic at levels up to 50 $\mu\text{g}/\text{kg}$ bodyweight/day, through high levels of fish consumption, had resulted in harmful effects. Therefore the total arsenic detected in arame, konbu, wakame and nori seaweeds is unlikely to constitute a hazard for health.

9 July 2004

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2004 Membership of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

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Ms J Salfield BSc(Hons) MSc MIFST CertED RPHN (up to 31 March 2004)
Public Interest Representative

Dr A G Smith BSc(Hons) PhD CChem FRSC (up to 31 March 2004)
Head of Molecular Toxicology, Medical Research Council Toxicology Unit, University of Leicester

Dr L Stanley BA PhD
Head of Operations, CXR Biosciences

Professor S Strobel MD PhD FRCP FRCPCH
Director of Clinical Education, Peninsula Postgraduate Health Institute, Peninsula Medical School, Plymouth

Dr M Tucker BSc(Hons) PhD FRCPath (up to 31 March 2004)
Independent pathologist

Dr Corrine de Vries MSc PhD (from 1 April 2004)
Senior lecturer in pharmacoepidemiology, University of Surrey

Miss A Ward
Public Interest Representative

Mrs A Williams OBE BA (Hons) (from 1 April 2004)
Public Interest Representative

SECRETARIAT

Dr D Benford BSc PhD	Scientific Secretary
Mr K Butler	Administrative Secretary
Mr J Battershill BSc MSc	Scientific – DH
Ms C A Mulholland BSc	
Dr D Gott BSc PhD	
Dr C Tahourdin BSc PhD	
Dr Sekai Ngarize BSc PhD (until October 2004)	
Dr S Sivapathasanduram BSc PhD (until October 2004)	
Dr N Thatcher BSc PhD	
Mr B Maycock BSc MSc	
Dr V Burgess BSc PhD	
Ms K Moizer BSc MSc (until January 2004)	
Ms A Adjei BSc MSc (from April until August 2004)	

Declaration of 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		
	BP Amoco	Daughter is an employee of this company	Academy of Med Sciences	Fellow
			Soc for Endocrinology	Member
	Topical Endocrinology	Editorial Board member	Royal College of Paediatrics and Child Health	Fellow, Senior Examiner, Regional Academic Adviser
			Medical Research Council	Member of Advisory Board
			Pfizer Aventis NovoNordisk Diabetes UK Wellcome Trust Juvenile Diabetes Fund	Funds received from all these sources for Departmental research and education in medicine and health related topics
Professor J Ashby	Syngenta	Employee, salary, share option	NONE	NONE
Dr D Bell	Alliance and Leicester	Shares	FSA	Research contract
	BAA	Shares		
	BG	Shares	Astrazeneca	BBSRC CASE studentship
	Centrica	Shares		
	HBOS plc	Shares		
	International Power RT Group	Shares		
	Rolls Royce	Shares		
	Scottish Power Thus	Shares		
	United Utilities	Shares		
	National Grid	Shares		
	Transco			

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Professor A R Boobis OBE	Abbey National	Shares	GlaxoSmithKline	Support by industry
	Scottish Power	Shares		
	Centrica	Shares	FSA	Research contract
	BG Group	Shares		
	Halifax	Shares	ILSI HESI	Member of Board of Trustees
	Barclays	Shares		
	National Grid	Shares		
	Transco	Shares	Elsevier	Editor-in-chief, Food and Chemical Toxicology
Dr P Carthew	Unilever	Employee	NONE	NONE
	Provalis	Shareholder		
Professor J K Chipman	Sequani	Training	AstraZeneca	Research Support
		Consultancy	GlaxoSmithKline	Research Support
	AstraZeneca	Consultancy	ICI	Research Support
	Inamed	Consultancy	HSE	Research Support
	Unilever	Consultancy	CEFIC-LRI	Research Support
	Syngenta	Lecture Fee	Dept of Health	Research Support
Dr R Dearman	Syngenta CTL	Salary	NONE	NONE
	Astrazeneca	Shareholder		
Dr P Greaves	Marix Drug Development Ltd	Consultancy	NONE	NONE
	Shire Pharmaceutical Development Ltd	Consultancy		
	Experimental Pathology Laboratory, USA	Consultancy		
	Sun Coast Tox, USA	Consultancy		
	Sanofi-Aventis	Consultancy		
	AstraZeneca	Pension		
Dr J Hinson	GlaxoSmithKline	Shareholder	NONE	NONE
Dr P Jackson	British Heart Foundation	Lecture Fees	Medtronic AVE	Research Grant
	Mitchell & Butlers	Share Ownership		
	Intercontinental Hotels	Share Ownership		
	Marks and Spencer	Share Ownership		
Dr M Joffe	NONE	NONE	NONE	NONE

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Professor J Lunec	NONE	NONE	Sciluent LLC.USA	Funding research group to investigate toxicology of soya-bean oil implants
Dr A Piersma	NONE	NONE	NONE	NONE
Professor D Ray	Medical Research Council	Employer	Consortium of: Bayer, DuPont, FMC, Syngenta, Valent	Research Grant
Professor I R Rowland	Alpro Foundation	Consultancy	Valio (Finland)	Partner in EC funded project
	Halifax	Shares	Alpro (Belgium)	Partner in EC Funded project
	Woolwich	Shares	Alpro	CAST PhD studentship
			VK Muehlen (Germany)	Partner in EC funded project
			Biohit (Finland)	Partner in EC funded project
			Danone (France)	Partner in EC funded project
			Orafti (Belgium)	Partner in EC funded project
			Unilever UK	CAST PhD studentship
			ILSI Europe	Partner in EC funded project
			Cerestar (Belgium)	Funded Research
			Geest	Funded Research
			Vitacress	Funded research
Dr L Rushton	Institute of Petroleum	Consultancy, Contracts and Grants – Completed	Concawe	Contracts to Institute for Environment and Health (IEH) Now Completed
	Transport and General Workers Union	Consultancy – Completed	European Silica Industry	Ongoing Cohort Study, Contract to IEH
	Friends Provident	Shares	International Manganese Institute	Contract to IEH to prepare criteria document
	Northern Rock	Shares	American Chemistry Council	Contract to IEH for systematic review and meta – analysis
	Unilever	Consultancy – advice on design of an epidemiological survey relating to dermatitis		

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Dr G Rylance	NONE	NONE	NONE	NONE
Ms J Salfield	NONE	NONE	NONE	NONE
Dr A Smith	Abbey National	Share Holder	Rhone Poulenc	Past Research
	British Telecom	Share Holder	Glaxo Wellcome	Support
	MMO2	Share Holder	CEFIC – LRI	Past Research
	HBOS	Share Holder		Support
	Latham & Watkins	Consultancy		Research Support
Dr L Stanley	CXR Biosciences	Salary	Alizyme	Company Contract
	Agan	Company Contract	Arrow	Company Contract
	Procter & Gamble	Company Contract	Bayer	Company Contract
	Toxel	Company Contract	Cyclacel	Company Contract
	Association of Plastics Manufacturers, Europe	Consortium Client	Entremed	Company Contract
	Eurochlor	Consortium Client	Etiologics	Company Contract
	European Council for Plasticisers and Intermediates	Consortium Client	Ferring	Company Contract
	Halogenated Solvents Industry Association	Consortium Client	Grupovita	Company Contract
	AstraZeneca	Research Collaboration	Guerbet	Company Contract
	GlaxoSmithKline	Research Collaboration	Ionix	Company Contract
	NovoNordisk	Research Collaboration	Nestle	Company Contract
	Pfizer	Research Collaboration	Neurosearch	Company Contract
	Wyeth	Research Collaboration	Oncosense	Company Contract
			Serono	Company Contract
			Stiefel	Company Contract
			Strakan	Company Contract
			Yamanouchi	Company Contract
Professor S Strobel	NONE	NONE	NONE	NONE
Dr M Tucker	Zeneca	Pension	NONE	NONE
Miss A Ward	NONE	NONE	Barking, Havering and Redbridge NHS Trust	Non-executive Director
Mrs A Williams	NONE	NONE	NONE	NONE
Dr C deVries	NONE	NONE	Schering AG	Research grant
			Yamanouchi	Research grant

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

Preface 2004



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 2004, the Committee provided advice on a wide range of chemicals which included 2,3-dichloropropan-1-ol, chromium picolinate, the effect of DNA repair on mutagenicity of genotoxic carcinogens and at low dose levels, malachite green/leucomalachite green and PAVA (pelargonyl vanillylamide)

The Committee was asked to contribute to two reviews in conjunction with its sister Committees on Toxicity (COT) and Carcinogenicity (COC). Advice was provided on the mutagenicity assessment of tobacco products (specifically tobacco-based Potentially Reduced Exposure Products) and the application of toxicogenomic methods to mutagenicity. 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 conduct of *in-vitro* micronucleus assay and the testing strategy advocated by the EC's Scientific Committee on Cosmetics and Non-Food Consumer Products (SCCNFP).

During 2004, the Committee also said farewell to Dr Robin Fielder who retired as scientific secretary to COM. I wish to record my thanks for his scientifically excellent contribution and commitment to the work of COM and public health during his 17 years of service for COM.

Professor P B Farmer Chair
MA DPhil CChem FRSC

2,3-Dichloropropan-1-ol (new mutagenicity data)

- 2.1 2,3-Dichloropropan-1-ol (2,3-DCP) is a member of a group of chemicals called chloropropanols. It was last considered by the COM and the COC in 2001, together with 1,3-dichloropropan-2-ol (1,3-DCP). COM concluded that it would be prudent to consider 1,3-DCP and 2,3-DCP as potentially genotoxic *in-vivo* but agreed that they should be tested for genotoxicity *in-vivo* using the approach set out in the COM guidelines. The COM had considered the results of *in-vivo* studies from a rat bone-marrow micronucleus test and a liver UDS assay conducted with 1,3-DCP at its May 2003 meeting and had reached a conclusion that there was no evidence for *in-vivo* mutagenicity in the tissues assessed. The Committee was asked to consider the data from two *in-vivo* mutagenicity assays (rat bone-marrow micronucleus test and a liver UDS assay) conducted with 2,3-DCP.

COM evaluation 2001

- 2.2 Members were aware that there was very little data on the absorption, distribution, and excretion of 2,3-DCP. Theoretically, 2,3-DCP could be metabolised to produce epichlorohydrin (and subsequently glycidol) and therefore there were structural alerts for genotoxicity and carcinogenicity.
- 2.3 The Committee noted 2,3-DCP was mutagenic in *Salmonella typhimurium* strains TA 100 and TA 1535 in a study with and without metabolic activation¹, and mutagenic in another Ames test. Positive results were also obtained for sister chromatid exchange with Chinese Hamster V79 cells both with and without metabolic activation. No *in-vivo* studies in mammals have been carried out.
- 2.4 The Committee concluded that in the limited studies conducted, 2,3-DCP was genotoxic *in vitro* with and without metabolic activation in bacterial and mammalian cells.

COM evaluation 2004

- 2.5 The Committee considered two new *in-vivo* genotoxicity studies at its February 2004 meeting. The Committee concluded that both the rat bone-marrow micronucleus test and the rat liver UDS test had been carried out to an acceptable standard and were negative. Thus the additional information recommended by the COM as being necessary to provide adequate reassurance that the mutagenic activity seen *in vitro* was not expressed *in vivo* had now been provided.
- 2.6 The Committee noted the uncertainties with regard to routes of metabolic activation of 2,3-DCP and agreed that the two new mutagenicity studies supported the view that reactive metabolites, if formed, did not produce genotoxicity *in vivo* in the tissues assessed.
- 2.7 The Committee concluded that 2,3-DCP can be regarded as having no significant genotoxic potential *in vivo*.
- 2.8 A statement is appended at the end of this report.

Chromium picolinate

- 2.9 The Food Standards Agency asked the COM to review the available information on the mutagenicity of trivalent chromium and specifically on chromium picolinate in order to support consumer advice and, if appropriate, to recommend what further studies would be required to draw definite conclusions. A US National Toxicology Program (NTP) carcinogenicity bioassay of chromium picolinate is underway but the final report is unlikely to be available for several years. Chromium picolinate is a widely available food supplement. The adverse effects of chromium had been reviewed by the Expert Group on Vitamins and Minerals (EVM). (<http://www.food.gov.uk/multimedia/pdfs/vitmin2003.pdf>) The reports of genotoxicity associated with chromium picolinate were noted and chromium picolinate was excluded from their recommendations for a safe upper level. Following the publication of the EVM report, the Food Standards Agency advised that consumers should use other forms of trivalent chromium supplements until more detailed advice is available.

Public Health Issue

- 2.10 Hexavalent chromium compounds are established human carcinogens on the basis of both animal studies and epidemiological evidence of carcinogenicity (i.e. considered Group 1 carcinogens by the WHO International Agency for Research on Cancer (IARC) (<http://monographs.iarc.fr/>). Trivalent chromium compounds have lower toxicity, which is generally attributed to their lower solubility and to their very limited ability to cross cell membranes. Trivalent chromium compounds were considered by IARC to be not classifiable with regard to carcinogenicity in humans (i.e. Group 3).
- 2.11 Trivalent chromium may be the ultimate carcinogen responsible for the effect of hexavalent chromium since it is able to bind DNA directly. However, it is unclear whether the reduction of hexavalent to trivalent chromium and the subsequent oxidative damage, or the direct binding of trivalent chromium to DNA, or both is responsible.

COM consideration

- 2.12 The COM undertook a preliminary discussion of available mutagenicity data at its October 2003 meeting. A request for additional information was forwarded to the manufacturers who supply chromium picolinate to the food supplement industry.
- 2.13 The evaluation of the mutagenicity of chromium picolinate is complex and the available data are conflicting. Chromium picolinate has given positive results in some *in-vitro* mutagenicity tests. The mechanism by which this occurs is unclear. However, in these studies the test material had been synthesised in the laboratory concerned and an adequate specification was not available. The Committee considered that the *in-vitro* studies had given some indication of mutagenic activity and recommended that the critical tests should be repeated using commercial grade material (the previously tested material had been synthesised by the testing laboratory) before any definite conclusions could be drawn. Thus a further *in-vitro* *hprt* assay and an *in-vitro* chromosome aberration

study were recommended with chromium picolinate (both tests using CHO cells and to international standards) to determine whether the published results could be confirmed with a commercial grade sample. It was also recommended that the *hprt* assay should include a 48-hour incubation experiment in the absence of S-9 in addition to the standard time points of analysis.

- 2.14 Replication of the tests using commercial grade material in tests conducted to internationally accepted protocols gave negative results. The Committee expressed some reservations regarding the conduct of these studies (possible limitations in sensitivity) and in particular regarding the repeat *in-vitro* chromosome aberration study in CHO cells. However, overall it can be concluded that the balance of the data suggest that chromium picolinate should be regarded as not being mutagenic *in vitro*.
- 2.15 The available *in-vivo* tests in mammals with chromium picolinate are negative. In view of the negative *in-vitro* results with commercial grade chromium picolinate, there is no further requirement for *in-vivo* testing at the current time.
- 2.16 The ongoing US NTP carcinogenicity bioassays of chromium picolinate will provide important *in-vivo* data in the future. The in-life phase of the NTP bioassays is due to end in July 2004. These data should be considered when the full results of the bioassay are available.
- 2.17 A statement is appended at the end of this report.

Genotoxic carcinogens and DNA repair at low doses

- 2.18 The Committee on Carcinogenicity had asked for advice on the effect of chemical induced DNA repair at low doses with regard to genotoxic carcinogens.

Background to request

- 2.19 The COC had considered a horizon scanning paper by the DH Toxicology Unit at its June 2003 meeting. One aspect considered was hormesis (the occurrence of “U” shaped dose-response curves). During its discussions the COC considered a paper by Calabrese EJ and Baldwin LA (*Nature*, vol 421, 691-692, 2003), which provided an argument for the occurrence of hormesis (i.e. the occurrence of “U” shaped dose-response curves at low doses). The COC conclusions included the recommendation that the evidence for the induction of increased DNA repair (above background levels and with the potential to reduce the number of spontaneous mutagenic lesions) following exposure to very low levels of genotoxic carcinogens warranted further review, as a potential mechanism that could result in a “U” shaped dose response curve; they had therefore asked the COM for advice.

COM evaluation

- 2.20 The COM undertook an initial evaluation based on a preliminary position paper at its February 2004 meeting. The Committee considered that it was still prudent to assume that there is no threshold for mutation for *in-vivo* mutagens and where a potential threshold mechanism could be identified, appropriate evidence should be provided on a case by case basis. The COM considered that it was important to keep the issue of the absence of a threshold under review. They felt that it would be useful to carry out a literature search targeted on low dose effects of a few direct acting chemical mutagens on DNA adduct formation, mutation rates, and the significance of DNA repair mechanisms. The search should concentrate on low molecular weight compounds such as ethylene oxide and ethyl or methyl methanesulphonate, (EMS, MMS) for which there were considerable amounts of data available. Members agreed that bacteria would most likely demonstrate the most sensitivity to low doses of mutagens and had reservations as to whether mammalian cell systems would have sufficient sensitivity to detect evidence for an effect of DNA repair induction on the dose-response relationship for mutation.
- 2.21 A further detailed review paper was considered at the May 2004 meeting. This had considered information on *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), EMS and MMS. In view of the amount of data on these three compounds, ethylene oxide had not been used as an example.
- 2.22 Most information was available on the O⁶-methyltransferase (O⁶-MT) repair system, mainly in bacterial systems but some information was also available in mammalian cells. Members agreed that these data provided some evidence for thresholds in the adaptive state that could be attributed to the induction of the O⁶-MT. The difficulty in establishing unequivocal evidence for a threshold was discussed, as opposed to a no-observable-effect-level depending on the sensitivity of the assay. The importance of some knowledge of the underlying mechanism to support the biological plausibility of a threshold (as emphasised in the current COM guidance) was acknowledged.
- 2.23 Only limited data were available on systems other than the O⁶-MT repair, and members considered that these did not provide any significant evidence regarding thresholds. In the general discussion it was noted that evidence regarding DNA adducts induced by bulky compounds such as aromatic amines was consistent with a linear dose response and no threshold. It may thus have been useful to have considered data on such compounds as well as simple alkylating agents. However, it was recognised that this would have introduced the added complication of the need for metabolic activation. It was also pertinent that the key issue was what happened to the DNA adduct with regard to error-prone or error-free repair, the latter being a potentially threshold related pathway.

- 2.24 Overall, the Committee agreed that there was no clear evidence for a J shaped dose response in any of the data considered. Data regarding the O⁶-MT induction suggested that an *in-vivo* threshold was likely, but not proven. No conclusions could be drawn from the limited data on other DNA repair mechanisms.
- 2.25 When considering whether any further work would be appropriate it was important to consider if this would be cost effective. The difficulty in designing experimental studies with adequate sensitivity to provide definite answers needed to be recognised.
- 2.26 The Committee agreed that these data considered in this paper do not warrant any reconsideration of the Committee's current advice that it is prudent to assume that *in-vivo* mutagens do not have a threshold, unless there is good evidence to the contrary.
- 2.27 This advice was passed to the COC.

Malachite green/Leucomalachite green

Background to request for advice

- 2.28 Malachite green is a cationic triphenylmethane dyestuff used in a number of industries, including fish farming. It is used in freshwater fisheries for the treatment of external fungal and other infections, its main use being to stop fungal growth on the eggs. The Food Standards Agency had asked for a toxicology review from the COT in 1999 who had in turn asked for advice on the mutagenicity of malachite green and the lipophilic metabolite leucomalachite green from the COM. The COM had reached an interim conclusion based predominantly on limited evidence which indicated *in-vitro* mutagenic activity of these two compounds and the formation of DNA adducts in rats and mice with both compounds indicated that it would be prudent to consider malachite green and leucomalachite green as potential *in-vivo* mutagens.
- 2.29 The COM had considered preliminary data from *in-vivo* mutagenicity studies in 2003. The studies had been undertaken with leucomalachite green using transgenic rats (Big Blue™) and indicated that leucomalachite green had *in-vivo* mutagenic activity in the liver. The Committee agreed that there was no need to alter its previous conclusions on malachite green and leucomalachite green.

Advice requested in 2004

- 2.30 The COM was asked in 2004 to consider the full published reports of *in-vivo* mutagenicity studies undertaken for the US National Toxicology Programme. The COC was asked to review the carcinogenicity data. A joint COM/COC statement would be prepared.

2.31. The COM agreed to revise their 1999 conclusions regarding the mutagenicity of MG and LMG as follows:

Malachite green

- i) There is one report of a *Salmonella* assay done to an acceptable protocol; MG oxalate (>90% pure) was shown to induce mutations in TA98 in the presence of an exogenous metabolic activation system. There is also some evidence of clastogenicity in Chinese Hamster Lung cells. MG has also been shown to produce DNA damage in CHO cells using the Comet assay. MG should thus be regarded as having mutagenic potential.
- ii) Negative results were obtained in a poorly reported bone marrow micronucleus test in mice using a single oral dose of malachite green oxalate (>90% pure) at the MTD (75% of the LD50). Members agreed that the high dose level of 37.5 mg/kg was adequate, but it was difficult to assess the value of the negative results in the absence of appropriate information on bone marrow toxicity or data to show that malachite green and/or metabolites reached the bone marrow.
- iii) ³²P post-labelling studies using a 28 day dietary exposure have indicated that MG induces DNA adduct(s) in the liver of both F344 rats and B6C3F₁ mice. This has been confirmed in a separate study using 16 weeks dietary exposure. MG did not induce micronuclei in peripheral lymphocytes nor *hprt* mutations in splenic lymphocytes in the 28 day study; the experimental design of these studies did not, however, optimise the chances of obtaining a positive response and no definite conclusions can be drawn.
- iv) In view of the demonstration of DNA adduct formation in samples from both rats and mice, MG should be regarded as an *in-vivo* mutagen.

Leucomalachite green

- i) Data are now available on the *in-vitro* mutagenicity of LMG from limited *Salmonella* and CHO/HGPRT assays, and also from a Comet assay in CHO cells. Negative results were obtained. However in view of the limitations of these studies, and the fact that no data are available on clastogenicity, it is not possible to make an adequate assessment of the mutagenic potential of LMG from these data.
- ii) ³²P Post-labelling studies using a 28 day dietary exposure have indicated that LMG induces a low level of DNA adduct(s) in the liver of F344 rats; only a marginal response was however seen in B6C3F₁ mice, and no conclusions can be drawn from these data. This has been confirmed in a separate unpublished study in mice using 16 weeks dietary exposure; there was no evidence for DNA adduct formation in the liver. LMG did not induce micronuclei in peripheral lymphocytes nor *hprt* mutations in splenic lymphocytes in the 28 day study, but the experimental design of these studies did not optimise the chances of obtaining a positive response and no definite conclusions can be drawn.

- iii) Gene mutation studies have indicated that LMG can induce mutations *in-vivo* in liver DNA. Studies in Big Blue F344 rats gave equivocal results. LMG produced an increase in *lacI* mutations only at the highest dose tested (543 ppm in diet) and at a single time point (16 weeks). No increase was seen after 32 weeks. Evidence was provided to suggest that this isolated positive may have been due to disproportionate expansion of spontaneous *lacI* mutations. However studies in female Big Blue B6C3F₁ mice indicated that LMG produced an increase in *cII* mutant frequency in liver DNA of mice treated with 408 ppm LMG in the diet. Furthermore it was shown that the spectrum of mutation seen in the DNA was distinct from that of the control mice. These data provide evidence of *in vivo* mutagenicity at the target site in the carcinogenicity bioassay.
- iv) In view of the demonstration of the induction of mutations in liver DNA of female B6C3F₁ mice, LMG should be regarded as an *in-vivo* mutagen

2.32 The COC. provided advice on carcinogenicity (see section 3.47-3.51 of this annual report). There was no definite evidence for carcinogenicity in long term animal bioassays with malachite green. The overall conclusion was that it would be prudent to regard leucomalachite green as a genotoxic carcinogen. A statement is appended at the end of this annual report.

PAVA (pelargonyl vanillylamide)

- 2.33 In 2001 the Home Office requested advice from the COT on the health effects of a chemical incapacitant spray based on pelargonyl vanillylamide (PAVA), a synthetic equivalent of capsaicin, present in pepper. It is a sensory stimulant and is also used both as a food flavour (at up to 10 ppm) and as a topical rubefaciant in human medicine. The COT requested the advice of the COM on a package of mutagenicity data that had been commissioned by Sussex Police to support the health risk assessment of PAVA. The COM considered these mutagenicity data at its meeting in February 2002. The COM agreed that some further reassurance was necessary, in addition to the negative *in vivo* bone marrow micronucleus tests that had already been conducted. The Committee requested an *in vivo* liver UDS assay to provide this necessary reassurance.
- 2.34 Sussex Police commissioned the requested study and a full report was provided in Annex B. An *in vivo* liver UDS assay was carried out in rats using oral exposure and dose levels of 625 and 1250 mg/kg body weight. The top dose was the maximum tolerated dose, as determined from a range-finding toxicity study.
- 2.35 Members agreed that the study conformed to the relevant OECD test guideline (OECD Guideline No. 486: UDS test with mammalian liver cells *in-vivo*) and was adequate. 1,2- Dimethylhydrazine dihydrochloride was used as the positive control. There was no evidence of the induction of DNA repair by PAVA, as measured by unscheduled DNA synthesis in this assay. The positive control clearly induced DNA damage at both time points.

- 2.36 The COM agreed that the information sought by the Committee to conclude that PAVA was not expected to be an *in-vivo* mutagen had now been provided. No further data were required on the mutagenicity of this compound.
- 2.37 These conclusions were forwarded to the COT and were included in a COT statement in the COT section of this annual report.

Significance of environmental mutagenesis

- 2.38 At the COM October 2003 meeting a paper in Nature by Professor Thilly (Biological Engineering Division, MIT, Nat Genet. 2003 Jul;34(3):255-9.) had been tabled. One member had drawn the attention of the secretariat to this paper. It was agreed that detailed discussion of the significance of the paper would take place at the February 2004 meeting. The paper argued that the evidence for environmental mutagens causing cancer in humans is very weak and was limited to sunlight and ionising radiation. Thilly suggested that environmental risk factors for cancer act through non-mutational processes, and the genetic changes that cause cancer in humans arise from endogenous processes. The paper questioned the value of mutagenicity assay in screening for potential carcinogens.
- 2.39 Members agreed that the alternative hypothesis proposed by Thilly, namely that environmental risk factors for carcinogenesis acted not by mutation but by selecting particular kinds of preneoplastic cells previously initiated by spontaneous mutation resulting in a positive net growth rate, was interesting and warranted consideration. Regarding the most important environmental risk factor for lung cancer, cigarette smoking, Thilly pointed out that there was little evidence that smoking increased point mutations in bronchial epithelial cells or in peripheral T cells in humans, where the *hprt* locus had been examined. Members felt that the *hprt* assay in human lymphocytes may not be sufficiently sensitive or appropriate for detecting smoking induced mutations. Additionally, Thilly had not carried out a comprehensive review of this evidence and at least one report showing a significant increase in mutations in the *hprt* locus in smokers had been omitted.
- 2.40 The Committee noted that although Thilly accepted ionising radiation and sunlight as exceptions to his alternative hypothesis, there was a considerable body of evidence supporting the somatic mutation mechanism for chemical carcinogenesis that had not been taken into account. Further, Thilly had concentrated on only one type of mutation (gene mutation) and had not considered other examples, such as structural or numerical chromosome changes. It was widely accepted that clastogenic events, for example, are seen in smokers, and that it is crucial to control for smoking habits in any cytogenetics study in humans. No mention was made of the dominantly inherited conditions (and the changes in genetic material involved) that predispose to cancer in humans. Thilly had ignored the fact that essentially all clear *in-vivo* mutagens in somatic cells had also been shown to be carcinogens in animals when adequately tested. The fact that the majority of chemicals recognised as being carcinogenic in humans are also *in-vivo* mutagens had also been ignored.

- 2.41 Members concluded that arguments in the Thilly paper were not convincing and there was no justification to depart from the somatic mutation theory of chemical induced cancer or to change the COM recommended strategy for testing chemicals for mutagenicity.

Reassessment of toxicology of tobacco products

- 2.42 The Committees (COT/COC/COM) were asked to provide advice on the toxicological assessment of tobacco products with reference to the assessment of Potentially Reduced Exposure Products (PREPS) and in particular tobacco-based PREPS which are smoked. The Committees agreed that it was important to state that the ideal way forward to reduce risks and hazards of tobacco smoke was to encourage smokers to stop or people not to start in the first place and any attempt to reduce toxicity should not be allowed to detract from that. Members acknowledged that the primary remit of the Committees' discussions was to provide advice based on the information provided in the discussion papers.
- 2.43 The COM concluded that, using suitable protocols, it was possible to compare mutagenicity *in-vitro* of different PREPS which could be useful to assess hazard. However, the results of such *in-vitro* tests had no predictive value for risk of *in-vivo* mutagenicity or cancer. No conclusions could be drawn on the approaches using toxicogenomic methods. The available biomonitoring approaches were too limited to draw any conclusions regarding a comparison of PREPS.
- 2.44 A statement providing details of all the conclusions reached by COT/COC/COM is appended to the COT section of this annual report.

Review of Committee Procedures

- 2.45 The Committee's publication scheme (prepared in accordance with Freedom of Information Act 2000) is available on the COM internet site (<http://www.advisorybodies.doh.gov.uk/foi/publicationscheme.htm>). The COM meetings are now held in open session. The procedures adopted by COM are equivalent to those used by COT. Details can be found on the COM internet site. (<http://www.advisorybodies.doh.gov.uk/foi/open.htm>)

Horizon scanning

- 2.46 The COM undertakes 'Horizon scanning' exercises at regular intervals to identify new and emerging issues which have the potential to impact on public health and which might require COM advice. Members considered a paper which focussed on target organ mutagenesis and carcinogenicity risk assessment, low dose DNA adducts, mutagenicity of micronised chemicals and effects of micronutrients on mutagenicity.

- 2.47 Target organ mutagenesis in relation to identifying cancer risk had been identified as a potential topic for future consideration following recent COM/COC evaluations of chloropropanols and malachite green. Key mutagenic data from cancer target organs can potentially assist in carcinogen risk assessment. However, tumours may be observed in an organ that has not been assessed during *in-vivo* mutagenicity tests. The development of a variety of methodologies, such as Muta MouseTM and Big BlueTM transgenic rodent assays, the Comet assay, mutation spectra data and microarray analysis, may help identify target organs. Thus, there was a potential to define more closely whether observed tumours were attributable to mutagenic events.
- 2.48 Members agreed that the link between target organ mutation and cancer had not been investigated in detail at present. The committee considered that factors that determined target organs for carcinogenicity were poorly understood and the process was complex. The COM agreed that it would be valuable to have a joint COM/COC meeting on the topic. A number of independent experts would be invited to attend.
- 2.49 Techniques to assess DNA adducts had improved in recent years and highly sensitive methods such as ³²P-postlabelling were available. The committee noted that ILSI/HESI had looked at the use of low dose DNA adducts in risk assessment at its meeting in April 2004. The COM agreed to await a review on this topic by a subgroup of ILSI/HESI before considering the matter further.
- 2.50 The COM agreed that genotoxicity testing of nanotechnology products was potentially important. There were a few examples where micronised particles of traditionally accepted non-mutagenic chemicals had given positive results in *in-vitro* tests (e.g. zinc oxide and titanium dioxide). Aspects to consider included whether nanoparticles could be more readily absorbed, whether conventional mutagenicity tests were suitable for particles and whether any genotoxicity could be secondary to toxic effects such as the generation of reactive oxygen species. Members agreed that a periodic review of this subject would be useful.

Test Strategies and Evaluation

In-vitro micronucleus assay

- 2.51 The Committee provided advice on the evaluation of the *in-vitro* micronucleus test. Members agreed that for observational purposes the unit was the cell; it is the number of cells with micronuclei that is recorded. However, the major source of variability is likely to be the culture, and this should be considered in the analysis. The following wording was agreed. 'The observational unit is the cell, but the unit for statistical analysis is the culture.' This advice would be forwarded for inclusion in the draft proposals for an OECD guideline.

SCCNFP Recommendations On A Strategy For Testing Cosmetic Ingredients For Mutagenicity

- 2.52 The Committee were asked to comment on the strategy recently proposed by the EC's Scientific Committee on Cosmetics and Non-Food Consumer Products (SCCNFP) for permitted cosmetic ingredients (Opinion SCCNFP/0755/03 adopted April 2004 http://europa.eu.int/comm/health/ph_risk/committees/sccp/sccp_en.htm). In particular the strategy proposed for hair dyes, which is based on their opinion of June 2003, is of particular concern as this differs from the COM recommended strategy both in the number of *in-vitro* tests being proposed, and the importance placed in the SHE cell transformation assay. The Committee were asked whether they were aware of any data that would warrant re-consideration of their current views that the SHE cell transformation assay should not be used for regulatory screening of chemicals for potential carcinogenicity, as outlined in their statement of April 2002.
- 2.53 The Committee agreed that the SCCNFP's general recommendations for *in-vitro* screening of cosmetic ingredients, based on an *in-vitro* gene mutation test in mammalian cells and an *in-vitro* micronucleus test were reasonable. However, the proposals for testing hair dye ingredients based on the SCCNFP's June 2003 recommendations, gave rise to concern. The requirement for both a metaphase analysis for clastogenicity and an *in-vitro* micronucleus test introduced unnecessary redundancy; the Committee has always felt that, as regards the clastogenicity end-point, these 2 assays should give equivalent data. Furthermore the Committee felt that there was little to be gained by the additional *in-vitro* assay for DNA damage.
- 2.54 Regarding the SHE Cell Transformation Assay, members were aware that considerable work was being carried out on the development of this assay, much of which would be discussed at a meeting of the Environmental Mutagen Society (EMS) later in the year. It was agreed that the COM should consider these data when they are published, with a view to revising their statement on this assay if necessary.

COT/COC/COM review of Toxicogenomics

- 2.55 The COT/COC/COM held a joint symposium on the use of genomics and proteomics in toxicology in October 2001. The Committees agreed to further consider toxicogenomics as part of the horizon scanning exercise initiated at the February 2004 COT meeting. It was noted that there was a considerable increase in the number of publications using toxicogenomic approaches. A number of discussion papers were subsequently prepared for the Committees which reviewed the available published literature. The data from 50 studies were considered during the review which also included available information from the HESI (Health and Environmental Sciences Institute of the International Life Sciences Institute (<http://www.ilsa.org/>)) collaborative scientific program on toxicogenomics. The current review considered information on use of metabonomics in toxicology for the first time. The COT requested a further paper and presentation on the use of statistics/bioinformatics in toxicogenomics. A presentation was given by Dr David Lovell (University of Surrey) to the COT at its meeting on the 7 September 2004.

[The Committees used the following definitions for the methods used in Toxicogenomics. Transcriptomics refers to gene expression as measured through cDNA or oligonucleotide or cRNA microarray based approaches, proteomics refers to determination of protein levels through gel or solid phase approaches and metabonomics refers to measurement of metabolites in tissues, plasma or urine.]

2.56 The COM reached the following conclusions after discussions held at its February and May 2004 meetings:

- i) No conclusions can be drawn from the preliminary results of the ILSI/HESI trial of mutagenesis in mouse lymphoma L5178Y tk^{+/−} cells. Further information on the detailed results from this trial and validation of the findings would be needed before conclusions can be drawn.
- ii) Mutagenicity may be associated with changes in expression of relatively few genes which might be potentially difficult to identify in high density arrays. The COM agreed there were considerable difficulties in developing *in-vitro* mutagenicity screening assays using toxicogenomic approaches with regard to selection of appropriate microarray platform, confirmation of microarray results using quantitative measures of mRNA levels, identification of appropriate fold change in gene expression, and development of appropriate statistical/bioinformatics approaches for assessment of studies. However it was possible that valid approaches to screening for mutagens might be developed in the future.
- iii) The COM identified the need for more research on time dependent changes in gene expression using mutagens and the application of integrated toxicogenomic approaches to evaluating changes in protein and metabolic pathways in response to exposure to mutagens. No adequate proteomic/metabonomic studies of mutagens had currently been identified.
- iv) The COM reviewed a number of published papers which presented data using mouse lymphoma L5178Y tk^{+/−} cells and agreed that no clearly defined pattern of gene expression changes which could logically be associated with mutagenesis had been identified. The COM reviewed a recent study which had used HepG2 cells and agreed that the authors had been able to distinguish between genotoxic and non-genotoxic carcinogens but only when a number of genotoxic compounds (predominantly methylating agents) were excluded. Overall this latter study provided some useful information but there was a need for considerable additional research involving multiple dose levels and sampling times before conclusions could be reached.
- v) The Committee considered that the limited available *in-vivo* studies using four hepatocarcinogens did provide some preliminary results which suggested genotoxic responses in gene expression could be identified *in-vivo*.
- vi) One preliminary investigation provided evidence to suggest that transcriptomics could provide information to aid in the interpretation of conventional *in-vitro* clastogenicity assays to assist in the evaluation of mutagenic or cytotoxic responses in these tests.

- 2.57 A statement providing details of all the conclusions reached by COT/COC/COM is appended to the COT section of this annual report.

Ongoing reviews

Biomonitoring studies for genotoxicity in pesticide applicators

- 2.58 The committee has been asked by the Medical and Toxicology Panel (MTP) of the Advisory Committee on Pesticides (ACP) to undertake a review of the available studies of genotoxicity in pesticide applicators and workers exposed to pesticides, particularly focussing on pesticide sprayers/applicators, floriculturists/greenhouse workers, agricultural workers and farmers and forestry workers. Advice from COM would be submitted to PSD as the registration authority for agricultural pesticide products and subsequently to the MTP and ACP. An initial overview paper of studies published in the European Union was considered at the October 2004 meeting. A further paper will be presented to the February 2005 meeting.

Statements

2,3-Dichloropropan-1-ol

Chromium picolinate

Malachite green/leucomalachite green

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

Introduction

1. 2,3-dichloropropan-1-ol (2,3-DCP) is a member of a group of chemicals called chloropropanols, which includes 3-monochloropropane-1,2-diol (3-MCPD) and 1,3-dichloropropan-2-ol (1,3-DCP). 2,3-DCP and 3-MCPD can be present as process contaminants in some polyamine flocculants used in water treatment and may therefore potentially be present in drinking water. 1,3-DCP is found as a process contaminant in food stuffs where acid-hydrolysed vegetable protein has been used as an ingredient in soy sauce or similar oriental sauces. It is not currently known if 2,3-DCP is present in food. 2,3-DCP was last considered by the COM in 2001. COM concluded that it would be prudent to consider 2,3-DCP as potentially genotoxic *in vivo* but agreed that it should be tested for genotoxicity *in vivo* using the approach set out in the COM guidelines.

COM evaluation 2001

2. Members were aware that there was very little data on the absorption, distribution, and excretion of 2,3-DCP. Theoretically, 2,3-DCP could be metabolised to produce epichlorohydrin (and subsequently glycidol) and therefore there were structural alerts for genotoxicity and carcinogenicity.
3. The Committee noted 2,3-DCP was mutagenic in *Salmonella typhimurium* strains TA 100 and TA 1535 in a study with and without metabolic activation¹, and mutagenic in another Ames test². Positive results were also obtained for sister chromatid exchange with Chinese Hamster V79 cells both with and without metabolic activation³. No *in-vivo* studies in mammals have been carried out.
4. The Committee concluded that in the limited studies conducted, 2,3-DCP was genotoxic *in-vitro* with and without metabolic activation in bacterial and mammalian cells.

COM evaluation 2004

5. The Committee considered two new *in-vivo* genotoxicity studies at its February 2004 meeting. These comprised a rat bone-marrow micronucleus test and a rat liver unscheduled DNA synthesis (UDS) assay, both of which are widely used to assess genotoxicity *in vivo*.

Rat in-vivo bone-marrow micronucleus test⁴

6. The assay followed the current OECD guideline (No 474). The highest dose used in the study was selected so that it would produce some signs of toxicity, but not severe effects, based on the results of a range-finding study. In the main study, 1,3-DCP was administered once daily for two consecutive days to groups of six male Han Wistar rats at doses of 70, 140 and 280 mg/kg. Bone marrow was harvested 24 hours after the final dose. A single sex study was considered adequate because no substantial difference in toxicity was observed between males and females in the range-finder.

7. Clinical signs of toxicity, including lethargy, eye closure and piloerection were observed at 140 and 280 mg/kg bw. A dose related decrease in the mean ratios of polychromatic to normochromatic erythrocytes (PCE/NCE) compared to vehicle control was documented indicating that the test material was toxic to the bone marrow.
8. There were no statistically significant increases in micronucleus frequency at any dose of 2,3-DCP. The positive control agent, cyclophosphamide, produced a clear increase in micronuclei.

Rat liver in-vivo UDS assay⁵

9. The UDS assay protocol conformed to the current OECD guideline (No 486). Based on the results of the range-finder for the micronucleus study, the study was conducted in male rats and the highest dose was 280 mg/kg. Single doses of 110 and 280 mg/kg were administered to groups of four male Han Wistar rats. Hepatocytes were isolated for analysis for UDS by the autoradiographic technique after 12-14 hours in the first study (3 rats per dose group) and at 2-4 hours in the second study (3 rats per dose group).
10. The investigators reported notable weight loss in animals dosed with 280 mg/kg bw at 12-14 hours post-dose. It was noted that for a one animal, at 110 mg/kg bw in the 12-14 hour trial, the number of cells scored was below the recommended number. However there were sufficient cells scored from all other animals in the study. There was clear evidence of toxicity at the top dose level used. There was no evidence of an increase in Net Nuclear Grain counts in treated animals at either time point. The positive control compounds 2-AAF and DMN both gave clear positive results.

COM discussion

11. Members agreed that the two new studies met the previously stated requirement that 2,3-DCP should be tested for genotoxicity *in-vivo* using the approach set out in the COM guidelines. The studies were adequately conducted and gave clear negative results, and therefore Members considered that these studies provided evidence that 2,3-DCP was not an *in-vivo* mutagen. Members then gave consideration as to possible mechanisms whereby mutagenic activity observed *in vitro* was not expressed *in vivo*.
12. Members agreed that 2,3-DCP was metabolised to 2,3-dichloroacetaldehyde and from this to the corresponding acid. One research group had provided some *in vitro* data to suggest that induction of CYP2E1 resulted in 2,3-DCP mediated hepatotoxicity and glutathione depletion⁶. Members noted the findings of Koga *et al*⁷ which suggested dechlorination/ hydroxylation occurred but agreed these authors had not provided evidence for the formation of an epoxide. Members concluded that there were insufficient data to draw conclusions on the metabolic activation of 2,3-DCP but overall the evidence suggested metabolic activation of 2,3-DCP differed from 1,3-DCP.

13. The COM considered that the metabolism of 2,3-DCP had not been fully elucidated. Metabolic activation *in vivo* to active metabolites had been postulated but had not been proven. The Committee agreed that 2,3-DCP was not mutagenic in the two tissues assessed which provided some assurance that active metabolites were not formed *in-vivo*.

Conclusions

14. The Committee concluded that both the rat bone-marrow micronucleus test and the rat liver UDS test had been carried out to an acceptable standard and were negative. Thus the additional information recommended by the COM as being necessary to provide adequate reassurance that the mutagenic activity seen *in vitro* was not expressed *in vivo* had now been provided.
15. The Committee noted the uncertainties with regard to routes of metabolic activation of 2,3-DCP and agreed that the two new mutagenicity studies supported the view that reactive metabolites, if formed, did not produce genotoxicity *in vivo* in the tissues assessed.
16. The Committee concluded that 2,3-DCP can be regarded as having no significant genotoxic potential *in vivo*.

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Statement on the mutagenicity of trivalent chromium and chromium picolinate

Introduction

Background to COM review

1. Chromium is a Group 6 metallic element which is ubiquitous in the environment where it generally occurs in the hexavalent or trivalent form. Chromium is an essential element, involved in carbohydrate metabolism. Chromium (III) picolinate is a food supplement which is widely available in the UK.
2. Chromium was one of the minerals recently reviewed by the Expert Group on Vitamins and Minerals (EVM) (<http://www.foodstandards.gov.uk/multimedia/pdfs/vitmin2003.pdf>). The EVM noted the reports of genotoxicity associated with chromium picolinate and excluded it from their recommendations for a Safe Upper Level for chromium. Following the publication of the EVM report, the Food Standards Agency advised that consumers should use other forms of trivalent chromium supplements.

Advice requested from COM

3. The Food Standards Agency has asked the COM to review the available information on the mutagenicity of trivalent chromium and specifically on chromium picolinate in order to support consumer advice and, if appropriate, to recommend what further studies would be required to draw definite conclusions. A US National Toxicology Program (NTP) carcinogenicity bioassay of chromium picolinate is underway but the final report is unlikely to be available for several years.

Public Health Issue

4. Hexavalent chromium compounds are established human carcinogens on the basis of both animal studies and epidemiological evidence of carcinogenicity (ie considered Group 1 carcinogens by the WHO International Agency for Research on Cancer (IARC) (<http://monographs.iarc.fr/>). Trivalent chromium compounds have lower toxicity, which is generally attributed to their lower solubility and to their very limited ability to cross cell membranes. Trivalent chromium compounds were considered by IARC to be not classifiable with regard to carcinogenicity in humans (i.e. Group 3).
5. Trivalent chromium may be the ultimate carcinogen responsible for the effect of hexavalent chromium since it is able to bind DNA directly. However, it is unclear whether the reduction of hexavalent to trivalent chromium and the subsequent oxidative damage, or the direct binding of trivalent chromium to DNA, or both is responsible.

Data considered by COM

Chromium picolinate and other chromium compounds

6. Chromium picolinate contains trivalent chromium bonded to three molecules of picolinic acid. The formation of additional chromium co-ordination complexes with each molecule of picolinic acid through the lone pair of electrons present on the nitrogen aids the stability of the molecule. Picolinic acid is an isomer of niacin (vitamin B₃) and a minor metabolite of tryptophan metabolism. Unlike other trivalent chromium compounds, chromium picolinate is soluble in water at neutral pH.

Absorption, distribution, metabolism and excretion

7. The data on the absorption, distribution, metabolism and excretion (ADME) of chromium picolinate are limited. The available data from human volunteer studies and from experimental studies in rats suggest that the gastrointestinal absorption of chromium picolinate is significantly greater than of other forms of trivalent chromium and is comparable to that of hexavalent chromium.
8. In a recent *in-vivo* ADME study, rats were given a daily intravenous dose of radiolabelled chromium picolinate (⁵¹Cr or ³H- picolinate) for 14 days¹. Retention of chromium was substantial, with daily urinary and faecal excretion of approximately 10% of the ⁵¹Cr at the beginning of the experiment, increasing to approximately 20% by the end. The majority of the radiolabel was found in the urine and when subject to column chromatography co-eluted with chromodulin. ³H-labelled material was largely excreted via the urine. At the end of the treatment period, both ⁵¹Cr and ³H labels were widely distributed in the tissues but were predominantly present in the liver, where the sub-cellular pattern of distribution differed to that in other tissues. Thus the absorption, distribution, metabolism and excretion of chromium picolinate is complex and includes some degree of dissociation.

Oxidative damage

9. Hexavalent chromium is readily reduced to trivalent chromium both *in vitro* and *in vivo*, resulting in oxidative and cytotoxic damage to cells. The evidence for oxidative damage *in vivo*, after treatment with chromium picolinate includes increased urinary excretion of 8-hydroxy-2' deoxyguanosine and increased lipid peroxidation in liver and kidney cells of rats². Evidence of *in vitro* oxidative damage includes damage to mitochondria from CHO cells and lipid peroxidation in cultured macrophage J774A.1 cells^{3,4}. However the only other *in-vivo* study available does not report any oxidative damage associated with chromium picolinate or with other forms of trivalent chromium⁵. Overall, it can be concluded that chromium picolinate induces oxidative damage *in vivo* to a lesser extent than hexavalent chromium compounds. However, *in-vivo* oxidative damage associated with chromium picolinate treatment has been reported by only one research group, where the test material was synthesised in the laboratory.

Interactions with DNA

10. Trivalent chromium compounds are able to bind DNA and RNA in cell free systems. While some studies with chromium picolinate suggest little direct interaction with DNA², Speetjens and colleagues⁶, have shown a dose-dependent relaxation of supercoiled plasmid DNA to the circular nicked form by trivalent chromium picolinate in the presence of ascorbic acid and air. The authors noted that chromium picolinate was stable and thus could be incorporated into cells intact. They further speculated that ascorbate could reduce trivalent chromium to divalent chromium, which could then enter Fenton or Haber-Weiss reaction cycles to produce hydroxyl radicals leading to oxidative damage. Members considered that more direct evidence was required to confirm this suggestion.
11. Chromium picolinate induces DNA fragmentation in cultured J774A.1 murine macrophages⁷.

In vitro mutagenicity

12. Trivalent chromium compounds are negative in a large number of *in-vitro* bacterial mutagenicity tests. In contrast, positive results have been documented for hexavalent chromium compounds. An adequate study using chromium picolinate was conducted in *Salmonella typhimurium* strains as part of the US NTP. This study reported negative results⁸. Other published bacterial tests with chromium picolinate have also yielded negative results^{9,10}.
13. Chromium picolinate has been reported to cause up to a 40 fold increase in mutation at the *hprt* locus in CHO AA8 cells in the absence of exogenous metabolic activation¹¹. However the results were from a single, unusually long, treatment time of 48 hours. The chromium picolinate used had been synthesised by the testing laboratory and there was uncertainty regarding the nature and quantities of impurities in test material used.
14. In response to recommendations made by the COM in October 2003, the ability of chromium picolinate to cause *hprt* mutations *in vitro* in CHO cells was tested using commercial grade material using a protocol adhering to International standards¹². Chromium picolinate was negative in both S9 activated and non-activated assays. In order to fully replicate the work of Stearns and colleagues¹¹, an additional experiment was conducted using a 48 hour incubation in the absence of S9 activation¹³. This was also negative. Additional information on dosing solution analysis and historical control data were reported to the COM in October 2004. The Committee was satisfied regarding the identity of the test material and dosing solution analysis. Overall it was agreed that the data should be considered as indicating a negative result.

15. Positive results have been documented for both hexavalent and trivalent chromium compounds in *in-vitro* clastogenicity tests in mammalian cells. The significance of the results with trivalent compounds is uncertain as the evidence of mutagenicity was documented at high cytotoxic concentrations and under prolonged exposure conditions where it was considered that endocytic uptake of test material had occurred. In a study by Stearns and colleagues¹⁴ chromium picolinate was tested in both a solubilised and a particulate form and resulted in a dose-related increase in chromosomal aberrations in CHO AA8 cells following incubation for 24 hours in the absence of exogenous metabolic activation. The magnitude of the response (3-18 times control) reported clearly suggested a clastogenic effect. The Committee noted that the test material used had been synthesised in the testing laboratory and commented that there was uncertainty regarding the nature and quantities of impurities in the test material used in this assay. The Committee also noted that chromium picolinate would have been expected to be poorly soluble in the solvent used (acetone). Overall it was agreed that there was a need for independent replication of the study undertaken by Stearns before any conclusions could be reached. In response to recommendations made by the COM in October 2003, the ability of chromium picolinate to cause chromosome aberrations in CHO cells *in vitro* was tested and reported to be negative¹⁵. The Committee considered that the submitted study had been adequately conducted according to internationally accepted guidelines but observed that there were limitations regarding this study (eg the wide historical positive control range for structural aberrations both in the absence and presence of exogenous metabolic activation). Additional information on dosing solution analysis and historical control data were reported to the COM in October 2004. The Committee was satisfied regarding the identity of the test material and dosing solution analysis. Overall it was agreed that although the committee noted some limitations in the study, the data should be considered as indicating a negative result.

In vivo mutagenicity

16. Chromium picolinate but not trivalent chromic chloride was active in a multi-generation *Drosophila* study where it was observed to delay pupation and decrease pupal viability. Further analysis indicated that chromium picolinate increased lethal mutations and dominant female sterility¹⁶. It is not possible to extrapolate such data to *in-vivo* exposure in mammals.
17. Three *in-vivo* mutagenicity studies of chromium picolinate have been reported; two in rats and one in mice. No evidence of chromosomal damage was reported in a study in which rats were given an oral dose of up to 2,000 mg/kg bw. The data were published in abstract form^{10,17} with further details being contained in the study report¹⁸.

18. No increase in micronuclei was reported in peripheral blood samples from F344 rats given gavage doses of up to 2,500 mg/kg chromium picolinate for 3 days¹⁹. This study has been undertaken as part of the preliminary studies of the US NTP prior to commissioning carcinogenicity bioassays. The ratio of polychromatic to normochromatic erythrocytes was not altered in this study. However it is not possible to conclude that the bone-marrow has been exposed to chromium picolinate in this study since previous work²⁰ had suggested that trivalent chromium may be retained in the fatty tissue surrounding the bone-marrow. There are no comparable absorption data available for chromium picolinate. As part of the US NTP study, B6C3F1 mice were given diets containing up to 50,000 ppm chromium picolinate for 13 weeks and micronuclei in peripheral blood erythrocytes counted²¹. There was no clear evidence for a mutagenic effect, although a statistical test for a dose-related trend had reported a positive finding in female mice ($P=0.005$). Pair-wise comparisons with control suggested no mutagenic effect. The NTP data have not yet been published in a peer-reviewed form.

Picolinic acid

19. An increase in *hprt* mutations and chromosomal aberrations in the absence of exogenous metabolic activation was also documented in CHO AA8 cells^{11,12}. These tests were undertaken concurrently with the tests using chromium picolinate described previously. Chromium picolinate was reported to be more mutagenic in the *hprt* system than the equivalent concentrations of either free picolinate or trivalent chromic chloride. Similarly, the clastogenic effects of chromium picolinate were apparent at lower equivalent concentrations than those associated with free picolinate.
20. Picolinic acid was also studied in a multigeneration *Drosophila* study¹⁶. Treatment with picolinic acid alone also increased the numbers of individuals arrested during pupation and reduced larval and adult viability. The significance of this with respect to the mutagenicity of picolinic acid is unclear. It is also not possible to extrapolate such data to *in-vivo* exposure in mammals.

Conclusions

21. The evaluation of the mutagenicity of chromium picolinate is complex and the available data are conflicting. Chromium picolinate has given positive results in some *in-vitro* mutagenicity tests. The mechanism by which this occurs is unclear. However, in these studies the test material had been synthesised in the laboratory concerned and an adequate specification was not available. Replication of the tests using commercial grade material in tests conducted to internationally accepted protocols gave negative results. The Committee expressed some reservations regarding the conduct of these studies (possible limitations in sensitivity) and in particular regarding the repeat *in-vitro* chromosome aberration study in CHO cells. However, overall it can be concluded that the balance of the data suggest that chromium picolinate should be regarded as not being mutagenic *in vitro*.
22. The available *in-vivo* tests in mammals with chromium picolinate are negative. In view of the negative *in-vitro* results with commercial grade chromium picolinate, there is no further requirement for *in-vivo* testing at the current time.

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23. The ongoing US NTP carcinogenicity bioassays of chromium picolinate will provide important *in-vivo* data in the future. The in-life phase of the NTP bioassays is due to end in July 2004. These data should be considered when the full results of the bioassay are available.

December 2004

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Joint COM and COC statement on the mutagenicity and carcinogenicity of malachite green (MG) and leucomalachite green (LMG)

Introduction

1. Malachite green (MG) is a cationic triphenylmethane dyestuff used in a number of industries including fish farming. Its use in fish for human consumption was banned in the EU in June 2002 but residues continue to be found in fish.
2. In 1999 the COM provided advice to the COT on the mutagenicity of malachite green and its lipophilic metabolite leucomalachite green (LMG). The COT had asked for advice in particular on the results from ^{32}P post-labelling studies investigating DNA adduct formation in the liver of rats and mice from 28 day repeated dose toxicity studies. These studies were part of the US National Toxicology programme, and were the range-finding studies prior to initiating carcinogenicity bioassays on MG and LMG.

Mutagenicity

COM Advice 1999

3. In 1999 the COM agreed the following conclusions regarding the mutagenicity of MG and LMG, which were incorporated in the 1999 COT statement on MG and LMG in farmed fish.¹

Malachite Green

- i) There is one report of a Salmonella assay done to an acceptable protocol; malachite green oxalate (>90% pure) was shown to induce mutations in TA98 in the presence of an exogenous metabolic activation system.² There is also some evidence of clastogenicity in Chinese hamster lung cells.³ MG should thus be regarded as having mutagenic potential.
- ii) Negative results were obtained in a poorly reported bone marrow micronucleus test in mice using a single oral dose of malachite green oxalate (>90% pure) at the MTD (75% of the LD50).² Members agreed that the high dose level of 37.5 mg/kg was adequate, but it was difficult to assess the value of the negative results in the absence of appropriate information on bone marrow toxicity or data to show that MG and/or metabolites reached the bone marrow.
- iii) Recent ^{32}P -post-labelling studies using a 28 day dietary exposure and carried out with NTP range – finding studies (used in designing carcinogenicity bioassays) indicate that MG induces DNA adducts in the liver of rats and mice.^{4,5}
- iv) The Committee concluded that although a limited negative *in-vivo* micronucleus test was available, the results of the recently conducted ^{32}P - post-labelling studies indicated that it would be prudent to assume that MG may be a potential *in-vivo* mutagen.

Leucomalachite green

- i) The Committee were concerned that only limited information was available on LMG. The lack of information on LMG prevents an adequate mutagenicity assessment for this compound.
- ii) The Committee recommended that *in-vitro* studies in bacteria for gene mutations, in mammalian cells for clastogenicity and a mammalian cell assay for gene mutation (preferably the mouse lymphoma assay), conducted according to current OECD guidelines, should be undertaken as an important step to evaluating the mutagenic potential of LMG.
- iii) The Committee, however, concluded that the results of the recently conducted post labelling studies^{4,5} indicated that it would be prudent to assume that LMG may also be a potential *in-vivo* mutagen.

Updated advice from COM 2004

- 4. The results of the NTP carcinogenicity bioassays on MG and LMG were published in 2004⁶. The NTP report in addition included the results of a number of new studies to investigate the mutagenicity of these 2 compounds, most of which had also been published separately. The COM reviewed these new data in 2004 with a view to updating their conclusions on the mutagenicity of these two compounds.
- 5. New data were now available from *in-vitro* studies to investigate the ability of MG and LMG to induce mutations in the Salmonella assay, the CHO/*hprt* assay, and an *in-vitro* comet assay^{6,7}. Negative results were obtained except for the comet assay with MG which was positive.
- 6. The ³²P post-labelling studies considered by the COM in 1999 have now been published in full. Male F344 rats and female B6C3F₁ mice were fed 9, 100 and 600 ppm MG or 0, 96 or 580 ppm LMG in the diet for 28 days⁸. ³²P post-labelling analysis of liver DNA indicated a single adduct, or co-eluting adducts, with both compounds, with the level of adducts increasing significantly as a function of dose. In rats the level of adducts seen was similar with MG and LMG. However in the mice MG gave a clear dose-related increase in binding which was slightly lower than that seen in the rats, whereas LMG produced only very low levels of adducts, of doubtful significance. In later studies with female Big Blue rats and mice LMG again produced evidence of DNA adduct formation in the rat but not the mouse.⁹
- 7. The ability of MG or LMG to induce micronuclei in peripheral blood or in *hprt* mutations in the spleen was also investigated in the above studies; negative results were obtained^{6,9}. The Committee noted that these studies, which used repeated exposure over 4-32 weeks, did not optimise the chances of detecting a mutagenic response.

8. Studies to investigate induction of *lacI* mutations by LMG in the liver of female Big Blue rats gave equivocal results¹⁰. Samples were obtained at 3 time periods (4, 16, 32 weeks) at 5 dose levels. An increase in *lacI* mutations was seen at only a single time point (16 weeks) and only at the top dose level. Since *lacI* mutations were not expected to decline with continued exposure to compounds the nature of the DNA sequence alterations was investigated in DNA from the isolated positive result. When corrected for clonality there was no significant difference between the LMG treated and the control frequency. The authors considered that this isolated positive was an artifact due to the disproportionate expansion of spontaneous *lacI* mutations.
9. Studies to investigate *cII* mutations induced by LMG in the liver of Big Blue B6C3F₁ mice did, however, give a positive result⁶. At a dietary level of 408 ppm LMG produced a statistically significant ($p < 0.05$) increase in mutations. Further analysis showed that these contained a spectrum of mutations that was distinct from that in the control animals. In contrast MG did not produce any significant increase in such mutations in the mice. In similar studies in Big Blue rats, LMG did not produce any increase in *cII* mutation frequency in liver DNA.
10. Members considered the lack of concordance between the results of studies investigation DNA adducts and mutations in transgenic mice and the observation of an apparently specific mutation in the *cII* transgene in mice represented an unusual data set and hence there was uncertainty in deriving conclusions from these studies.
11. The COM agreed to revise their 1999 conclusions regarding the mutagenicity of MG and LMG as follows:

Malachite green

- i) There is one report of a *Salmonella* assay done to an acceptable protocol; MG oxalate (>90% pure) was shown to induce mutations in TA98 in the presence of an exogenous metabolic activation system². There is also some evidence of clastogenicity in Chinese Hamster Lung cells³. MG has also been shown to produce DNA damage in CHO cells using the Comet assay⁶. MG should thus be regarded as having mutagenic potential.
- ii) Negative results were obtained in a poorly reported bone marrow micronucleus test in mice using a single oral dose of malachite green oxalate (>90% pure) at the MTD (75% of the LD50)². Members agreed that the high dose level of 37.5 mg/kg was adequate, but it was difficult to assess the value of the negative results in the absence of appropriate information on bone marrow toxicity or data to show that malachite green and/or metabolites reached the bone marrow.
- iii) ³²P post-labelling studies using a 28 day dietary exposure have indicated that MG induces DNA adduct(s) in the liver of both F344 rats and B6C3F₁ mice⁷. This has been confirmed in a separate study using 16 weeks dietary exposure¹. MG did not induce micronuclei in peripheral lymphocytes nor *hprt* mutations in splenic lymphocytes in the 28 day study; the experimental design of these studies did not, however, optimise the chances of obtaining a positive response and no definite conclusions can be drawn.

- iv) In view of the demonstration of DNA adduct formation in samples from both rats and mice, MG should be regarded as an *in-vivo* mutagen.

Leucomalachite green

- i) Data are now available on the *in-vitro* mutagenicity of LMG from limited Salmonella and CHO/HGPRT assays, and also from a Comet assay in CHO cells⁷. Negative results were obtained. However in view of the limitations of these studies, and the fact that no data are available on clastogenicity, it is not possible to make an adequate assessment of the mutagenic potential of LMG from these data.
- ii) ³²P Post-labelling studies using a 28 day dietary exposure have indicated that LMG induces a low level of DNA adduct(s) in the liver of F344 rats; only a marginal response was however seen in B6C3F₁ mice, and no conclusions can be drawn from these data⁸. This has been confirmed in a separate unpublished study in mice using 16 weeks dietary exposure; there was no evidence for DNA adduct formation in the liver⁶. LMG did not induce micronuclei in peripheral lymphocytes nor *hprt* mutations in splenic lymphocytes in the 28 day study⁹, but the experimental design of these studies did not optimise the chances of obtaining a positive response and no definite conclusions can be drawn.
- iii) Gene mutation studies have indicated that LMG can induce mutations *in vivo* in liver DNA. Studies in Big Blue F344 rats gave equivocal results. LMG produced an increase in *lacI* mutations only at the highest dose tested (543 ppm in diet) and at a single time point (16 weeks). No increase was seen after 32 weeks. Evidence was provided to suggest that this isolated positive may have been due to disproportionate expansion of spontaneous *lacI* mutations^{9,10}. However studies in female Big Blue B6C3F₁ mice indicated that LMG produced an increase in *cII* mutant frequency in liver DNA of mice treated with 408 ppm LMG in the diet⁶. Furthermore it was shown that the spectrum of mutation seen in the DNA was distinct from that of the control mice. These data provide evidence of *in-vivo* mutagenicity at the target site in the carcinogenicity bioassay.
- iv) In view of the demonstration of the induction of mutations in liver DNA of female B6C3F₁ mice, LMG should be regarded as an *in-vivo* mutagen.

Carcinogenicity

COC Advice 2004

12. The COC considered the results of the NTP carcinogenicity studies on MG and LMG in June 2004. They noted that prior to the publication of the NTP bioassay data on MG and LMG there had been no data available to make any meaningful assessment of the carcinogenicity of these compounds.

13. The carcinogenicity of MG was investigated in female F344 rats and in female B6C3F₁ mice, compound being given in the diet for 104 weeks. It was noted that the rationale for testing only in females was that this had been shown in range finding studies to be the most sensitive gender. In the study in rats groups of 48 female rats were given diets containing 0, 100, 300 and 600 ppm MG (equivalent to average daily doses of approximately 0, 1, 21 and 43 mg/kg bw/day). Survival in all groups was comparable, but mean body weight gain was slightly lower at 300 and 600 ppm (approximately 10% reduction compared to controls). At autopsy it was noted that the relative liver weights were increased at the top dose. In the study in mice groups of 48 animals were fed diets containing 0, 100, 225 and 450 ppm MG (equivalent to average daily doses of approximately 0, 15, 33 and 67 mg/kg bw/day). Survival was again comparable in all groups but there was a slight reduction in body weight gain (5-10%) at the top dose. Effects on relative kidney weight were noted at autopsy.
14. The carcinogenicity of LMG was investigated in both male and female F344 rats and in female mice, the compound being given in the diet for 104 weeks. Groups of 48 male and female rats were fed diets containing 0, 91, 272 or 543 ppm LMG (equivalent to daily doses of 0, 5, 15 and 30 mg/kg bw/day in the males and 0, 6, 17 and 35 mg LMG/kg bw/day in the females). Survival was comparable in all groups (except for an increase in males given 272 ppm) but there was a reduction in body weight gain at the top dose (25% in females, 10-15% in males). At autopsy liver weight were noted to be increased in the males at the two higher doses, as were relative liver weights in the females.
15. In the study in female mice groups of 48 were given diets containing 0, 91, 204 or 408 ppm LMG (equivalent to average daily doses of 0, 13, 31 and 63 mg LMG/kg bw/day). Survival and body weight gain were comparable in all groups. At autopsy a decrease in relative kidney weight was noted.
16. Regarding the evidence for carcinogenicity in these bioassays the Committee agreed with the NTP conclusions, as modified by their peer review panel. They agreed the following conclusions regarding the carcinogenicity of MG and LMG.

Conclusions regarding carcinogenicity of malachite green

- i) There was equivocal evidence of carcinogenicity in female F344 rats based on an increase in thyroid gland tumours (adenoma or carcinoma combined) hepatocellular adenomas and mammary gland carcinomas.
- ii) There was no evidence of carcinogenic activity in female B6C3F₁ mice.
- iii) Overall there was no convincing evidence for any carcinogenic effect with malachite green in these studies.

Carcinogenicity of leucomalachite green

- i) There was evidence of carcinogenic activity in female B6C3F₁ mice based on an increase in hepatocellular adenoma or carcinoma combined.
- ii) There was equivocal evidence of carcinogenic activity in male F344 rats based on an increase in interstitial cell adenoma of the testes and the occurrence of thyroid gland follicular cell adenoma or carcinoma (combined).
- iii) There was equivocal evidence of carcinogenic activity in female F344 rats based on an increased incidence of hepatocellular adenoma or carcinoma (combined).

Overall conclusions regarding mutagenicity and carcinogenicity of MG and LMG

- 16. The COM concluded that both MG and LMG should be regarded as *in vivo* mutagens.
- 17. The COC concluded that the only convincing evidence for any carcinogenic effect of MG or LMG in the NTP bioassays was for LMG in female mice, based on an increase in hepatocellular adenoma or carcinoma combined.
- 18. The COC considered the possible mechanisms by which LMG induced tumours in the liver of the female mice. It was noted that the overall tumour profile was not that which would be expected of a genotoxic carcinogen, with activity being limited to effects in the liver of the female mouse; furthermore this was mainly due to an increase in adenomas. However it was also noted that there was no evidence from the NTP studies to support any non-genotoxic mechanism. In view of this, and taking into account the views of the COM, the Committee agreed that it was not possible to discount a genotoxic mechanism for the induction of the liver tumours in female mice and it would therefore be prudent to regard LMG as a genotoxic carcinogen.

December 2004

COM/04/S4 & COC/04/S7

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2004 membership of the Committee on Mutagenicity of Chemicals in Food, Consumer products and the Environment

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Dr D Benford BSc PhD Scientific Secretary – Food Standards Agency

Mr K Mistry Administrative Secretary

Mr J Battershill BSc MSc

Declaration of interests during the period of this report

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof P B Farmer (Chairman)	Abbey National	Share Holder	American Chemistry Council CEFIC	Research Support
	Bradford & Bingley	Share Holder		Research Support
	Celltech	Share Holder		
	Foreign & Colonial	Share Holder		
	Friends Provident			
	Health Effects Institute	Share Holder Research Committee Member		
	Torotrak	Share Holder		
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	Share Holder		
	AstraZeneca	Share Holder		
	Diagco	Share Holder		
	HBOS	Share Holder		
	Marks & Spencer	Share Holder		
Dr J Clements	Covance	Salary Share Option Share Holder	NONE	NONE
Dr D Gatehouse	Covance	Salary Consultant	NONE	NONE
	Friends Provident	Share Holder		
	GlaxoSmithKline	Pension Share Option Holder		
		Share Holder		
Mrs R Glazebrook	Dr Foster Ltd	Salary	NONE	NONE
	BT Group	Share Holder		
	Lloyds TSB	Share Holder		
	National Grid	Share Holder		

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

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

Preface



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

During the year 2004, the Committee provided advice on a wide diversity of topics including alcohol and breast cancer, 1,3-dichloropropan-2-ol, 2,3-dichloropropan-1-ol (1,3-DCP, 2,3-DCP), organochlorine insecticides and the possible association with breast cancer, malachite green/leucomalachite green, and polycyclic aromatic hydrocarbons in air pollution. The Committee also provided generic advice on oesophageal and prostate cancer.

in relation to potential chemical exposures which might be associated with these particular cancers, and on the observation of olfactory neuroblastoma in a small number of dentists/dental nurses. The COC epidemiologists provided advice to the Advisory Committee on Pesticides on the Ontario College of Physicians Report. Finally the COC (along with COT and COM) provided an input to the reassessment of tobacco products.

The Committee has an ongoing responsibility to provide Government Departments and Regulatory Authorities with advice on developments in procedures for the evaluation and risk assessment of carcinogens. During this year, the Committee provided advice to the Health and Safety Executive on the development of its proposed priority programme on occupational exposures to carcinogens and the risk assessment of genotoxic carcinogens and the effect of DNA repair at low doses.

During 2004, the Committee also said farewell to Dr Robin Fielder who retired from the COC secretariat. I wish to record my thanks for his excellent contribution and commitment to the work of the COC and to the improvement of public health in general during his many years of service for the COC.

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

Alcohol and breast cancer

- 3.1 Breast cancer is the most common cancer in women and the most common cause of cancer mortality in women. Each year there are approximately 41,000 cases (2000 data) registered and 13,000 deaths (2001 data) in the U.K. The most clearly established risk factors for breast cancer are reproductive (e.g. age at first full term pregnancy, parity, age at menarche and menopause). Other known risk factors for breast cancer include age, ethnic group, family history of the disease, history of benign breast disease, socioeconomic status, use of oral contraceptives and hormone replacement therapy and, in postmenopausal women, obesity. The reason for the interest in the association between alcohol and breast cancer is that even a small risk, if causally associated with alcohol, may have serious public health implications. In addition, drinking alcoholic beverages may be one of the few risk factors for breast cancer where intervention might offer some scope for prevention. An extensive literature on the association between alcohol and breast cancer was reviewed by the World Health Organisation's International Agency for Research on Cancer in 1988 and by this Committee for the Inter Departmental Working Group on Alcohol in 1995 but neither group was able to advise that there is a causal association between drinking alcoholic beverages and breast cancer.
- 3.2 A further review was undertaken by the COC in 1999 (<http://www.doh.gov.uk/alcbrst.htm>). The Committee concluded there was sufficient evidence to associate drinking alcoholic beverages with an increased risk of breast cancer but agreed that a systematic review (meta-analysis) of all the epidemiology studies and further evaluation of potential mechanisms were required before definite conclusions could be reached. The Department of Health commissioned a systematic review of the epidemiology from the Department of Epidemiology and Public Health at Imperial College, London.
- 3.3 The COC considered a draft report from the Imperial College London research group at a meeting in November 2002, an update review of mechanisms from the DH Toxicology Unit at Imperial College, and a published evaluation of the literature prepared by the Oxford Collaborative Group on Hormonal Factors in breast cancer (British Journal of Cancer, 87, 1234-1245, 2002). The COC considered further draft reports from Imperial College and updated searches of the published literature up to June 2004 when a COC statement (and non-technical summary) were finalised. The overall conclusions (as taken from the non-technical summary) reached by the COC are given below.
- 3.4 The new research estimates that a woman drinking an average of two units of alcohol per day* has a lifetime risk of developing breast cancer 8% higher than a woman who drinks an average of one unit of alcohol per day. The risk of breast cancer further increases with each additional drink consumed per day. There was no evidence for variation in the association with any specific type of alcoholic drink. (Key J, Hodgson S, Omar R, Kold-Jensen T, Thompson S, Boobis A, Davies D, Elliott P (2003). Alcohol and Breast Cancer: A meta-analysis. In-confidence paper submitted to COC and for publication).

* A standard 'unit' of alcohol contains 8grams of ethanol, the amount usually found in half a pint of normal strength beer, or cider, a single measure of spirits, or one small glass of ordinary wine. In recent years the average amount of alcohol in some drinks has increased and maybe up to 10grams ethanol.

- 3.5 The research also concludes that approximately 6% (between 3.2% and 8.8%) of breast cancers reported in the U.K. each year could be prevented if drinking was reduced to a very low level (i.e. less than 1 unit/week). This approximates to between 1290 and 3560 cases of breast cancer out of a total of approximately 41,000 new cases registered each year.
- 3.6 The risk of breast cancer associated with drinking alcohol increased with the amount of alcohol consumed. Thus, if a woman increased her drinking from the U.K average level of 1 unit per day by an extra 1, 2, or 3 units a day then the incidence of additional cases of breast cancer expected at 60 years and 80 years can be calculated.

The following table summarises the results:

Cumulative Incidence per 1000 women

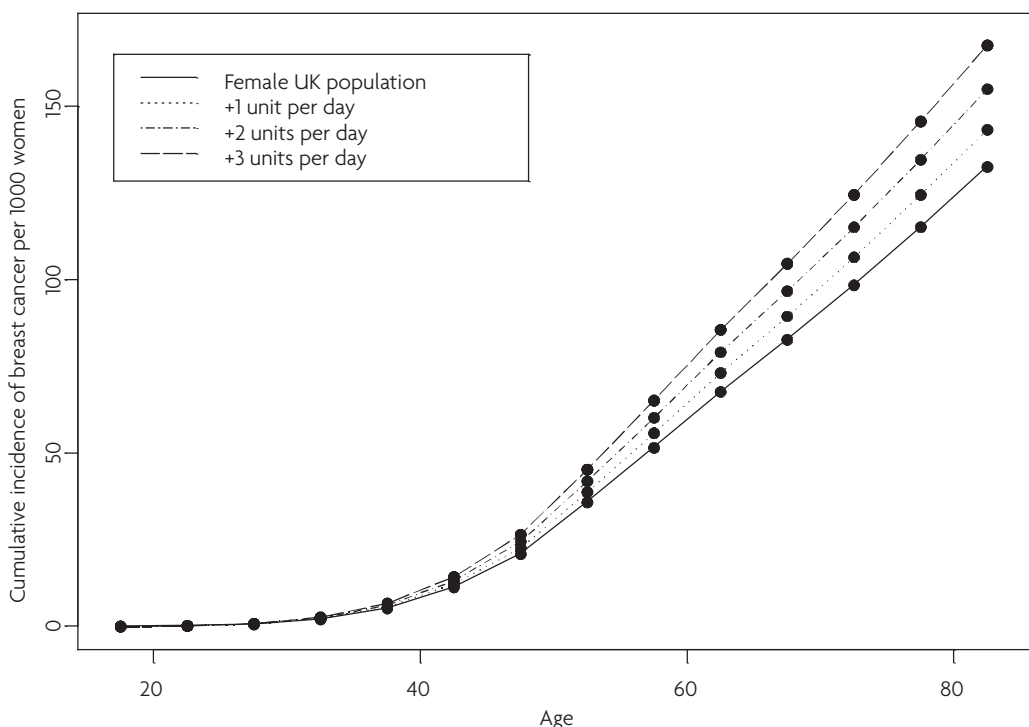
	Current consumption	+1 unit per day	+2 units per day	+3 units per day
Age 60	60	65	70	75
Age 80	125	134	145	157

- 3.7 It is not known precisely how drinking alcohol can lead to breast cancer. The most likely explanation is that drinking alcohol can produce biochemical effects in the liver (such as changes to oestrogen metabolism and effects on growth factors) which if drinking alcohol is prolonged (i.e. over decades) could lead to breast cancer.
- 3.8 There is not enough information available to assess whether drinking alcohol can interact with the use of oral contraceptives or hormone replacement therapy by women to increase further the risk of breast cancer. More research on these aspects is required.
- 3.9 The Committee was concerned that recent evidence** demonstrated that the consumption of alcohol is increasing mainly in young women. If the increased consumption of alcohol is maintained over most of their lifetime, then the number of alcohol-related breast cancer cases may be even higher in these women than reported in paragraph 3.6 above. The Committee concluded that it was important to raise awareness of the potential risks of drinking alcoholic beverages, particularly amongst young women.

**There is a lot of information on the consumption of alcoholic beverages regularly obtained as part of the General Household Survey (GHS). (<http://www.statistics.gov.uk/lib2001/index.html>) and the Health Survey for England (HSfE) (<http://www.doh.gov.uk/public/summary1.htm>) Detailed information can be obtained from these sources. These studies examined alcohol in a variety of age groups. The youngest age group was 16-24 y.

Graph of cumulative incidence of breast cancer and effect of drinking additional units of alcohol.

The bold line indicates cumulative incidence at current average intakes alcohol (1 unit/day). The dotted lines show the effect of increasing intakes by additional 1,2, or 3 units per day.



3.10 A full statement is included at the end of this report.

1,3-Dichloropropan-2-ol, 2,3-Dichloropropan-1-ol

3.11 1,3-Dichloropropan-2-ol (1,3-DCP) and 2,3 dichloropropan-1-ol (2,3-DCP) are contaminants of some foodstuffs and of polyamine flocculants used in the treatment of drinking water. Both the COC and COM have previously published statements during 2000 on the closely related compound 3-chloro-1,2-propanediol (3-MCPD). 1,3-DCP and 2,3-DCP were considered by the COC and COM in 2001. In 2001, the COM recommended that appropriate in-vivo mutagenicity studies should be undertaken with 1,3-DCP and 2,3-DCP in accordance with the COM guidelines. In 2001, the COC came to the following conclusions:

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

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

- 3.12 Both of these compounds have been recently considered by the COM which has updated its advice on the mutagenicity of 1,3-DCP and 2,3-DCP in the light of results from new *in-vivo* mutagenicity studies on these two compounds. An updated COM statement on 1,3-DCP was published in October 2003 and an updated statement on 2,3-DCP was published in June 2004.
- 3.13 The COC reviewed the available carcinogenicity data and the recent conclusions reached by COM. The following overall conclusions were reached:

1,3-DCP: The COC concurs with its previous advice that 1,3-DCP should be regarded as a genotoxic carcinogen. It is not possible to exclude a genotoxic mechanism for the induction of the tumours of rat tongue seen in a long-term drinking water study with 1,3-DCP. The Committee recommended that further investigations regarding the mechanism of 1,3-DCP carcinogenicity in the rat tongue should include information on contact-irritancy, cell proliferation and formation of adducts in tongue tissue using ³²P-postlabelling in animals treated with suitably high doses of 1,3-DCP.

2,3-DCP: The available evidence is consistent with the conclusion that 2,3-DCP does not possess genotoxic activity *in-vivo*. There are no appropriate carcinogenicity bioassays of 2,3-DCP available. No conclusions regarding carcinogenicity of 2,3-DCP can be reached.

- 3.14 A full statement is included at the end of this report.

Genotoxic carcinogens and DNA repair at low doses

- 3.15 The COC had asked the COM to provide advice regarding an approach to evaluating the significance of DNA repair induction at low doses of genotoxic carcinogens in the context of the hormesis hypothesis. The COM recommended a literature search targeted on low dose effects of a few direct acting chemical mutagens on DNA adduct formation, mutation rates, and the significance of DNA repair mechanisms. The COM had also recommended that the search should concentrate on low molecular weight compounds such as ethylene oxide and ethyl or methyl methanesulphonate, for which there was a rich database. Members had agreed that bacteria would most likely demonstrate more sensitivity to low doses of mutagens than mammalian cells.

- 3.16 Overall, the COM had concluded that there was no convincing evidence for a 'J' shaped dose response relationship in any of the data considered. The COM agreed that the data considered in the paper did not warrant reconsideration of the COM's advice that it is prudent to assume that *in-vivo* mutagens do not have a threshold for mutagenicity, unless there is good evidence to the contrary.
- 3.17 The COC was asked whether any further consideration of DNA repair at low doses of mutagens should be undertaken and whether any further consideration of the concept of hormesis was warranted at the present time.
- 3.18 COC Members agreed with the COM conclusions and did not recommend further work at present, but considered that a watching brief should be kept on this topic. The COC felt that it was possible that DNA repair processes could be induced at low levels of exposure to mutagens, but that it would be very difficult to observe such effects experimentally in *in-vitro* mutagenicity assays. The committee concluded that for the purposes of carcinogen risk assessment it remained prudent to assume that there was no threshold for genotoxic carcinogens.

HSE Strategic Programme on Occupational Disease Reduction: Project on Chemical Carcinogens

- 3.19 Occupational carcinogens are a priority for HSE in a new strategic programme to reduce the incidence of occupational ill health. In June 2004, the COC was given a short introduction to the initial project plan for the work on carcinogens. The general objective of the project was to gather and analyse toxicological and occupational hygiene data on carcinogens, and then to formulate a strategy by mid 2006 that included recommendations for risk reduction. In November 2004, the COC was invited to comment on two early activities.
- 3.20 HSE had begun to collate available toxicological evidence on chemical carcinogens, their use and information on the adequacy of exposure controls in the workplace. The COC was asked to consider an initial list of 10 carcinogens that HSE was especially keen to learn more about. Members noted the difficulty of using currently available data on carcinogens and exposures to select priority lists. A number of suggestions were made which would be further considered by HSE during the review process.
- 3.21 HSE planned to hold two workshops of experts and undertake research to update the estimate of cancer mortality due to occupation published in 1981 by Doll and Peto (Doll R, and Peto R., J. National Cancer Inst, 66, 1191-1308, 1981). This much cited study estimated the proportion of cancer mortality in the USA due to occupational causes to be around 4%, uncertainty range 2-8%. The new work to be undertaken by HSE would provide an estimate of the burden of cancer that can be attributed to occupation in Great Britain.

- 3.22 Members felt that this was a worthwhile and ambitious project. It was noted that the Doll Peto analysis had analysed cancer attributed to occupation in the U.S.A. Members commented that industrial exposures to chemicals had changed rapidly over the past two decades and the estimate of cancer burden associated with chemicals in use could not be assessed on the available current cancer mortality data.
- 3.23 The Committee asked to be kept informed on developments arising from the workshop and review process.

Oesophageal cancer

- 3.24 The COC had asked for a review of oesophageal cancer during its discussion of the 2003 horizon scanning paper. The DH Toxicology Unit had drafted an overview paper. Oesophageal cancer is broadly classified into two histological types, squamous cell carcinoma (occurring mainly in the upper region of the oesophagus) and oesophageal adenocarcinoma (occurring mainly in the lower region of the oesophagus). Members agreed that the incidence of oesophageal adenocarcinoma (EAC) was increasing whilst the incidence of squamous cell carcinoma (SCC) had essentially stabilised.
- 3.25. Tobacco smoking and alcohol consumption are considered to be the predominant risk factors for SCC with about 90% of cases being attributable to these factors. There was no convincing evidence for an association between alcohol consumption and EAC. Members were aware of some studies which documented results supporting an association between tobacco smoking and EAC but agreed the evidence was less convincing than for tobacco smoke and SCC. Members agreed there was convincing evidence for an association between body mass index and gastro-oesophageal reflux disease (GERD) and EAC whilst the incidence of SCC did not appear to be affected by these two factors.
- 3.26 Members considered the evidence for particular chemical exposures and SCC and EAC. It was agreed that the limited evidence for an association between the rubber industry and an increased risk of oesophageal tumours was not convincing. In particular, it was noted that the available studies did not account for potential confounding by alcohol consumption or tobacco usage. Members commented that evidence for increased risk associated with occupational exposure to nitrosamines, PAHs, various dusts or other chemicals was limited. However many of these studies had not adjusted for confounding by alcohol consumption or tobacco usage and were therefore of limited value.
- 3.27 The Committee considered the available information on dietary nitrosamines in more detail. Nitrosamines were known to be oesophageal carcinogens in rodents and evidence suggesting similar metabolic pathways leading to activation was available for both rodents and humans. The metabolism and potential metabolic activation of nitrosamines in rats and monkeys were reported in the DH Toxicology Unit paper to be modified by ethanol. Nitrosamine exposure can occur through smoking and the diet and any effect on oesophageal cancer due to nitrosamines could potentially be influenced by alcohol.

- 3.28 There is some evidence from studies in China linking dietary nitrosamines and oesophageal cancer. However, no conclusions on the importance of nitrosamine exposure on oesophageal cancer in the UK could be drawn from these studies.
- 3.29 The Committee agreed with the overall conclusions in the DH paper, namely that lifestyle factors are likely to predominate in the aetiology of both types of oesophageal cancer. The available evidence did not clearly suggest any potential occupational or environmental exposure to chemicals that should be further considered at this point in time.
- 3.30 The Committee felt that a further review of the dose-response for alcohol and oesophageal cancer and a consideration of mechanism was warranted at this point in time.

Olfactory neuroblastoma and dentists/dental nurses.

- 3.31 Olfactory neuroblastoma (ONB. The alternative name is esthesioneuroepithelioma) is estimated to comprise approximately 3% of nasal neoplasms excluding benign polyps. The incidence in North America/Western Europe is estimated to be approximately 0.15/million/year. There is no evidence for a sex difference in incidence. It occurs in all ages (but is rare below 10 years and over 70 years). It has been reported to have bimodal incidence, with peaks in the 2nd -3rd decade and later in the 6th and 7th decades of life. It has also been estimated there have only been 950 cases cited in the scientific literature from 1924, when ONB was first cited in the literature, up to 1997. Thus the available evidence suggests that ONB is a very rare tumour.
- 3.32 ONB is described as a neuroectodermal neoplasm showing predominantly neural features. The most common symptoms in patients presenting with ONB are nasal obstruction (93%), epistaxis (55%) and rhinorrhea (30%). Other symptoms such as headache and anosmia occur at an incidence of below 10%. Diagnoses is based on clinical presentation, CT/MRI* screening and histology with the need for a battery of immunohistochemical stains to differentiate from other closely related head and neck cancers.
- 3.33 The Committee heard presentation from the Institute of Laryngology and Otolaryngology, London, on details of four individuals with ONB, two of whom had worked as dentists, and two who had been employed as dental nurses. Members heard that two pathologists had independently verified the diagnoses. Full details of these case reports had been submitted to a peer reviewed journal.
- 3.34 The Committee reviewed the available pathology literature and agreed that it was highly improbable that the researchers investigating wood workers would have misdiagnosed ONB as adenocarcinoma of the sinuses. The Committee considered available information on potential chemical exposures of dentists/dental nurses (eg to metallic mercury, oil of cloves (principle ingredient eugenol) and

* Computerised Topography/Magnetic Resonance Imaging

methymethacrylate). It was agreed that there was no evidence to associate exposure to these chemicals with ONB. The Committee considered that the first priority for further work would be to consider additional epidemiological investigations to confirm the finding reported by the Institute of Laryngology and Otolaryngology. This might include evaluation of case-reports of ONB from other countries or detailed evaluation of information held by centres of excellence (for head and neck tumours) and pathology departments from the UK, Europe and elsewhere.

3.35 A full statement is included at the end of this report.

Organochlorine insecticides and breast cancer

3.36 Organochlorine insecticides (OCIs) are a group of synthetic chemicals that were widely used in agriculture (and for the control of malaria) during the 1940s up until the 1960s. They were commonly used because they were very effective and were also relatively cheap. However, following evidence that they persist in the environment and in humans, concern about their use increased and they were subsequently withdrawn from use. Currently there are no approved pesticide formulations in the UK that contain OCIs.

3.37 People may be exposed to OCIs as environmental contaminants in their diet. In 1995, the Committee was asked to review studies where humans were exposed to OCIs to advise whether it was possible that environmental exposure to OCIs could be linked to the development of breast cancer in women.

3.38 Breast cancer is a very complex disease and the risk factors are well documented. They include the age at first birth, menarche and menopause, as well as obesity, parity and use of oral contraceptives and hormone replacement. A common feature associated with these various risk factors is the fact that they result in increased amounts of the female sex hormone oestrogen in the body. In women, oestrogen is normally secreted by the ovary and is responsible for producing typical female sexual characteristics. Some synthetic chemicals, including OCIs, may have a very weak ability to act like oestrogens in the body i.e. have 'oestrogen-like effects'.

3.39 Therefore in 1995 (and again in 1999) the Committee was asked to consider evidence that suggested exposure to certain OCIs i.e. DDT, dieldrin, β -HCH and lindane, might be associated to breast cancer.

3.40 To help determine whether OCIs could possibly be involved in breast cancer the following areas must be considered:

- a) Does the chemical have oestrogen-like effects in animals?
- b) How does the oestrogen-like effects of OCIs compare to other chemicals with these effects?
- c) Does the presence of more than one OCI in a mixture change the way each component behaves?
- d) Do OCIs actually persist in breast tissue?

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- 3.41 The OCl_s reviewed here had at most a very weak ability to act like oestrogen in the body, although the majority had no oestrogen-like effects. Even if added together these chemicals would still be very weak compared to the use of oral contraceptives, hormone replacement therapy (HRT) or flavonoids in food.
- 3.42 Furthermore, from the available studies the COC concluded that there is currently no evidence to support the view that OCl_s can alter the behaviour of other OCl_s at the levels humans are exposed to environmentally.
- 3.43 One way in which OCl_s are thought to contribute to the development of breast cancer is by their capacity to accumulate in fatty (adipose) tissue. Under these circumstances the breast receives a continuous supply of oestrogen-like substances over a prolonged period. Various studies conducted across the UK, Europe and USA measured the concentrations of OCl_s in human adipose tissue or breast milk. Upon reviewing data published by the UK Pesticides Residues Committee (PRC) collected over 37 years since the 1960s, the levels of OCl_s in human tissue are decreasing.
- 3.44 Upon reviewing studies of humans exposed to OCl_s, there is currently no convincing evidence that OCl_s are associated with the development of breast cancer.
- 3.45 The Committee reached a number of specific conclusions regarding the individual chemicals under review, which are tabulated below for ease of reference.

Conclusions on the individual chemicals considered in the 2003/4 review

OCI	Does the chemical have oestrogen-like effects in animals?	Are the levels detected in human tissue significant?	What is the relationship between human exposure to particular OCI and breast cancer?	Are people who are exposed to environmental levels of a particular OCI at increased risk of developing breast cancer?
DDT	Yes, although its effects are very weak.	Levels of DDT are known to be declining.	There is no evidence for link.	No
Dieldrin	No.	Levels of dieldrin are known to be declining.	Overall there is Insufficient information to draw any conclusions.	No definite conclusions drawn. To be kept under review.
β HCH	Yes, although its effects are very weak.	Levels of HCH are known to be declining.	Overall there is no evidence for a link.	No.
Lindane	No.	No.	Overall there is insufficient information to draw any conclusions.	No.

Ontario College of Family Physicians: Report on pesticides

3.46 The Advisory Committee on Pesticides asked for advice from COC epidemiologists on the evaluation of this report. A letter cleared by the chairman explaining COC epidemiologists views was forwarded to the ACP secretariat.

Malachite green/Leucomalachite green

3.47 Malachite green (MG) is a cationic triphenylmethane dyestuff used in a number of industries including fish farming. Its use in fish for human consumption was banned in the EU in June 2002 but residues continue to be found in fish. The Food Standards Agency had asked for a review from the COT in 1999. The COM provided advice to the COT on the mutagenicity of malachite green and its lipophilic metabolite leucomalachite green (LMG) at that time. The COM had provided further advice in 2003 on preliminary data from *in-vivo* mutagenicity studies.

3.48 The COM had now provided up to date advice on the mutagenicity of MG and LMG. The COM had concluded that malachite green and leucomalachite green should be regarded as *in-vivo* mutagens (see section 2. of the COM annual report).

3.49 The COC considered the results of the NTP carcinogenicity studies on MG and LMG in June 2004. They noted that prior to the publication of the NTP bioassay data on MG and LMG there had been no data available to make any meaningful assessment of the carcinogenicity of these compounds.

Conclusions regarding carcinogenicity of malachite green

- i) There was equivocal evidence of carcinogenicity in female F344 rats based on an increase in thyroid gland tumours (adenoma or carcinoma combined) hepatocellular adenomas and mammary gland carcinomas.
- ii) There was no evidence of carcinogenic activity in female B6C3F₁ mice.
- iii) Overall there was no convincing evidence for any carcinogenic effect with malachite green in these studies.

Carcinogenicity of leucomalachite green

- i) There was evidence of carcinogenic activity in female B6C3F₁ mice based on an increase in hepatocellular adenoma or carcinoma combined.
- ii) There was equivocal evidence of carcinogenic activity in male F344 rats based on an increase in interstitial cell adenoma of the testes and the occurrence of thyroid gland follicular cell adenoma or carcinoma (combined).
- iii) There was equivocal evidence of carcinogenic activity in female F344 rats based on an increased incidence of hepatocellular adenoma or carcinoma (combined).

3.50 The COC considered the possible mechanisms by which LMG induced tumours in the liver of the female mice. It was noted that the overall tumour profile was not that which would be expected of a genotoxic carcinogen, with activity being limited to effects in the liver of the female mouse; furthermore this was mainly due to an increase in adenomas. However it was also noted that there was no evidence from the NTP studies to support any non-genotoxic mechanism. In view of this, and taking into account the views of the COM, the Committee agreed that it was not possible to discount a genotoxic mechanism for the induction of the liver tumours in female mice and it would therefore be prudent to regard LMG as a genotoxic carcinogen.

3.51 A full statement is included at the end of the COM section of this annual report.

Polycyclic aromatic hydrocarbons in air pollution

3.52 Polycyclic aromatic hydrocarbons (PAHs) are a large class of organic compounds that are present as pollutants in air, food and drinking water. PAHs are formed by incomplete combustion of organic matter. The main sources of PAHs are industrial processes (e.g. aluminium production, coal gasification, coke production and iron and steel founding), road traffic emissions and domestic fuel combustion (WHO, 1998). Other sources include natural fires and open agricultural burning, wood treatment and natural processes such as carbonisation. Cigarette smoke is also a major source of PAHs for individual

exposure. In food, PAHs may be formed during processing and domestic food preparation. Some complex mixtures containing PAHs, such as coal tar pitch and tobacco smoke, are considered by the International Agency for Research on Cancer (IARC) to be known carcinogens in humans (i.e. group 1) and a number of individual PAHs are considered to be probably (Group 2A) or possibly (Group 2B) carcinogenic to humans.

- 3.53 The Committee had been asked in 2003 to evaluate the relative carcinogenic potency of dibenzo(a,l)pyrene compared to benzo(a)pyrene. The COC had agreed the following overall conclusion:

“Dibenzo(a,l)pyrene should be considered as a highly potent genotoxic carcinogen in experimental animals. There is a need for further consideration of the potential importance of exposure to dibenzo(a,l)pyrene and other highly potent carcinogenic polycyclic aromatic hydrocarbons in air pollution.

- 3.54 The COC agreed a suggested approach of using DNA adducts for ranking PAHs as a suitable surrogate for measuring carcinogenic potency by inhalation. It was noted that the potential for induction of PAH metabolism and differential repair of DNA adducts needed to be considered when interpreting results. However the approach represented a pragmatic method of providing ranking into broad groups. The DH Toxicology Unit drafted a paper which presented information on possible biomonitoring approaches for evaluating the potential contribution of high potency PAHs to air pollution related lung cancer. It had been suggested that data from biomonitoring studies might aid in the evaluation of which PAHs were more likely to pose the greatest risk of carcinogenicity from air pollution. The approach utilised the COC agreed position that DNA adducts may serve as a useful approach to ranking PAHs.
- 3.55 Several occupational and environmental PAH biomonitoring studies were reviewed, with particular focus on identifying appropriate exposure groups, study design, sample tissue, in particular the use of nasal tissues, and biomarkers used in each study. A proposal was developed which used a novel biomonitoring approach to evaluate exposure, uptake and the role of high potency PAHs in air pollution-related lung cancer. This is based upon an occupational study examining specific DNA adducts for DBA and DB[a,l]P in nasal cells to evaluate the extent to which these high potency PAHs might contribute to the increased risk of developing lung cancer from air pollution. A paper describing a proposed biomonitoring approach was submitted to a peer reviewed journal and has recently been accepted for publication.

Prostate cancer

3.56 Prostate cancer is the most common cancer in men in the UK, with over 24,700 new cases a year (2000 data). Prostate cancer is the second largest cause of death from cancer in the UK. There were 9,900 deaths reported in 2002 accounting for around 13% of cancer deaths in men. Around 70% of these deaths are in men aged over 70 years. The mortality rate for prostate cancer peaked in the early 1990s and has now fallen to 25 per 100,000 population at risk. The lifetime risk for being diagnosed with prostate cancer is 1 in 14. The cancer develops from cells within the prostate gland. The majority of prostate cancers are slow growing and many men are unaware that they have this cancer. However, a small number of prostate cancers grow more quickly and may spread to other parts of the body. Cancer Research UK reported a 57% increase in prostate cancer incidence in Great Britain between 1991 and 2000. The Committee was asked to review the available epidemiological and other research to identify if there were any potential chemical exposures which might be associated with prostate cancer.

3.57 The conclusions reached by COC are given below:

- i) The increase in incidence of prostate cancer reported over the past 2-3 decades is largely accounted for by improved identification of cases due to increased numbers of individuals undergoing surgery for benign prostatic conditions and the use of Prostate Specific Antigen Screening.
- ii) The Committee concluded that there was some limited evidence to suggest an association between farmers/farm workers, exposure to pesticides and increased risk of prostate cancer. The possibility of such an association being causal could not be discounted and the published literature should continue to be monitored for further studies. Members commented on the need for improved measures of exposure to pesticides and in particular herbicides. It was considered that the potential association between herbicide use by farmers and farm workers should be kept under review.
- iii) The information from the available epidemiological studies are consistent with the view that overall, there is no convincing evidence of an increased risk of prostate cancer in rubber workers as a whole.
- iv) There is no convincing evidence to associate other occupations with prostate cancer.
- v) There is no convincing evidence to associate occupational exposure to cadmium with cancer of the prostate. The possibility that cadmium might induce androgen imbalance and thus might potentially be associated with prostate cancer should be monitored and relevant new information considered in the future.

- vi) The one available epidemiological study on dietary zinc supplementation and risk of prostate cancer dose found increased risk of prostate cancer at high levels of supplementation (>100 mg/day). Further epidemiology studies are unlikely to provide sufficient numbers of individuals regularly consuming high doses of supplements for a study to be undertaken in the UK. The Committee agreed that it could not identify a biologically plausible rationale as to why zinc should be associated with prostate cancer.

3.58 A full statement is included at the end of this report.

Reassessment of tobacco products

- 3.59 The Committees (COT/COC/COM) were asked to provide advice on the toxicological assessment of tobacco products with reference to the assessment of Potentially Reduced Exposure Products (PREPS) and in particular tobacco-based PREPS which are smoked. The Committees agreed that it was important to state that the ideal way forward to reduce risks and hazards of tobacco smoke was to encourage smokers to stop or people not to start in the first place and any attempt to reduce toxicity should not be allowed to detract from that. Members acknowledged that the primary remit of the Committees' discussions was to provide advice based on the information provided in the discussion papers.
- 3.60 The COC commented on the complexity of tobacco induced cancer and noted that the mechanism(s) and information on the chemical agents responsible for tobacco induced cancer in humans had not been fully elucidated. In addition members noted the importance of the interaction between chemical carcinogens and susceptibility factors regarding the pathogenesis of tobacco-induced cancer. The COC concluded there is no strategy which could be used to compare PREPS for carcinogenic potency and that the approaches used are not informative on the risk of tobacco induced carcinogenicity. The COC agreed that it was not possible to draw conclusions on the carcinogenic risk of tobacco-based PREPS on the available biomarker studies reviewed. The COC commented on the need to examine a wide range of biomarkers for carcinogenicity and their interaction with susceptibility factors.
- 3.61 A statement providing details of all the conclusions reached by COT/COC/COM is included in the COT section of this annual report.

Review of Committee Procedures

- 3.62 The Committee's publication scheme (prepared in accordance with Freedom of Information Act 2000) is available on the COM internet site (<http://www.advisorybodies.doh.gov.uk/foi/publicationscheme.htm>). The COM meetings are now held in open session. The procedures adopted by COC are equivalent to those used by COT. Details can be found on the COC internet site. (<http://www.advisorybodies.doh.gov.uk/foi/open.htm>)

Horizon scanning

3.63 The COC undertakes “horizon scanning” exercises at regular intervals to identify new and emerging issues which have the potential to impact on public health and which might require COC advice. The DH Toxicology Unit at Imperial college had drafted a discussion paper. Members discussed the following topics.

3.64 *Target organ mutagenesis and implications for carcinogen risk assessment.*

This topic was suggested by the recent consideration of malachite green and leucomalachite green. COM had agreed it would be useful to have a joint COC/COM symposium to provide guidance on how data from mutagenicity in target organs can be fed into the risk assessment process. Members agreed this was an important topic to follow-up in order to provide advice on the assessment of multi-site carcinogens.

3.65 *Use of transgenic animal models in carcinogen risk assessment.*

Members recalled the conclusions reached in 2002 by COC with regard to the proposals from ILSI regarding use of hemizygous p53 and Tg.AC and other mouse models to replace the conventional mouse long-term bioassay. Members agreed there was a value in developing specific transgenic animal models for mechanistic studies but overall considered this topic should be given low priority for further COC consideration.

3.66 *Risk assessment of non-genotoxic carcinogens.*

This subject was extensively reviewed during the preparation of the COC guidelines (see paragraphs 3.71-3.76 below of this Annual report). An update of the research which was funded by DH on investigation gap junction function for inclusion in possible screens for non-genotoxic carcinogenesis is provided. Members considered that further consideration of some worked examples of evaluation using the IPCS mode of action and the ILSI Human Relevancy Framework would be valuable for the Committee. It was noted that further developmental work which aimed to amalgamate these two approaches was being considered in international fora.

3.67 *Single exposure carcinogens.*

A short overview was provided regarding definitions of single and short duration exposures and the identification of single exposure carcinogens. Members agreed that this suggestion should be given high priority. Members also asked whether there was any evidence that non-genotoxic carcinogens could induce tumours over a short duration of exposure.

3.68 *Potential mechanisms of metal induced carcinogenesis.*

A very short overview of the literature had been provided. Members agreed that the research initiatives would be valuable with regard to elucidating the mechanisms of metal-induced carcinogenesis but considered overall that further work on this subject was not merited at this point in time.

3.69 *Epidemiology.*

Members agreed to take forward the review of alcohol consumption and oesophageal cancer but asked the secretariat to undertake some initial work to define the key outcomes of the review.

3.70 *Nanotechnology.*

COM considered it would be worthwhile undertaking a review of this subject. A similar short overview section had been provided with regard to carcinogenicity. Members agreed that this was a potentially important and also interesting area of work. It was agreed that COC would be interested to see papers on this topic in the future.

Test Strategies and Evaluation

COC guidance on a strategy for risk assessment of carcinogens

- 3.71 The COC first published guidelines for the evaluation of chemicals for carcinogenicity in 1982. These dealt in the main with the design, conduct and interpretation of long-term animal bioassays and provided guidance to the relevant government departments and agencies on best practice for testing at that time. The need for guidelines to be periodically updated, to reflect advances in development and validation of methods, was recognised and revised guidelines were published in 1991, which addressed the evaluation of chemicals as potential carcinogens. The revised strategy published during 2004 concentrated on one section of the aforementioned 1991 guidelines, namely the risk assessment of chemical carcinogens, with reference to new approaches such as 'minimum risk levels'. However, the detailed approaches to the evaluation of epidemiological studies used in the risk assessment of carcinogens are to be considered later in a separate document.
- 3.72 The Committee on Carcinogenicity evaluates carcinogenicity data on chemicals on a case-by-case basis, taking into account the weight of all the available evidence. The range of data considered may differ with circumstances, for instance, it will not always be possible to obtain epidemiological data and each assessment will be considered on its own merits. It is not possible to provide a universally applicable list of data that will be needed for a carcinogenic assessment. However, it is hoped that this document will provide some guidance on a suitable strategy that could be adopted.

- 3.73 The Committee recommends a four stage evaluation strategy for the risk assessment process of carcinogenic hazard. Initial identification of a carcinogenic hazard at stage 1 should be based upon a review of the toxicity data, the results of toxicity testing, and any knowledge of effects on human health. The Committee considers it essential to determine whether carcinogens act via a genotoxic or non-genotoxic mechanism, therefore the Committee fully endorse the strategy published by the Committee on Mutagenicity (<http://www.doh.gov.uk/com/guidance.pdf>). Hazard characterisation (stage 2) should determine the dose-response relationship from epidemiological or animal data. During this stage it is important that factors such as interspecies variation in susceptibility, and information on mode of action are considered. Exposure assessment (stage 3) should estimate probable human exposure, routes of entry and levels of potential exposure taking into account the limitations of exposure models. The final stage (stage 4) should characterise the carcinogenic risk by summarising the previous stages and developing appropriate approaches to genotoxic and non-genotoxic carcinogens.
- 3.74 If a putative carcinogen is found to be non-genotoxic, the Committee recommends the adoption of a threshold approach. Thus a method based on the identification of a NOAEL and the use of uncertainty factors is appropriate, as is used in other areas of chemical risk assessment.
- 3.75 If a putative carcinogen is found to be potentially genotoxic, the Committee recommends a non-threshold approach for risk assessment. The assumption of no threshold, together with the practical difficulties of using low doses of human relevance in animal carcinogenicity studies, has led to the development of mathematical models that attempt to provide a 'best estimate' of the likely extrapolation of the dose-response curve below the lowest experimental data points. These models may give an impression of precision, which cannot be justified from the approximations and assumptions upon which they are based. Therefore, the Committee on Carcinogenicity recommend that the ALARP (as low as reasonable practicable) approach should be adopted. This can be supplemented in specific situations e.g. low exposures to contaminants or impurities by the setting of a minimum risk level. This approach should be based on expert judgement of available data. The use of potency estimates can be used to rank priorities for genotoxic carcinogens within a particular class of compounds (e.g. polycyclic aromatic hydrocarbons). The Committee agrees that it should always remain important to keep any exposure to genotoxic carcinogens as low as reasonably practicable (ALARP).
- 3.76 The Committee emphasises the importance of further research in order to refine the process of risk assessment. This includes the development of toxicological methods to refine extrapolation between animals and humans. In addition, biomarkers of effect need to be further investigated to aid in the extrapolation of low doses and exposure. Continued research on carcinogenic mechanisms with the ultimate aim of developing appropriate models for low dose extrapolation is also required.

- 3.76 A detailed guidance document can be obtained from the COC internet site.
<http://www.advisorybodies.doh.gov.uk/coc/guideline04.pdf>

COT/COC/COM review of Toxicogenomics

- 3.77 The COT/COC/COM held a joint symposium on the use of genomics and proteomics in toxicology in October 2001. The Committees agreed to further consider toxicogenomics as part of the horizon scanning exercise initiated at the February 2004 COT meeting. It was noted that there was a considerable increase in the number of publications using toxicogenomic approaches. A number of discussion papers were subsequently prepared for the Committees which reviewed the available published literature. The data from 50 studies were considered during the review which also included available information from the HESI (Health and Environmental Sciences Institute of the International Life Sciences Institute (<http://www.ils.org/>)) collaborative scientific program on toxicogenomics. The current review considered information on use of metabonomics in toxicology for the first time. The COT requested a further paper and presentation on the use of statistics/bioinformatics in toxicogenomics. A presentation was given by Dr David Lovell (University of Surrey) to the COT at its meeting on the 7 September 2004.

[The Committees used the following definitions for the methods used in Toxicogenomics. Transcriptomics refers to gene expression as measured through cDNA or oligonucleotide or cRNA microarray based approaches, proteomics refers to determination of protein levels through gel or solid phase approaches and metabonomics refers to measurement of metabolites in tissues, plasma or urine.]

- 3.78 The COC reached the following conclusions after discussions held at its June 2004 meeting. The COC reached a number of general conclusions on toxicogenomic studies in experimental animals regarding dose-response evaluation, investigations of reversibility, statistical handling of data and bioinformatic developments which are consistent with those reached by COT. COC members also commented on the need for “pathway mapping” for the identification of toxicologically relevant gene changes. The COC agreed with the COM conclusion that a gene expression pattern had been reported in studies in rodents using genotoxic hepatocarcinogens.
- a) A number of studies in rodents using model carcinogens had reported on toxicogenomic approaches to investigate the process leading to neoplasia from initiation to tumour formation and growth. However no conclusions could be drawn from these limited studies. It was noted from the preliminary evidence considered by the committee that it was difficult to distinguish between chemical induced changes in gene expression from those occurring as a result of the neoplastic process.

- b) It was not possible in studies in animals using model non-genotoxic carcinogens to identify common gene expression changes which might be of value in developing an approach to early detection of non-genotoxic carcinogen. The available study identified more distinct than common changes in studies in mice using two model non-genotoxic hepatocarcinogens.
- c) Potentially valuable information on mechanisms of carcinogenesis could be derived from experiments designed to investigate particular specific mechanisms. Some preliminary information on non-genotoxic liver carcinogenesis in mice was available.
- d) Comparison of gene expression changes in stomach tumours in rodents induced by a model genotoxic carcinogen had shown similarities with gene expression profiles from human stomach cancers. These preliminary data could be used for hypothesis generation regarding the aetiology of stomach cancer. However caution was required in interpreting the studies considered by the committee as the range of toxicological effects in animals given relatively high doses of model carcinogens did not reflect the likely effects in humans exposed to much lower doses in the environment.

3.79 A statement providing details of all conclusions reached by COT/COC/COM is included in the COT section of this annual report.

Ongoing reviews

Childhood cancer

3.80 A preliminary discussion overview paper had been drafted as the result of a horizon scanning exercise for 2003 to evaluate the published literature on the possible 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 i.e. CNS tumours; acute lymphocytic leukaemia (ALL); germ cell tumours (GCT) and neuroblastomas (NBT). The review would also consider the possible role of transplacental carcinogens, paternal exposure and the significance of animal models in the risk assessment of childhood cancer. The Committee has also recommended that a review of the epidemiology literature on environmental exposure and childhood leukaemia with residence near to petrol stations and garages and roads should be undertaken.

Joint Symposium with COM on use of target organ mutagenicity in carcinogen risk assessment

- 3.81 The COC and COM have agreed to hold a joint symposium on this subject on the 9 June 2005 at the Department of Health, Skipton House, Elephant and Castle. It is hoped that a peer reviewed publication would result from this meeting.

Biobank

- 3.82 COC has corresponded with the Biobank research team regarding approaches to exposure evaluation. It is anticipated that there would additional exchanges of views and collaboration in the future.

Statements

Alcohol and Breast Cancer

Breast cancer risk and exposure to organochlorine insecticides: consideration of the epidemiology data on dieldrin, DDT and certain hexachlorocyclohexane isomers

Carcinogenicity of 1,3-dichloropropan-2-ol (1,3-DCP) and 2,3-dichloropropan-1-ol (2,3-DCP)

Olfactory Neuroblastoma: Evidence For An Elevated Incidence Among Dentists And Dental Nurses?

Prostate cancer

The Joint statements with COT/COM on Toxicogenomics and reassessment of tobacco products are included in the COT section of this annual report.

The joint statement with COM on malachite green and leucomalachite green is included in the COM section of this annual report.

Consumption of alcoholic beverages and risk of breast cancer in women: consideration of significance to public health

Introduction

Breast Cancer in U.K

1. Breast cancer is the most common cancer in women and the most common cause of cancer mortality in women. Each year there are approximately 41,000 cases (2000 data) registered and 13,000 deaths (2001 data) in the U.K^{1,2}. The most clearly established risk factors for breast cancer are reproductive³ (e.g. age at first full term pregnancy, parity, age at menarche and menopause). Other known risk factors for breast cancer include age, ethnic group, family history of the disease, history of benign breast disease, socioeconomic status, use of oral contraceptives and hormone replacement therapy and, in postmenopausal breast cancer, obesity. The reason for the interest in the association between alcohol and breast cancer is that even a small risk, if causally associated with alcohol, may have serious public health implications. In addition, drinking alcoholic beverages maybe one of the few risk factors for breast cancer where intervention might offer some scope for prevention. An extensive literature on the association between alcohol and breast cancer was reviewed by the World Health Organisation's International Agency for Research on Cancer in 1988⁴ and by this Committee for the InterDepartmental Working Group on Alcohol in 1995⁵ but neither group was able to advise that there is a causal association between drinking alcoholic beverages and breast cancer.
2. A further review was undertaken by the COC in 1999 (<http://www.doh.gov.uk/alcbrst.htm>)⁶. The Committee concluded there was sufficient evidence to associate drinking alcoholic beverages with an increased risk of breast cancer but agreed that a systematic review of all the epidemiology studies and further evaluation of potential mechanisms were required before definite conclusions could be reached. The conclusions reached following the 1999 review are summarised in paragraphs 11-14 below. The Department of Health commissioned a systematic review of the epidemiology from the Department of Epidemiology and Public Health at Imperial College, London. The Committee agreed to undertake a further review of all the available information when the report of the systematic review became available. The Committee was also aware that additional relevant data on alcohol consumption and risk of breast cancer was expected from the Oxford Collaborative Group on Hormonal Factors in Breast Cancer and should also be reviewed when available. The draft report of the systematic review undertaken by Imperial College and a copy of the published report by the Oxford Collaborative Group on Hormonal Factors⁷ both became available in November 2002 and thus a further review was initiated.

Consumption of alcoholic beverages in the U.K.

3. Estimates of the consumption of alcoholic beverages are generally reported in terms of units of alcohol or grams of ethanol consumed per day. One unit of alcohol is approximately equivalent to half a pint of normal strength beer, lager, or cider, a single measure of spirits, one small glass of ordinary strength (9% by volume) wine or one small glass of port, sherry or other fortified wine. This is approximately equivalent to 8 grams by weight or 1 centilitre (10 ml) by volume of pure alcohol (ethanol)⁸. One research publication has reported that the average amount of ethanol in a standard drink in the U.K ranges between 8-10 grams with an average of 9.5 grams⁹. This later figure has been used by the Imperial College research group in its systematic review.
4. The Department of Health for England advises that women should drink no more than 2-3 units of alcohol per day (i.e. 16 g – 24g ethanol/day). This daily benchmark applies whether individuals drink every day, once or twice a week, or occasionally. This guidance on sensible drinking was derived from an Interdepartmental Working Group (IDWG) report published in 1995. The IDWG considered all of the evidence relating to potential health benefits to women from drinking 1-2 units per day and the evidence for progressive health risk from consistently drinking 3 or more units per day¹⁰. Prior to 1995 the sensible drinking message had been expressed in terms of a weekly intake of alcohol units (i.e. less than 14 units/week was unlikely to damage a woman's health). The effect of the change in advice from a weekly limit to a daily benchmark has only recently been investigated in routine surveys which evaluate drinking patterns among women (see paragraph 6 below).
5. There is a lot of information on the consumption of alcoholic beverages regularly obtained as part of the General Household Survey (GHS). (http://www.statistics.gov.uk/ssd/surveys/general_household_survey.asp) and the Health Survey for England (HSfE) (<http://www.official-documents.co.uk/document/deps/doh/survey02/summ03.htm>) Detailed information can be obtained from these sources and therefore only a very brief review of the main conclusions on drinking patterns amongst women in the U.K. is presented below. (No comment on regional or socio-economic influences on drinking patterns has been included in this statement.) The GHS is a face-to-face interview survey conducted with a sample of 13,200 households across Great Britain and gathers a large amount of information on social, economic and health-related topics. The GHS has reported annually since 1971 (with breaks in 1997, 1999 and 2000 when the survey was re-developed) The HSfE is an annual survey which has provided information on consumption of alcoholic beverages since 1991. Both surveys have been adapted in recent years to take into account the change to expressing the sensible drinking message in terms of daily benchmark intake of alcohol units. The Committee also had access to an evaluation of the HfSE undertaken by Dr Paola Primatesta and colleagues from University College London¹¹.

6. The available information from these surveys provides similar findings. The average consumption of alcoholic beverages (expressed either as weekly or, where available as daily intakes) in women of all ages has increased over the last decade. Thus the weekly average intake from the HSfE was 6.2 units/week in 1993, 7.1 units/week in 1998 and 8.4 units/week in 2002. This trend was most noticeable in women aged 16-24 years where consumption rose from about 8 units/week in 1993/4 to almost 12 in 2001 and 13.3 units/week in 2002. The GHS reported a similar finding for women aged 16-24 across Great Britain with average weekly consumption reported to be 7.3 units in 1992 and 14.1 units in 2002. Primatesta and colleagues reported a strong correlation between mean or median intake and proportion of women exceeding the Sensible Drinking Limit (expressed as 14 units/week). A marked increase in women aged 16-24 years reporting consumption in excess of 14 units/week is noticeable from 1992 to 2002. The GHS reports this increase to be from 17% to 33%. The HSfE reported similar findings (from 20% in 1992 to 33% in 2002). Information on daily consumption of alcoholic beverages collected from 1998 in the GHS documented that the proportion of women aged 16-24 years who had drunk 6 or more units on at least one day in the previous week rose from 24% to 28% between 1998 and 2002. The equivalent proportion among women aged 25-44 years of age was 11% in 1998 rising to 14% in 2001, and 13% in 2002.
7. Thus, overall, the evidence supports the view that consumption of alcoholic beverages among women is increasing with the predominant increase in young women aged 16-24. The evidence suggests that increased consumption among women aged 45 years or more is spread evenly across the week whilst the increase in intakes in younger women, and particularly those aged 16-24 years predominantly occurs on one or two days per week. The GHS survey authors did note that there were too few data on daily consumption patterns to reach any conclusions about long term trends in daily consumption of alcoholic beverages.

Background to COC consideration

COC Statement for the Interdepartmental Working Group on Alcohol (1995)

8. The Committee first considered the epidemiological evidence for an association between alcohol and breast cancer in 1995 at the request of the Interdepartmental Working Group (IDWG) on Sensible Drinking¹⁰ as part of the review of medical and scientific evidence and its interpretation of the long term effects of drinking alcoholic beverages. The Committee provided a statement to the IDWG on the evidence for alcohol and cancer at all sites and concluded that drinking alcoholic beverages causes a dose-related increase in the risk of squamous carcinomas of the upper aerodigestive tract as a whole, and for cancers of the oral cavity, pharynx, larynx, and oesophagus which was independent of the effect of smoking. There was a substantial amount of information available to members who were able to draw conclusions on dosimetry, duration and frequency of drinking alcoholic beverages and the effect of abstinence and of smoking⁵.

9. A substantial amount of research was available to the Committee on drinking alcoholic beverages and breast cancer in 1995. Members reviewed the 1988 IARC monograph, which provides an evaluation of four large prospective and 13 case-control studies. The Committee also reviewed seven additional prospective studies¹²⁻¹⁸, 17 new case control studies¹⁹⁻³⁵ and two systematic reviews^{36,37}. In addition a number of reviews of the available information were also considered³⁸⁻⁴⁰. The Committee agreed that the adequacy of control for confounding by known and/or alleged risk factors for breast cancer varied in the different accounts. A dose-related association was reported in most cohort studies and in some hospital-based case-control studies. The results of population-based case-control studies did not generally support an association. A statistically significant dose-related increase in relative risk (RR) was reported in the two systematic reviews (RR at 3 drinks/day 1.38 (95% CI 1.23-1.55)). The Committee noted that the small increases in relative risk documented in epidemiological studies ranging between approximately 1.2-3 were associated with highly variable estimates of consumption (*ca* 1-60g ethanol/day). It was agreed that clear evidence of causality had not been demonstrated⁵.
10. The Committee concluded “...that while there is no decisive evidence that breast cancer is causally related to drinking alcoholic beverages, the potential significance, for public health, of even a weak association between alcohol and breast cancer is such that we recommend, in particular, that this matter be kept under review”⁵. The Interdepartmental Working Group endorsed the COC’s conclusions and the recommendation that the relationship between alcohol and breast cancer should be kept under review¹⁰.

COC review of information published between 1995-1999
<http://www.advisorybodies.doh.gov.uk/coc/index.htm>).

11. The Committee considered review papers prepared by the DH Toxicology Unit at Imperial College on the published epidemiology studies and investigations into potential mechanisms by which drinking alcoholic beverages could increase the risk of breast cancer⁴¹⁻⁴³. The epidemiological evidence included three prospective studies⁴⁴⁻⁴⁶ and a further 22 case-control studies⁴⁷⁻⁶⁸. The results were in accordance with the conclusions reached in the 1995 review in that most studies reported a small association between drinking alcoholic beverages and increased risk of breast cancer with evidence for a dose-response in the majority of studies examined. A pooled analysis of six prospective studies also reported a significant trend between increasing alcohol consumption and increased risk of breast cancer⁶⁹. The Committee agreed that no conclusions on the influence of menopausal status, type of beverage, frequency of drinking could be reached from the available information.
12. The DH Toxicology Unit paper⁴² identified sparse evidence for a number of potential mechanisms by which alcohol could induce breast cancer including enhanced metabolism of carcinogens⁷⁰⁻⁷², increased cellular permeability to potential carcinogens⁷³, impaired immune responsiveness⁷⁴, and abnormal differentiation of mammary tissue⁷⁵. A further published paper presented a hypothesis that alcohol could induce tissue and DNA damage via the formation of reactive oxygen species in breast tissue⁷⁶. However, most of the available studies on mechanism examined the effects of drinking alcoholic beverages on oestrogen metabolism in humans. There was evidence from both cross-

sectional and intervention studies that alcohol consumption affected oestrogen metabolism in premenopausal^{77,78} and postmenopausal⁷⁹⁻⁸⁵ women. Some recent research provided evidence that drinking alcoholic beverages affected serum oestradiol concentrations in premenopausal women using oral contraceptives⁸⁶. The Committee considered that the data on effects of drinking alcohol on hormones were complex and asked for a further tabulation of data on plasma and urinary sex hormones following consumption of alcohol. Overall the available data from the 1999 review suggested a plausible mechanistic link between consumption of alcohol and breast cancer involving effects on hormones. The interpretation of these data was particularly complicated and difficult; for example, the influence of confounding effects of other possible breast cancer risk factors such as obesity, use of oral contraceptives and hormone replacement therapy and their potential interaction with drinking alcoholic beverages needed to be considered carefully.

13. The Committee assessed all of the available data using the Bradford-Hill criteria as a guide to consideration of causality. The Committee concluded there was considerable evidence to support an association between drinking alcoholic beverages and risk of breast cancer but the magnitude of the association was small (i.e. the relative risk is modest and, even for heavy drinkers, in most studies does not exceed 3 (i.e. 3 times that of non-drinkers). The Committee also considered that it was difficult to ascertain the nature of the dose-response relationship from the available information. The small magnitude of the association between drinking alcoholic beverages and risk of breast cancer and the complex aetiology (i.e. there are weak associations with a number of other risk factors) of breast cancer are the main reasons for the difficulty in reaching a definite conclusion. The association could be due to systematic biases in the studies or to confounding by other risk factors. The Committee concluded that a rigorous systematic review (including appropriate meta-analyses) was needed in an attempt to identify and evaluate potential biases, confounding and heterogeneity so that a fuller assessment of causality and the magnitude of the risk associated with drinking alcoholic beverages could be made. It would also be important for any further analyses to provide an estimate of the Population-Attributable Risk (PAR). A systematic review was subsequently commissioned with the Department of Epidemiology and Public Health, Imperial College London.
14. The Committee had also asked its sister committee, the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) to update its 1995 review on the evidence regarding the potential for alcoholic beverages to induce mutagenicity *in vivo*. The COM considered the available evidence up to November 2000. The COM reaffirmed its 1995 conclusion that consumption of alcoholic beverages does not present any significant concern with respect to mutagenic potential. The statement can be found on the COM internet site. (<http://www.advisorybodies.doh.gov.uk/com/index.htm>)

Introduction to current review

15. An initial draft report of the systematic review was considered at the November 2002 COC meeting. Further drafts were considered at meetings during 2003. The Department of Health also commissioned a further review of evidence on possible mechanisms by which drinking alcoholic beverages could induce breast cancer from the DH Toxicology Unit at Imperial College. The Committee also received a copy of the published paper from the Oxford Collaborative Group on Hormonal Factors in Breast Cancer just prior to its November 2002 meeting. The Committee agreed that it would be important to compare the approaches used and results reported in the commissioned systematic review study by the Imperial College group with results published by the Oxford Collaborative Group as this might help it to draw conclusions about causality. The Committee also reviewed additional epidemiological studies on the association between drinking alcohol and breast cancer retrieved up to June 2003. The primary objectives of the current COC review were;
 - a) To evaluate the report of the systematic review undertaken by the Department of Epidemiology and Public Health, Imperial College and the investigations undertaken by the Oxford Collaborative Group study and to consider whether the association between drinking alcoholic beverages and increased risk of breast cancer can be explained by bias or confounding and the extent of heterogeneity in the reported association.
 - b) To review the available evidence for a mechanistic basis for the observed association between drinking alcoholic beverages and breast cancer.
 - c) To assess whether the association between drinking alcoholic beverages and risk of breast cancer can be considered causal.
 - d) To evaluate quantitative estimates for population attributable risk (PAR).

Review of new information

Uncertainties in evaluation

16. Potential uncertainties that might affect the interpretation of results obtained in the two systematic reviews considered by the Committee (i.e from Imperial College and the Oxford Collaborative Group) could include misclassification of cases and controls, misclassification of exposure, misreporting of alcohol consumption, the evaluation of dose-response, and the evaluation of potential effects of confounding factors for breast cancer on estimated risks associated with drinking alcohol. The Committee considered that the different approaches used by the two groups complemented each other. Thus the evaluation of individual subject level data by the Oxford group would allow for a more consistent classification of exposure and adjustment for confounding factors. The evaluation of study quality by the Imperial group would aid in the assessment of the sensitivity of findings to study design.

Systematic review undertaken by Department of Public Health and Epidemiology⁸⁷⁻⁸⁹ (Draft reports reviewed at November 2002 and meetings during 2003). Finalised report submitted to peer reviewed journal and considered by COC at June 2004 meeting¹²⁰.

17. The objectives of the systematic review and subsequent meta-analyses undertaken by Imperial College were to determine the magnitude of any association between drinking alcohol and primary breast cancer, to explore the dose-response relationship, to examine whether any association was related to specific beverages or to consumption of all alcoholic beverages, to explore possible heterogeneity, bias and confounding and to estimate the population attributable risk. All publications, in any language, between January 1st 1966 and 31st December 2003 were eligible for inclusion. The results from studies were examined after data on study design and methods had been abstracted and reviewed independently by two members of the team. Duplicate reports of the same study were carefully evaluated to include only a single and the most complete dataset. A simple scoring scheme was used: suboptimal design (1), good design but insufficient control for confounding (2), good design and adequate control of confounding (3). Meta-analyses were undertaken for least, at-least-age, and multivariate-adjusted odds ratios (where possible) separately for all reports, those scoring 2 or 3 and, finally those scoring 3. Dose response modelling used standardised exposures (converted to grams/day (g/day)), the mid-point estimates for consumption, and a linear model with a variable intercept and meta-analysis of dose-response using a random effects model. The authors assumed that an average drink in the U.K. contained approximately 9.5g ethanol and used this as a conversion factor in reporting their analysis of risk of breast cancer associated with drinking alcoholic beverages.
18. A total of 298 papers were identified. Data from 111 were considered appropriate for inclusion in the review. These related to 98 unique studies. The number of studies that provided data that could be included in the ever versus never analysis was 89 and was based on 75,728 cases. Using all these studies and least adjusted odds ratios, a statistically significant risk associated with drinking alcohol of 1.11 (95% CI 1.06-1.17) was reported. Combining least adjusted odds ratios estimates from studies scoring 2 or 3, the risk associated with drinking was 1.12 (95% CI 1.06-1.18). The odds ratio for studies with a score of 3 and multivariate adjustment was 1.22 (95% CI 1.09-1.37). The use of a linear dose-response model with a variable intercept allowed for the presence of drinkers/exdrinkers in the referent group and also avoided the assumption that if a linear dose-response relationship existed then it would be linear through the origin. It was reported that when the adjusted dose-response slopes from studies of good design only (multivariate adjustment for confounders with a score of 3) were combined, the odds ratio was found to be 1.10 (95% CI 1.05-1.15) associated with drinking an extra 1g of ethanol (in alcoholic beverages) per day amongst drinkers. The Imperial research group reported that a woman drinking on average two drinks per day (assuming each drink contains 9.5 g ethanol) has a lifetime risk of breast cancer estimated to be 10% (95% CI 5-15%) higher than a woman who drinks an average of one drink per day. The relative risk can also be expressed in terms of units of alcohol consumed (where, as noted in paragraph 3 above each unit contains 8 g of ethanol). This is important since intakes in the U.K. are usually expressed in terms of unit of alcohol consumed. Thus a woman consuming on average two units per day has a lifetime risk of breast cancer estimated to be 8% (95%CI 4%-12%) higher than a woman who drinks on average one unit per day. There was no evidence for stronger or weaker associations with any particular type of beverage.

19. The Imperial research group found considerable unexplained statistical heterogeneity between the studies they reviewed. Thus meta regression with random effects was used to investigate heterogeneity. The following study characteristics were included: data collected before or after disease onset, hospital or community controls, pre and post menopausal participants and country of study population. It was noted that there was a significant difference in relative risk between case-control studies using hospital or community controls, but the association between drinking alcohol and breast cancer was still statistically significant in studies using either of these control groups. Otherwise none of the variables included in the meta regression significantly reduced heterogeneity between studies. Funnel plots were used to investigate the possibility of publication bias, and they did not indicate that this had been a problem. Bias and confounding could not be dismissed as an explanation of the results, but the study methods minimised their impact as far as possible.
20. A Population Attributable Risk (PAR) for the U.K had been calculated using Cancer Statistics for 1999 and information on drinking patterns derived from the Health Survey for England from 1993 and 1998. Assuming causality and that 1 unit of alcohol contains 8 g ethanol, the PAR calculated from the best quality studies was 6.0% (95% CI 3.2%-8.8%) (i.e the fraction of breast cancer cases that could be prevented if drinking was reduced to a very light level (i.e below 1 unit per week). Using 2000 cancer registration data for the U.K, this would suggest approximately 2430 cases each year (95% CI 1290-3560) could be prevented.

COC Comments on draft and final reports of systematic review undertaken by Imperial College

21. The Committee considered that the work had been thoroughly undertaken and was the largest and most comprehensive systematic review available.
22. The scoring system allowed examination of study quality and, further analyses had been undertaken to examine for bias and confounding. The Committee noted that the majority of analyses reported statistically significant positive associations. The investigators had acknowledged that the definition of non-drinker, use of mid point estimates of alcohol consumption and aggregate (study) data instead of individual data had limited the evaluation. Members noted there were limited data available on assessment of the influence of menopausal status and agreed no conclusions could be drawn on this aspect. The Committee agreed that the evaluation of dose-response was difficult, particularly at higher levels of drinker where there were comparatively fewer data available. Members accepted the rationale for adopting a linear model with a variable intercept.
23. It was noted that the available mechanistic data supported the possibility of a threshold for carcinogenesis. The Committee considered that the estimate of Population Attributable Risk (PAR) was potentially one of the most important outcomes of the systematic review with regard to presentation of the public health significance of the analyses. Members noted that there was no significant alteration in PAR when non-drinkers were excluded. The Committee concluded that the PAR estimate based on best studies and most adjusted model using intake data for England and cancer registration data for the UK represented an acceptable analysis. They recommended that the PAR estimate could be used in the consideration of policy options.

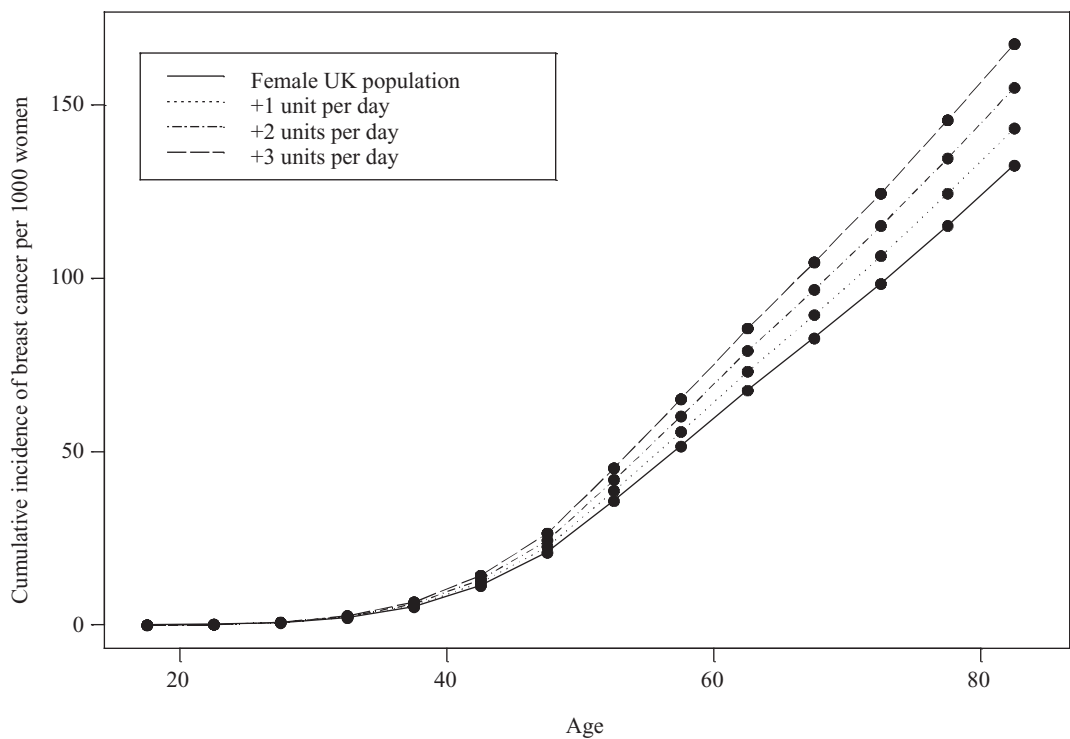
Publication by Oxford Collaborative Group on Hormonal Factors in Breast Cancer⁷. (Report reviewed at November 2002 meeting)

24. The Oxford Collaborative Group has collated individual subject data from epidemiology studies where the relationship between breast cancer and hormonal, reproductive and other factors (including alcohol consumption) had been investigated. Case-control studies were eligible if they included at least 100 women with incident invasive breast cancer and recorded appropriate information on the potential risk factors for breast cancer. Cohort studies were included using a nested case-control design, in which four controls were selected at random, matched on follow-up to age of diagnosis and, where appropriate broad geographical regions. There were 53 studies (two unpublished) that contributed data on alcohol and tobacco usage. There were a total of 58,515 cases where individual data were obtained and 95,067 controls. Relative risks of breast cancer were estimated after stratifying for age, study centre, parity, and where appropriate, women's age when their first child was born, and by tobacco use. A relative risk of breast cancer of 1.32 (95%CI 1.19-1.45) was reported for an intake of alcoholic beverages equivalent to 35-44 g ethanol per day (i.e. approximately 4-5 drinks/day) compared to non-drinkers. The relative risk of breast cancer increased by 7.1% (95% CI 5.5%-8.7%) for each additional 10 g/day intake of ethanol in alcoholic beverages. The authors estimated that approximately 4% of breast cancers in developed countries were attributable to drinking alcoholic beverages.
25. The Committee noted that the Oxford Collaborative Group had access to individual data from 58,515 women, including some from unpublished studies. They had been able to determine median intakes of alcohol. The dose response data reported suggested some evidence for a threshold below a median intake of 8 g/day. Overall it was felt that the Imperial College group had examined significantly more cases than the Oxford Collaborative group.

Discussion of Oxford Collaborative Group research and Imperial College review (March 2003 – June 2004 meetings)

26. The Committee noted the percentage of non-drinkers was 36% in the Oxford Collaborative study and 28.6% in the Imperial College report. The estimate of PAR reported by the Oxford Collaborative Group was 4 % compared to 6% by the Imperial research team. The Committee considered that this difference between the two studies was minor and probably resulted from different proportions of non-drinkers included in the respective calculations. Members considered that the approach used to determine cumulative incidence of breast cancer with age per 100 women at 2, 4 and 6 drinks per day was a useful way to present risks (see figure 5 of the Oxford Collaborative Group report⁷) and suggested the Imperial research group undertake a similar analysis based on their data. Taken together, the results of the two systematic reviews considered by the Committee (i.e. the Imperial research group report commissioned by the Committee and the published Oxford Collaborative Group paper) indicate that the association between drinking alcohol and risk of breast cancer is very unlikely to be due to chance.

27. Following the November 2003 meeting, the Imperial research group provided the figure given below which reports the cumulative incidence of breast cancer per 1000 women for each additional unit of alcohol drunk per day¹²⁰. The solid curve shows the cumulative incidence for the female population in the U.K. (where the average consumption of alcohol is 1 unit per day). The dotted curves show the estimated cumulative incidence if women drank an extra 1, 2, or 3 units per day, where a unit of alcohol contains 8 g ethanol. Studies included in the systematic review undertaken by Imperial College do permit an assessment of breast cancer in non-drinking women in the U.K.



28. The estimated cumulative incidence of breast cancer for women aged 60 and 80 assuming daily consumption of alcohol throughout the majority of a life has been tabulated below.

Cumulative Incidence per 1000 women

	Current consumption	+1 unit per day	+2 units per day	+3 units per day
Age 60	60	65	70	75
Age 80	125	134	145	157

Additional published epidemiology studies retrieved after November 2002

29. The Committee reviewed a number of additional epidemiological papers at the March 2003 meeting retrieved after the November 2002 meeting⁹⁰⁻⁹⁶. The Committee noted new evidence from the Nurses Health Study I cohort that self reported diagnosis of Benign Breast Disease (BBD) might potentially be a useful marker for breast cancer and noted the dose-response relationship between BBD and alcohol intake⁹³. This association might be explored in further research. Members noted the recent studies of receptor status in the association between drinking alcohol and breast cancer and agreed no conclusions could be drawn from the conflicting data. Members commented on one small case-control study which suggested an elevated risk of breast cancer in African-American women who drank alcohol and noted the claim that there was increased mortality in African-American women following diagnosis of breast cancer which might be attributable to drinking alcohol or represent a particular susceptibility of African-American women⁹¹. The Committee asked for additional literature review work on this topic to be undertaken.
30. Some additional papers were submitted to the 26 June 2003 COC meeting⁹⁷⁻¹⁰⁰. The information available, which included a review of all relevant studies published up to April 2001 suggested that there is no association between drinking alcohol and increased mortality from breast cancer following diagnosis^{101,102}. A further review of the claimed variation in risk of breast cancer between different ethnic groups revealed that any association is unlikely to be due to consumption of alcohol. It was noted that, for women, alcohol consumption by ethnic minority groups in the U.K is lower than the estimate for the whole population^{103,104}. One small study found that heavy drinking of alcohol did not modify the risk of early onset breast cancer in young women⁹⁸. Two studies reported on the potential role of genetic polymorphisms of metabolising enzymes but the results suggested that any modifying effect on alcohol induced breast cancer was minimal^{99,100}.

Further paper from DH Toxicology Unit on mechanisms¹⁰⁵

31. The DH Toxicology Unit noted in its paper that alcohol may not be carcinogenic to the breast *per se*, but may facilitate carcinogenesis through a variety of mechanisms. Several pathways have been proposed, including effects on the permeability of cell membranes in the breast, induced hepatic metabolism of carcinogens by ethanol-inducible enzymes, inhibition of DNA repair mechanisms, effects on hormone metabolism and interactions of alcohol with other host and environmental factors. The paper has been published on the COC internet site (<http://www.doh.gov.uk/coc/index.htm>)
32. The Committee was aided in its deliberations by expert advice from Professor H S Jacobs (Emeritus Professor of Reproductive Endocrinology, University College Medical School, London) who attended the November 2002 meeting of the COC. The DH Toxicology Unit report highlighted evidence to support the view that alcohol induced hyperinsulinaemia and increased Insulin-like Growth Factors (IGFs) which subsequently induced an increase in breast tissue density through increased cell division¹⁰⁵⁻¹⁰⁸. It was noted that there were additional studies to support an association between drinking alcohol and effects on oestrogen metabolism¹⁰⁹. There were considerably less convincing data

for a number of suggestions such as alcohol induced suppression of melatonin excretion products and aromatisation of androgens to oestrogens. The Committee agreed that it would be appropriate to focus the review on the effects of alcohol on oestrogens, hyperinsulinaemia and effects on IGFs. The evidence supporting other proposed mechanisms was preliminary and no conclusions could be drawn.

33. Evidence to support the association of alcohol with increased oestrogen levels has been documented in a number of studies^{110,111} not previously reviewed by COC. The Committee considered a cross sectional study by Verkassalo PK et al¹¹² and agreed that sufficient numbers of premenopausal (n= 636) and postmenopausal (n = 456) women had been included. The results were inconsistent with the data previously reviewed by the committee in 1999 and suggested an effect of cigarette smoking, but not drinking alcohol, on levels of oestrogens. These results were not consistent with the available epidemiological data on breast cancer. The COC reviewed a study by Dorgan JF et al¹¹³ in postmenopausal women and agreed that a satisfactory crossover design had been used for this intervention study, although there were some reservations about potential compliance of study participants. It was noted that there was some evidence for a small increase in oestrone sulphate and dehydroepiandrosterone sulphate (DEHA sulphate) following the consumption of 15 g or 30 g ethanol/day over an eight week period. There was no effect on oestradiol levels (free or bound) in this study. Members agreed the data supported a small effect of drinking alcohol on adrenal output of hormones. This study suggested the effect of drinking alcohol on hormone levels was milder than that suggested by the cross sectional studies previously reviewed by the COC in 1999.
34. The Committee agreed that the weight of evidence available suggested that drinking alcohol produced a number of biochemical effects in the liver which resulted in changes to oestrogen metabolism and IGF levels which, over a prolonged period of time, i.e. decades, could induce breast cancer. Both of these suggested mechanisms would potentially have a threshold with regard to induction of breast cancer.

Consideration of Causality

35. The Committee had previously considered the available evidence in accordance with the Bradford-Hill criteria¹¹⁴ during its review in 1999. The Committee agreed it would be appropriate to undertake a further review using these criteria as an aid in the assessment of the potential causation of breast cancer by drinking alcoholic beverages as there were new epidemiological and mechanistic data available. An assessment of the evidence has been tabulated below.

Criterion	Evidence regarding alcohol and breast cancer	Comments
Strength	Limited	The RR* in alcohol drinkers is modest and, even for heavy drinkers, in most studies rarely exceeds 3 (i.e. risk in drinkers is 3 times that of non-drinkers). However the RR for most other identified breast cancer risk factors also rarely exceed this value.
Consistency	Yes.	Literature largely points towards a small positive association but there is still unexplained heterogeneity. The systematic review by Longnecker MP ³⁷ also reported significant heterogeneity. The pooled analysis of prospective studies published by Smith-Warner SA et al ⁶⁹ found evidence of heterogeneity in results for pre menopausal women but not postmenopausal women. Heterogeneity was only partly explained in the systematic review report from the Imperial College group ⁸⁷⁻⁸⁹ . However the approach used by the Oxford collaborative group which used individual data from the same cases, gave substantially similar results to the Imperial College group ⁷ .
Specificity	Not relevant.	Cancer risk attributed to alcohol is not specific to breast cancer (e.g. prolonged alcohol consumption can induce cancers of the head and neck and oesophagus and liver).
Temporality	Yes	Association demonstrated in prospective studies where alcohol consumption can be studied before the occurrence of disease.
Biological gradient	Yes	There is evidence for a monotonic dose-response curve in the submitted systematic reviews from Imperial College and in the Oxford Collaborative group analysis.
Plausibility	Yes	Evidence for effect of alcohol consumption and elevations in blood levels of oestrogen metabolites documented ^{77-86,110-112} . Raised oestradiol is a risk factor for breast cancer. ⁵ The evidence therefore suggests a plausible mechanism. Further studies have also suggested that an effect of drinking alcoholic beverages could affect liver biochemistry and hence could affect insulin levels and Insulin dependent Growth Factors (IGFs) and thus induce increased cell numbers in breast tissue.
Coherence	Limited	Evidence for an increased risk of breast cancer in alcoholics ¹¹⁵ and for a relatively low rate of breast cancer incidence among populations abstaining from alcohol (e.g. Mormons) ¹¹⁶ .
Experiment	Limited.	Evidence from one limited study where ICR mice were given ethanol via the drinking water (at 10% or 15%) for 25 months ¹¹⁷ . No evidence that alcohol is carcinogenic in a large number of carcinogenicity studies including some conducted to acceptable standards. ⁵ Some evidence that alcohol affects breast tissue differentiation in animals ¹¹⁸ .
Analogy	Yes	Other causes of significantly increased oestradiol levels in exposed populations are suggested risk factors for breast cancer (e.g. use of oral contraceptives and HRT) ⁵ . IGFs may be involved in development and progression of breast cancer ^{107,108} .

*RR = Relative risk

36. There was evidence to satisfy most of the criteria. The Committee agreed that, overall, there was no consistent evidence that alcohol is carcinogenic from experimental studies in animals. The isolated finding of mammary tumours in ICR mice¹¹⁷ given extremely high doses of ethanol in the drinking water in excess of the Maximum Tolerated Dose level had not been demonstrated in other studies in rats and mice which also used high doses of ethanol. The Committee was aware of some preliminary findings which suggested that *in-utero* exposure to ethanol in rats may be associated with increased mammary tumourigenesis, but agreed that no conclusions could be based on the preliminary results of this work¹¹⁹. The Committee considered that the criterion of specificity was not relevant to the assessment of breast cancer risk.
37. The Committee agreed that the available evidence indicated there is a modest association between drinking alcohol and increased risk of breast cancer which was consistently demonstrated. The small magnitude of the association between drinking alcoholic beverages and risk of breast cancer and the complex aetiology of breast cancer (i.e. there are weak associations with a number of other risk factors) are the main reasons for the difficulty in reaching a definite conclusion. However the most recent review of mechanisms provided evidence for a number of plausible mechanisms which focused on a potential effect of drinking alcohol on liver function. Overall, the Committee considered it was prudent to assume a causal association exists.

Significance to Public Health

38. The Committee agreed that if, for practical purposes, a causal association is assumed, then it was important to consider the magnitude of the association between drinking alcohol and breast cancer in terms of potential impact on public health. The Population Attributable Risk (PAR) is an estimate of the proportion of cancer cases which might be prevented if the levels of alcohol consumption were reduced to very light levels of drinking (below 1 unit/week). The calculation of PAR from epidemiological data requires information on the rate of breast cancer in the population, the estimate of relative risk, and data on intake of alcoholic beverages. There are some uncertainties in all of these and, hence in the estimate of PAR produced. The estimate of PAR from the Imperial College group which takes some of the uncertainties into account in the estimate of relative risk is 6.0% (95% CI 3.2%-8.8%). Based on the 2000 data for breast cancer registration in the U.K this would indicate that approximately 2430 cases/year (95% CI 1290-3560) may be attributable to drinking alcoholic beverages.
39. The estimate of PAR from the Oxford Collaborative Group was slightly lower. The Committee agreed that it would be prudent to base its evaluation on the calculations proposed by Imperial College since these were based on intake data for England and could be readily applied to the U.K. The Committee noted that the systematic review undertaken by the Imperial College group had reported inconclusive results for the effect of Hormone Replacement Therapy on risk of breast cancer associated with drinking alcohol. The Oxford Collaborative group had reported that stratification for use of oral contraceptives and HRT had not affected the estimation of risk of breast cancer associated with drinking of alcoholic beverages. The Committee felt that the potential for oral contraceptive and HRT use to influence the association between drinking alcohol and risk of breast cancer had not been researched in detail and recommended further epidemiological studies to assess any potential interactions.

40. The Committee agreed with the reported assessment of cumulative risk submitted by the Imperial College group and noted that lifetime drinking of an extra 3 units of alcohol per day above the national average for the female population of 1 unit/day would result in an additional 15 cases of breast cancer/1000 women at 60 years of age. An extra 32 cases of breast cancer/1000 women would occur at 80 years of age at this increased level of drinking. This needs to be compared to background rates of 60 cases of breast cancer/1000 women at 60 years of age and 125 cases of breast cancer/1000 women at 80 years of age where the average intake of alcohol is 1 unit per day. The Committee agreed there was a progressive increase in the risk of breast cancer associated with increasing amounts of alcoholic beverages drunk and duration of drinking. A review of the sensible drinking message would have to balance the increasing risk of breast cancer against the benefit attributed to drinking alcoholic beverages of reduced mortality due from coronary heart disease. Such an evaluation would be outside the terms of reference of the COC.
41. The Committee noted the evidence for increasing consumption of alcoholic beverages by women in the U.K, and particularly amongst younger women and was concerned that if the increased intake of alcoholic beverages reported amongst this group were maintained over most of their lifetime then it would result in an increase in the number of alcohol-related breast cancer cases. The surveys of alcohol consumption reported to the Committee specifically reported drinking in various age groups. The youngest age group studied included women aged 16-24 years. It was therefore important to raise awareness of the potential risks associated with drinking alcoholic beverages particularly amongst young women.

Conclusions of current review

42. The Committee reached the following conclusions based on an evaluation of all the data available up to the end of June 2003 and a finalised report of a systematic review undertaken by Imperial College submitted to the June 2004 COC meeting.
 - a) Taken together, the results of the two systematic reviews considered by the Committee (i.e. the Imperial College research group report, commissioned by the Committee, and the published Oxford Collaborative Group paper) indicate that the association between drinking alcohol and risk of breast cancer is very unlikely to be due to chance (**paragraph 26**).
 - b) From the Imperial College review, the best estimate for the relative risk of breast cancer associated with each additional gram of ethanol consumed was 1.01 (95% CI 1.005-1.015). This means that a woman drinking an average of two units of alcohol (each unit containing 8 g ethanol) per day has a lifetime risk estimated to be 8% higher compared to a woman who drinks an average of one unit of alcohol per day. There was no evidence for variation in the association with any specific type of alcoholic drink (**paragraph 18**).

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- c) The Population Attributable Risk (i.e. percentage of breast cancers which could be prevented if drinking were reduced to a very low level of less than 1 unit/week) using U.K. cancer registry data and intake data from the Health Survey for England is 6% (95% CI 3.2%-8.8%). This equates to approximately 2430 cases of breast cancer per year (95% CI 1290-3560) **(paragraph 20)**.
 - d) The assessment of cumulative risks suggests that lifetime drinking of an extra 3 units of alcohol per day would result in an additional 15 cases of breast cancer/1000 women at 60 years of age and an extra 32 cases of breast cancer/1000 women at 80 years of age compared to current rates of 60 cases of breast cancer/1000 women at 60 years and 125 cases of breast cancer/1000 women at 80 years where the average intake is 1 unit per day **(paragraphs 28 and 40)**.
 - e) The Committee agreed that the weight of evidence available suggested that drinking alcohol produced a number of biochemical effects in the liver which resulted in changes to oestrogen metabolism and IGF levels, which over a prolonged period of time, i.e decades, could induce breast cancer. Both of these suggested mechanisms would potentially have a threshold with regard to induction of breast cancer. **(paragraph 34)**.
 - f) The Committee concluded it prudent to assume that drinking alcoholic beverages may cause breast cancer in women. **(paragraph 37)**.
 - g) The Committee agreed that more research into the potential for interaction between use of oral contraceptives and use of Hormone Replacement therapy and the induction of breast cancer by drinking alcoholic beverages was appropriate. **(paragraph 39)**.
 - h) The Committee was concerned to note that if the increased consumption of alcoholic beverages by young women were maintained over most of their life-time then it would result in an increase in the number of alcohol-related breast cancer cases in the future. It is therefore important to raise awareness of the potential risk of breast cancer in women associated with drinking alcoholic beverages. **(paragraph 41)**.

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Glossary of terms/phrases used in statement.

Aetiology: The study of causation.

Benign Breast Disease: A proliferation of breast tissue which is not malignant. However some forms are indicative of an elevated risk of breast cancer.

Breast Cancer: A malignant tumour of breast tissue, usually arising from ductal or lobular epithelial cells. The great majority of breast cancers occur in women. Breast cancer is rare in men.

Causal Association: Describes the relationship between two factors which are associated where it can be established that one of the factors causes the other, i.e smoking cigarettes and lung cancer.

Collaborative Group On Hormonal Factors in Breast Cancer: A large international collaborative group of researchers. The secretariat is based at the Cancer Research UK Epidemiology Unit, Gibson Building, Radcliffe infirmary, Oxford OX2 6HE. The group had access to raw data from 65 epidemiology studies in its evaluation of the association between drinking alcoholic beverages and increased risk of breast cancer.

Epidemiology studies: Epidemiology is the study of the distribution and determinants of diseases in human populations. All of the studies included in the COC review of the association between drinking alcoholic beverages and increased risk of breast cancer are called Analytical studies. Very briefly, the basic outline of the types of studies included in this review are;

- A. Case-control studies where drinking patterns are gathered from individuals with breast cancer and compared to patterns in control individuals who don't have breast cancer.
- B. Cohort studies where information on drinking patterns is gathered from individuals who are then followed for a period of time (often decades) until the occurrence of breast cancer or death.

General Household Survey (GHS): The GHS is conducted on a yearly basis by the Social Survey Department of the Office for National Statistics (ONS). It has charted the changes in British households and society since 1971. Questions about drinking habits were included every other year from 1978-1998 and every year from 2000 onwards. Questions regarding maximum daily amount drunk last week and weekly drinking habits have been included every year have been included since 1998.

Heterogeneity: A variation in an estimate which exceeds the expected.

Hormone Replacement Therapy: (HRT) HRT consists of oestrogen given continuously to women during the menopause to manage symptoms associated with loss of ovarian function. Cyclical progestogen is given to women who have not had a hysterectomy. HRT may also help to prevent osteoporosis.

IGFs: Insulin-like Growth Factors.

International Agency for Research on Cancer (IARC): The International Agency for Research on Cancer (IARC) is part of the World Health Organisation. IARC's mission is to co-ordinate and conduct research on the causes of human cancer, the mechanisms of carcinogenesis, and to develop scientific strategies for cancer control. The Agency is involved in both epidemiological and laboratory research and disseminates scientific information through publications, meetings, courses, and fellowships.

Interdepartmental Working Group on Alcohol (IDWG): The IDWG was established in 1994 and consisted of a group of officials. Its remit was to review current medical and scientific evidence and its interpretation on the long term effects of drinking alcohol and, to consider whether the sensible drinking message should be reviewed in the light of this, also taking into account Government policies on the short term effects of drinking alcohol and any other factors considered relevant by this group. The IDWG produced a report entitled "Sensible Drinking" in December 1995.

Mechanisms (by which alcohol could induce breast cancer): A term describing the effects of alcohol drinking on biochemistry and physiology which could, if sustained over a period of time, ultimately lead to induction of breast cancer. Evidence regarding mechanisms can come from a variety of sources including studies in cell culture (in-vitro), studies in animals, and investigations in human epidemiology or volunteer studies. A proposed mechanism for induction of cancer cannot be verified through statistical evaluation of cancer data and requires scientific judgement to assess plausibility.

Menarche: The beginning of menstruation.

Menopause: The cessation of menstruation, occurring usually around the age of 50y. Pre-menopausal (before menopause). Post- menopausal (after menopause).

Meta-Analysis: A meta-analysis study is a specialised statistical analysis which combines the results of individual studies producing a quantitative summary across all studies of the effect of interest. This type of study can provide valuable information to help in estimating the strength and consistency of the association between drinking alcohol and breast cancer.

Meta-Regression: A statistical analysis which aims to investigate how the size of an effect varies with characteristics of the studies in a meta-analysis

Oral contraceptive: A compound, usually hormonal, taken usually by the oral route in order to block ovulation and prevent pregnancy. Most oral contraceptives available in the U.K contain both an oestrogen and a progestogen.

Parity: Condition of a women with respect to having borne viable offspring.

Random Effects: A statistical model which assumes that an underlying strength of association can vary between studies.

Recommended level of drinking: The recommended number of units of alcohol which can be consumed without long term adverse effects. This has been set as daily benchmarks which are appropriate for regular and irregular drinkers. For women this is 2-3 units/day. Drinking in excess of 3 units/day accrues progressive health risks.

Risk factors for breast cancer: The aetiology (see above definition) of breast cancer is complex. An association has been demonstrated for many different factors including heredity, reproductive, hormonal, and lifestyle factors. A role for environmental factors can be deduced from geographical variation in rates of breast cancer and changes in rates among migrants toward those of the host country. Thus drinking alcohol is one of several risk factors for breast cancer.

World Health Organisation: The World Health Organisation, the United Nations specialised agency for health, was established on 7 April 1948. WHO's objective, as set out in its Constitution, is the attainment by all peoples of the highest possible level of health. Health is defined in WHO's Constitution as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity. The WHO has established agencies to assist in its work. One of which is IARC (see above for definition).

Breast cancer risk and exposure to organochlorine insecticides: consideration of the epidemiology data on dieldrin, DDT and certain hexachlorocyclohexane isomers

Introduction

1. In 1995, the COC reviewed the available epidemiological studies on three chemicals (DDT and isomers/metabolites, and the hexachlorocyclohexane isomers γ -HCH (lindane) and β -HCH. The Committee agreed that the available evidence indicated no clear association. It was felt, however, that the matter should be kept under review. The Committee on Carcinogenicity was asked by the Department of Health in 1999 to review the relevant information on four organochlorine insecticides (OCIs) in respect of the potential for an association with breast cancer. The additional chemical included was dieldrin, for which new epidemiological data had become available at the time of that review. The main conclusions of the 1999 review are given below;

DDT Some DDT isomers and metabolites should be regarded as having weak *in-vivo* oestrogenic activity. The stable metabolite p, p' DDE, a marker for exposure to DDT, can be found in samples of fat from most individuals. There is however, good evidence from investigations undertaken in the UK that concentrations of p, p' DDE in human fat samples have been declining for several decades. There are now 14 epidemiological studies which have considered p, p' DDE using both case-control and prospective study designs. There is no convincing evidence for an association with an increased risk of breast cancer. Overall the available data do not suggest that environmental exposure to DDT (and isomers/metabolites) is a cause for concern as a risk factor for human breast cancer.

Dieldrin Dieldrin is not considered to have *in-vivo* oestrogenic activity. There is thus no rationale to consider that exposure to this chemical should be associated with an increased risk of breast cancer. There is good evidence from investigations undertaken in the UK that concentrations of dieldrin in human fat samples have been declining for several decades. There is no convincing evidence from the five available epidemiological studies for an elevated risk of breast cancer in association with exposure to dieldrin. Overall the available data do not suggest that environmental exposure to Dieldrin is a cause for concern as a risk factor for human breast cancer.

β -HCH β -HCH should be regarded as having weak *in-vivo* oestrogenic activity. β -HCH can be found in samples of fat from most individuals. There is evidence from investigations undertaken in the UK for a decline in β -HCH concentrations in human fat samples after 1982/3. There is no convincing evidence from the five available epidemiological studies for an elevated risk of breast cancer in association with exposure to β -HCH. It is recommended that the published literature on this chemical should be kept under review.

Lindane Lindane (γ -HCH) is not considered to have any *in-vivo* oestrogenic activity. There is thus no rationale to consider that exposure to this chemical should be associated with an increased risk of breast cancer. The available evidence for environmental exposure to lindane suggests that body burdens of this chemical in the UK are very small, being undetectable in most individuals. None of the five available epidemiological investigations found evidence for an association with breast cancer. Overall the available data do not suggest that environmental exposure to lindane is a cause for concern as a risk factor for human breast cancer.

2. The Committee was aware of a number of research investigations that were either planned or had been instigated at the time of the 1999 review and agreed to review relevant publications in the scientific literature at some point in the future. A large number of publications have become available and it is now timely to review the evidence.

Introduction to current review

3. The Committee considered a review paper presented to its 22 September 2003 meeting. Further review papers and tabulated summaries of epidemiology studies were considered at the 6 November 2003 and 1 April 2004 meeting. All papers can be accessed through the COC internet site (either via links from agendas or in the section entitled papers).
4. During the review process, members agreed that it was important to consider all of the epidemiology studies together rather than focus on studies published after the last review in 1999. However no additional relevant information has been retrieved on lindane (γ -hexachlorocyclohexane, (γ -HCH) since the 1999 review. The COC concluded in 1999 that there was no rationale that γ -HCH could be associated with breast cancer. A summarised tabulation of the studies available at the time of the review has been published as an Annex to this statement. A summary of the main results from epidemiology studies has been presented in a number of graphs which are also available as an Annex to this statement. The tables and graphs present information for DDT (and its isomers and metabolites), β -hexachlorocyclohexane (β -HCH) and dieldrin.
5. The Committee was aware that none of the OCIs included in this review are approved for use in pesticide formulations in the U.K.
6. The Committee agreed in 1999 that a number of observations and assumptions had led some observers to suggest the hypothesis that OCIs and other organochlorine compounds may be associated with an increased risk of breast cancer. The format of the statement agreed in 1999 presented a review of the biological plausibility that OCIs may be associated with an increased risk of breast cancer and then a review of the available epidemiology for each OCI under consideration. The conclusion reached for each of the OCIs under consideration in 1999 took account of the potential for an oestrogenic response *in-vivo*, the evidence for persistence in humans and the available epidemiological investigations. The Committee agreed that the format of the current statement should adopt the same procedure used for the 1999 review. Thus a brief overview of the proposed hypothesis that OCIs maybe associated with an increased risk of breast cancer is given below;

Overview of hypothesis that OCl's may cause breast cancer

7.
 - i) Many of the known or proposed risk factors for breast cancer are related to endogenous or exogenous hormones (in particular oestrogen). These factors include age at first birth, at menarche, and at menopause, and obesity, parity and use of oral contraceptives and hormone replacement.
 - ii) there is some evidence available to suggest that some of the OCl's under consideration may have weak oestrogenic activity^{2,7}.
 - iii) these OCl's have been shown to induce tumours (predominantly of the liver) in experimental animals⁸.
 - iv) these OCl's persist in the environment and exposure of the population has occurred mainly via the diet^{4,9}.

Consideration of biological plausibility.

8. The Committee reviewed the evidence that dietary exposure to environmental levels of these OCl's might induce an oestrogenic response *in vivo* through the consideration of three questions, namely;
 - i) these OCl's have oestrogenic activity *in vivo* and if so what is their potency relative to other sources of oestrogens?
 - ii) Is there any evidence for synergistic effects?
 - iii) Do these compounds persist in breast tissue?

Do these OCl's have oestrogenic activity in vivo and if so what is their potency relative to other sources of oestrogens?

9. A tabulation of the Committee's assessment of the evidence for oestrogenic activity of the OC insecticides under consideration is given overleaf. The new data published since the 1999 COC review provided additional information on the *in-vitro* oestrogenic effects of β -HCH^{37,38} and DDT isomers/metabolites³² and evidence to show that dieldrin had no effect on human placental aromatase activity *in vitro*³⁵. An *in-vivo* study with dieldrin showed that very high lethal doses administered to rats did alter oestrogen metabolism³⁶. However the Committee concluded that the data from this latter study were irrelevant with regard to assessment of the very low levels exposure experienced by the general population.

Table 1: Assessment of oestrogenic activity of OC insecticides

OCI	Evidence of oestrogenicity <i>in-vitro</i> through effects on			<i>In-vivo</i> data	Conclusion
	i) Oestrogen receptors	ii) Oestrogen metabolism	iii) or by other mechanisms		
β-HCH	Evidence of very weak oestrogenic activity, ^{5,10} (ca 40,000 x weaker than oestradiol)	No data available.	Yes ^{5,10,37,38} Data suggest a number of mechanisms possible but relevance to <i>in-vivo</i> situation is uncertain.	Uterotrophic effects documented in rats and mice. ^{11,12}	Regard as a weak <i>in-vivo</i> oestrogen. Mechanism unknown
Lindane	No evidence of oestrogenic activity. ^{7,13}	Effects noted in MCF-7 cells ^{14, 15} , but other investigators have been unable to identify xenoestrogens using this test system. ¹⁷	No data available	Difficult to interpret but equivocal evidence for oestrogenic and anti-oestrogenic effects in rats. ¹⁷⁻²⁰ . No evidence of uterotrophic effect in reproduction studies in rats and mice ²¹	Considered not to have <i>in-vivo</i> oestrogenic activity
DDT isomers/ metabolites (in particular p,p DDE)	Evidence of weak oestrogenic activity ^{4,22,32} (ca 1000 x weaker than oestradiol)	Effects noted in MCF-7 cells ^{14, 15} , but other investigators have been unable to identify xenoestrogens using this test system. ¹⁶	Yes ^{10, 22-24} relevance to <i>in-vivo</i> situation is uncertain	Oestrogenic effects in rodents reported with certain DDT isomers (e.g. o,p' DDT and o,p DDD) but not with p,p' DDE ⁴	Regard some DDT metabolites and isomers as weak <i>in-vivo</i> oestrogens. Several mechanisms possible involved
Dieldrin	Evidence of very weak oestrogenic activity in the majority of studies ca 10,000-50,000 less potent than oestradiol ^{8,26-32}	No effect on human placental aromatase activity. ³⁵	Yes ³⁹ relevance to <i>in-vivo</i> situation is uncertain	No evidence of uterotrophic effects in a number of studies in either rats or mice ^{27,30,32} . High oral doses given to rats (equivalent to LD50) induced cytochrome P450 enzymes and altered oestrogen metabolism ³⁶	Does not to have <i>in-vivo</i> oestrogenic activity. Effects on oestrogen metabolism at lethal oral doses is irrelevant to consideration of environmental exposure

10. The Committee reaffirmed the conclusions reached in 1999 that the evidence supported the conclusion that β -HCH and DDT (in particular some metabolites and isomers) could have weak oestrogenic activity *in vivo* which most probably occurs by several different mechanisms. Members agreed that dieldrin appeared to have weak oestrogenic activity *in vitro*, but no evidence of an effect had been documented *in vivo* in a number of studies in rats and mice. There was no convincing evidence that lindane had oestrogenic activity *in vivo*. The Committee reaffirmed its conclusion that there was no plausible reason for including lindane as a xenoestrogen for examination in epidemiological studies of breast cancer.
11. The Committee concurred with its conclusion reached in 1999 that in comparison to other potential sources of exposure to oestrogenic substances (e.g birth control regimes, post-menopausal hormone therapy, flavenoids in foods⁴⁰) that OCIs represent an extremely small proportion of the potential oestrogenic burden.

Is there any evidence for synergistic effects?

12. The Committee undertook a detailed review of the potential for synergism between OCIs during the 1999 review. The evidence for synergism between xenoestrogens, and in particular in respect of OCIs, arose from the results obtained in *in-vitro* experiments undertaken by one research group in the USA and published in 1996 which concerned mixtures of dieldrin with endosulfan or methoxychlor^{41,42}. The authors subsequently retracted their data in 1997 after they were unable to replicate the original experiments⁴³. Many other research groups were unable to repeat the finding of synergism using a number of *in-vitro* ^{20,21,24,31} and *in-vivo* tests^{4,24,31}. The results of the available experiments suggested, at most, an additive effect. The Committee also considered a claim by one group of authors⁴⁴ regarding evidence of synergism between certain xenoestrogens and concluded that any significant synergistic interactions in mammals should have been identified by the available published experiments.
13. A further six papers, retrieved since 1999, report the findings of a number of *in-vitro* studies using mixtures of OCIs or mixtures of OCIs with other xenoestrogens⁴⁵⁻⁵⁰. These studies used yeast or mammalian cell lines and a variety of different reporter systems for measuring activation of oestrogen receptors and were relatively complex in design particularly as dose-response effects for a range of combinations of chemicals under test were investigated. A separate investigation investigated the potential interactions of xenoestrogens but did not include any of the OCIs under consideration in this statement⁵¹. Most of these studies found no evidence for interaction between OCIs or between OCIs and other xenoestrogens^{45,46,48,49}. The Committee noted several studies from one research group based at the School for Pharmacy, London had reported significant interactions between xenoestrogens *in-vitro* using either a recombinant yeast system⁵¹ or human breast cancer cells (MCF-7 cells)⁵⁰ which were evident when individual xenoestrogens were included in a mixture at concentrations below the No-observed concentration level when tested individually. The Committee considered that the results obtained in these latter experiments were consistent with an additive effect. A further study which used activation of luciferase linked to oestrogen-receptor activation in T47D breast cancer cells reported evidence for an additive effect between dieldrin and endosulfan⁴⁷.

14. One recently published *in-vivo* study using the immature rat uterotrophic assay has been published which investigated mixtures of seven well established xenoestrogens at dose levels where individual chemicals were ineffective found a uterotrophic response when the mixture was tested. Further investigations by the authors confirmed that the observed response was below that predicted by simple addition of the observed or predicted activities⁵².
15. The Committee was aware that a Working Group of its sister Committee, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) had undertaken a detailed consideration of the risk assessment of mixtures of pesticides and similar substances and a report was published on 15 October 2002⁵³.
(<http://www.food.gov.uk/science/ouradvisors/toxicity/reports/cocktailreport>) The Working Group on Risk Assessment of Pesticides and Similar Substances (WiGRAMP) considered the available evidence regarding all toxic endpoints including endocrine disruption effects such as oestrogenic effects. The overall conclusion of all the available evidence is given below;

“Several studies claim to have identified synergistic interactions of some mixtures. However, for the most part, these studies have been inadequately designed and based on incomplete understanding of the concepts involved, but a few well designed studies have demonstrated both synergistic and antagonistic interactions, as well as additive effects in mixtures, usually at high concentrations or high experimental exposure levels, which are probably unrepresentative of exposure doses.”
16. The information available to the Committee regarding OCIs is consistent with the conclusion reached in 1999 that there is no evidence to support the view that low levels of mixtures of xenoestrogens induce a biologically significant oestrogenic effect in mammals by acting synergistically. The possibility of a simple additive effect cannot be excluded but at the likely exposure levels for the OCIs concerned this will represent a negligible risk of any oestrogenic effect *in vivo*.

Do these compounds persist in breast tissue?

17. The observation that residues of certain OCIs persist in adipose tissue leading to bio-accumulation which might result in continuous exposure of breast tissue to weak oestrogenic substances has been cited as an essential part of the hypothesis that such compounds may cause breast cancer³⁶. In 1999, the COC reviewed the available evidence from surveys of concentrations of OCIs in human adipose tissue or milk from a number of different sources including U.S.A. and countries in Europe. The most complete and appropriate data on adipose tissue concentrations of OCIs in the U.K. had been published by the Pesticides Residues Committee (formerly the Working Party on Pesticides Residues, WPPR) (<http://www.pesticides.gov.uk/committees/PRC/prc.htm>) The data collected from the early 1960s up to 1997 by the PRC which is retabulated below for ease of reference, indicated that body burdens of OCIs have been decreasing.

18. The results published by the Pesticides Residues Committee show that p, p' DDE, and β -HCH can be detected in samples of fat or milk from most individuals studied whilst dieldrin was detected in fewer individuals. Lindane was infrequently found in human fat (3%) and milk (1.8%) samples, mainly at low levels (i.e. only one human fat sample contained > 0.01 mg/kg). The available literature shows that lindane is more rapidly metabolised and eliminated in mammals⁴⁴ than other OCIs such as dieldrin⁴⁵ and thus one possible explanation for the low frequency of detectable lindane residues in humans could be due to its metabolism^{38,39}.

Table 2: Concentrations of OC insecticides in human fat

OC	Mean concentration (mg/kg) in human fat. (Percentage of first reported residue level)				
	1963/4	1969-71	1976/7	1982/3	1995-7
Dieldrin	0.26 (100)	0.16 (61.5)	0.11 (42)	0.08 (30.7)	0.02 (7.6)
p,p'DDE	2.0 (100)	1.8 (90)	2.1 (100)	1.3 (65)	0.71 (35.5)
β -HCH	No Data	0.28 (100)	0.27 (96.4)	0.31 (110)	0.12 (42.8)

19. No new information has been retrieved since the 1999 review. However since it is evident that tissue levels of OCIs were declining, and none of these chemicals is approved for use in pesticide formulations in the U.K. and they have also been subjected to widespread restrictions on use in other countries, it would be appropriate to conclude that concentrations in human adipose tissue and milk have continued to decline.

Conclusions about Biological Plausibility

20. The Committee concluded that the OCIs considered, were, at most, very weak *in vivo* oestrogens and agreed that there was no evidence of any synergistic effects between these chemicals. The impact of exposure to oestrogenic chemicals would be the product of oestrogenic potency and bioavailability. The possibility of an additive effect of OCIs could not be discounted. However as OCIs are of low potency and occur at low concentrations, it is most unlikely that the effect of current exposures individually or collectively will significantly add to total oestrogenic burden in women and will not present any significant risk with regard to breast cancer.

Epidemiology

21. In 1995 when the Committee first reviewed the subject of organochlorine insecticides and the potential association with breast cancer, the available epidemiological data on breast cancer and exposure to OC insecticides were limited, comprising 6 case-control studies which investigated a total of 301 women with breast cancer using a variety of exposure analyses (in serum, plasma and breast adipose tissue)⁶²⁻⁶⁷. In 1999 a further eight additional epidemiological studies had been published⁶⁸⁻⁷⁵, which considerably increased the number of women studied (for p,p', DDE this was estimated to be around 1500). By January 2004 (the deadline for this statement), over 80 estimates of odds

ratios/relative risks had been documented from a wide range of epidemiological investigations using different study designs involving both prospective and retrospective approaches, and analyses of OCIs in blood, serum or adipose tissue in women. A number of these investigations have also included an evaluation of hormone receptor status of breast cancer as part of the assessment⁷⁷⁻¹⁰⁸. However most of these studies have examined DDT or its metabolite p,p'DDE whilst relatively few have reported data for dieldrin and β -HCH and none for lindane (β -HCH). One meta-analysis of 22 investigations of the potential association between p,p'DDE and risk of breast cancer has been published⁷⁶. It is not possible to provide a narrative summary of all these studies in this statement. A tabulated summary and graphical representation of the main results from the studies are appended to this statement as separate Annexes. The overall conclusions reached by the Committee on each OCI are given below.

DDT (and isomers/metabolites p,p'DDE)

- i) 1999, the Committee concluded that overall, there was no convincing evidence from epidemiology studies for an elevated relative risk of breast cancer in association with DDT (as measured by pp' DDE). With regard to DDT, the available studies are overwhelmingly negative apart from one prospective cohort study⁸⁶ and one retrospective case-control study⁸⁰. With regard to p,p'DDE a number of positive studies have also been published^{65,73,95,96,103}. The Committee considered the meta-analysis study of published studies investigating the association between p,p'DDE and risk of breast cancer had been adequately undertaken and the summary risk estimate of 0.97 (95% CI 0.87-1.09) suggested there was no evidence for an association¹¹⁰. Overall the Committee considered there was no compelling evidence that DDT or its metabolite pp'DDE were associated with an increased risk of breast cancer.

Dieldrin

- ii) In 1999 the Committee noted that there was relatively little epidemiological data on dieldrin. Overall, the Committee concluded there was no convincing evidence from epidemiological studies for an elevated relative risk of breast cancer associated with dieldrin. There have been relatively few studies published since 1999 on the potential association between dieldrin and risk of breast cancer. Hoyer and colleagues have reported positive findings in a number of analyses^{72,87,88}. However the COC has previously stated there are methodological problems with these studies which prevent definite conclusions from being drawn¹⁰⁹. Thus overall the Committee consider there are no convincing data available regarding an association between dieldrin and risk of breast cancer.

(It was not appropriate to calculate an overall estimate of the strength of association from the available data)

β -HCH

- iii) In 1999 the Committee concluded that there was very little epidemiological information available on β -HCH and its possible association with breast cancer. The studies available at that time had all provided negative findings. None of the studies retrieved for the current review reported statistically significant positive findings^{77,82,86,92,100,103}. Thus overall there is no evidence to associate β -HCH with an increased risk of breast cancer.

Lindane (γ -HCH)

- iv) In 1999, the Committee noted there was very little epidemiological information on the potential association between lindane and risk of breast cancer. The available studies did not suggest an association. The Committee concluded that it was unlikely that further epidemiological investigations of breast cancer based on assessment of levels of lindane in adipose tissue, blood, or breast tissue would provide additional relevant information. There have been no additional epidemiological investigations retrieved. There is therefore no evidence to associate lindane with an increased risk of breast cancer.

Overall conclusion

- 22. The Committee noted the hypothesis that OCl_s might increase the risk of breast cancer by virtue of their claimed oestrogenic effects (**para 2**). The Committee reaffirmed conclusions reached in 1999 namely;
 - a) that the oestrogenic effects (if any) of these xenoestrogens were likely to be small in magnitude, especially compared with those of oral contraceptives or HRT, which entail much higher exposures to oestrogens (**para 7-11**)
 - b) there is no convincing evidence of oestrogenic synergy in mammals between different OCl_s or OCl_s with other xenoestrogens. The possibility of a simple additive effect cannot be excluded but at the likely exposure levels for the OCl_s concerned this will represent a negligible risk of any oestrogenic effect *in-vivo*. (**para 15**).
 - c) concentrations in human fat of the OCl_s considered in this statement are decreasing in humans which provides some additional reassurance with regard to any potential risk of breast cancer (**para 18**).
- 23. The following overall conclusions were reached.

DDT (and isomers and metabolites *p,p'*DDE)

There is evidence that DDT and some of its isomers/metabolites such as *p,p'*DDE have weak xenoestrogenic activity *in vivo*. Concentrations of *p,p'*DDE in human fat have been declining for

decades. There is extensive epidemiological data on the potential for an association between DDT its isomers and metabolites such as p,p'DDE and increased risk of breast cancer. There is no convincing evidence for an association with an increased risk of breast cancer. Overall the available data do not suggest that environmental exposure to DDT (and isomers/metabolites) is a cause for concern as a risk factor for human breast cancer.

Dieldrin

Dieldrin does not have any oestrogenic activity *in vivo*. There is evidence to show that concentrations in human fat are decreasing. There is no convincing epidemiological evidence for an association between dieldrin and increased risk of breast cancer. However the available epidemiological evidence on dieldrin and risk of breast cancer is limited and it is suggested that the relevant literature on dieldrin is kept under review.

β -HCH

β -HCH should be regarded as having weak *in-vivo* oestrogenic activity. There is evidence from investigations undertaken in the UK for a decline in β -HCH concentrations in human fat samples after 1982/3. The available epidemiological studies do not suggest any evidence for an association between β -HCH and increased risk of breast cancer. Overall the available data do not suggest that environmental exposure to β -HCH is a cause for concern as a risk factor for human breast cancer.

Lindane

Lindane (γ -HCH) does not have any *in-vivo* oestrogenic activity. It is not approved for use as a pesticide in the U.K. Exposure is likely to be negligible. The Committee have previously concluded that there is no biological rationale for including lindane in any epidemiology studies on risk of breast cancer. The Committee concluded there is no reason to undertake any further reviews of the association of this chemical with increased risk of breast cancer.

COC/04/S3 August 2004.

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Carcinogenicity of 1,3-dichloropropan-2-ol (1,3-DCP) and 2,3-dichloropropan-1-ol (2,3-DCP)

Introduction

1. 1,3-Dichloropropan-2-ol (1,3-DCP) and 2,3 dichloropropan-1-ol (2,3-DCP) are contaminants of some foodstuffs and of polyamine flocculants used in the treatment of drinking water. Both the COC and COM have previously published statements on the closely related compound 3-chloro-1,2-propanediol (3-MCPD)^{1,2} 1,3-DCP and 2,3-DCP were considered by the COC and COM in 2001^{3,4}. In 2001, the COM recommended that appropriate in-vivo mutagenicity studies should be undertaken with 1,3-DCP and 2,3DCP in accordance with the COM guidelines^(3,5). In 2001, the COC came to the following conclusions;

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

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

2. Both of these compounds have been recently considered by the COM who has updated its advice on the mutagenicity of 1,3-DCP and 2,3-DCP in the light of results from new *in-vivo* mutagenicity studies on these two compounds. An updated COM statement on 1,3-DCP was published in October 2003 and an updated statement on 2,3-DCP was published in June 2004^{6,7}.
3. The available carcinogenicity and other relevant toxicological information on these two compounds including the recent conclusions reached by the COM are considered below. 1,3-DCP and 2,3-DCP have been considered separately in the following sections.

1,3 DCP

4. Available toxicology, mutagenicity and carcinogenicity data for 1,3 DCP has been summarised by the Joint FAO/WHO Committee on Food Additives (JECFA)⁸ although much of the key data remain unpublished. From a 13-week oral toxicity study, a NOAEL of 1mg/kg/day had been identified. Limited information on the metabolism of 1,3 DCP indicates that it may be metabolised to form epichlorohydrin, which may, via glycidol, be conjugated to form mercapturic acid derivatives.⁹ *In-vitro* investigations with hepatocyte cultures indicate also a pathway involving CYP2E1 to dichloroacetone (a directly acting cytotoxic compound) leading to glutathione depletion¹⁰⁻¹⁴.

Mutagenicity

Updated Advice from COM 2003

5. The metabolism of 1,3-DCP was likely to produce a reactive epoxide intermediate that could damage DNA. Members were aware that 1,3-DCP had been found to be mutagenic to *Salmonella typhimurium* strains TA1535 and or TA 100¹⁵⁻²². Studies with mammalian cells have produced increased frequencies of sister chromatid exchanges and chromosome aberrations^{23,24}. A positive result has been obtained in a mouse lymphoma assay^{25,26}. 1,3-DCP was negative in the wing spot test in *Drosophila melanogaster* (a somatic mutation and recombination test)²⁷.
6. The Committee considered two new *in-vivo* genotoxicity studies at its May 2003 meeting^{28,29}. These comprised a rat bone-marrow micronucleus test²⁸ and a rat liver unscheduled DNA synthesis (UDS) assay²⁹, both of which are widely used to assess genotoxicity *in vivo*.
7. The Committee concluded that both the rat bone-marrow micronucleus test and the rat liver UDS test had been carried out to an acceptable standard and were negative. Thus the additional information recommended by the COM as being necessary to provide adequate reassurance that the mutagenic activity seen *in vitro* was not expressed *in vivo* had now been provided. The COM noted the uncertainties with regard to routes of metabolic activation of 1,3-DCP and agreed that the two new mutagenicity studies supported the view that reactive metabolites, if formed, did not produce genotoxicity *in vivo* in the tissues assessed. The COM concluded that 1,3-DCP can be regarded as having no significant genotoxic potential *in vivo*.

Carcinogenicity

Advice from COC 2001.

8. A 104-week toxicology and carcinogenicity study with 1,3 DCP in Wistar rats was previously considered by COC in 1991³⁰. At the time COC concluded that 1,3 DCP was genotoxic and carcinogenic, although a formal committee statement was not issued. Additional information on the study was presented to the COC in 2001. The COC concluded that the spectrum of tumours observed in the 104-week rat study (which are reproduced below for ease of access), particularly in the liver and tongue was evidence of a clear carcinogenic effect of 1,3 DCP. It was possible that the tumours in the male kidney could be associated with the high rate of chronic progressive nephropathy observed in the study and additionally, the thyroid follicular cell tumours could be associated with hyperplasia, a toxic finding commonly seen in male rats, although no specific mechanism data were available.

9. A brief summary of the evidence for carcinogenicity of 1,3-DCP in the rat carcinogenicity study provided to COC in 2001 is given below.
- In the *liver*, combined incidences of hepatocellular adenoma and carcinoma, showed a statistically significant dose-related increase ($p < 0.001$) in both males and females. (eg males – controls 1/50, high dose 8/50; females – controls 1/50, high dose 41/50).
 - In the *tongue*, combined incidences of squamous cell papilloma and carcinoma showed a statistically significant dose-related increase ($p < 0.001$) in both males and females. (eg males – controls 0/50, high dose 12/50; females – controls 0/49, high dose 11/49).
 - In the *thyroid* combined incidences of follicular cell adenoma and carcinoma showed a statistically significant dose-related increase ($p < 0.001$) in both males and females (eg males – controls 0/50, high dose 4/50; females – controls 1/49, high dose 5/49).
 - In the *kidney*, combined incidences of renal tubular adenoma and carcinoma, showed a statistically significant dose-related increase ($p < 0.001$) in males only (eg controls 0/50, high dose 9/50).

Updated Advice from COC 2004

10. The COC reaffirmed its previous opinion that 1,3-DCP induced tumours of the kidney and thyroid could have been secondary to sustained cell proliferation. Members also agreed that there was evidence of a hepatotoxic effect at doses below those producing a significant increase in combined hepatocellular adenoma and carcinoma. The Committee agreed that the evidence of hepatotoxicity, together with negative results from the *in-vivo* rat liver UDS assay, provided evidence of non-genotoxic mode of action in the liver.

Further consideration of tumours of the tongue (November 2003 and April 2004 meetings)

11. The Committee then considered possible modes of action of 1,3-DCP in inducing tumours of the tongue. The Committee considered that the finding of 2% papillary carcinoma in the mid-dose female group might not be treatment related but the incidence of tongue papilloma (14.3%) and carcinoma (8.2%) in high dose females was clearly treatment related. In male rats the incidence of tongue tumours in the high dose group was 12% (for both papilloma and carcinoma). There were no tongue tumours in males at the low and mid dose groups. The high dose level clearly exceeded the Maximum Tolerated Dose level in that there was an increase in treatment related mortality and hepatotoxicity. The Committee agreed that 1,3-DCP was an irritant and had produced irritant effects in gastric mucosa of treated rats, but there were no suitable data on the potential for 1,3-DCP irritation of the tongue. Members noted that at the time of conduct of the bioassay (1986) it was not routine to examine the tongue histologically. It was agreed however, that since the compound had been given in the drinking water in the bioassay, chronic irritation was a plausible hypothesis for the induction of the tumours in

the tongue. Members discussed the suggestion that bacteria metabolised 1,3-DCP to the genotoxic carcinogen epichlorohydrin but agreed there was no specific evidence to support this proposal.

12. The COC discussed future research to investigate whether the tumours of the tongue occurred via a genotoxic mechanism and agreed that information on contact-irritancy, cell proliferation and formation of DNA adducts in tongue tissue using ³²P-postlabelling in animals treated with suitably high doses of 1,3-DCP was needed.
13. The COC concluded that until such data were available, it was not possible to exclude the possibility of a genotoxic mechanism for the tumours of rat tongue seen in a long-term drinking water study with 1,3-DCP.

2,3-DCP

14. There are very little data on the absorption, distribution, and excretion of 2,3-DCP. Theoretically, 2,3-DCP could be metabolised to produce epichlorohydrin (and subsequently glycidol) and therefore there were structural alerts for genotoxicity and carcinogenicity. One research group had provided some *in vitro* data to suggest that induction of CYP2E1 resulted in 2,3-DCP mediated hepatotoxicity and glutathione depletion³¹. The findings of Koga et al³¹ suggest dechlorination/ hydroxylation of 2,3-DCP may occur but the evidence for epoxide formation was not conclusive. There are insufficient data to draw conclusions on the metabolic activation of 2,3-DCP but overall the evidence suggested metabolic activation of 2,3-DCP differs from 1,3-DCP. The COM considered these data and agreed that the metabolism of 2,3-DCP had not been fully elucidated. Metabolic activation *in vivo* to active metabolites had been postulated but had not been proven.

Mutagenicity

15. The COM concluded in 2001 that 2,3-DCP was mutagenic *in vitro* in *Salmonella typhimurium* strains TA 100 and TA 1535 in a study with and without metabolic activation¹⁸ and mutagenic in another Ames test¹⁹. Positive results were also obtained for sister chromatid exchange with Chinese Hamster V79 cells both with and without metabolic activation²³.

Updated advice from COM 2004

16. The COM considered two new *in-vivo* genotoxicity studies at its February 2004 meeting. These comprised a rat bone-marrow micronucleus test³² and a rat liver unscheduled DNA synthesis (UDS) assay³³, both of which are widely used to assess genotoxicity *in vivo*.
17. The Committee concluded that both the rat bone-marrow micronucleus test and the rat liver UDS test had been carried out to an acceptable standard and were negative. Thus the additional information recommended by the COM as being necessary to provide adequate reassurance that the mutagenic activity seen *in vitro* was not expressed *in vivo* had now been provided. The Committee noted the

uncertainties with regard to routes of metabolic activation of 2,3-DCP and agreed that the two new mutagenicity studies supported the view that reactive metabolites, if formed, did not produce genotoxicity *in vivo* in the tissues assessed. The Committee concluded that 2,3-DCP can be regarded as having no significant genotoxic potential *in vivo*.

Carcinogenicity

18. Further advice on the carcinogenicity of 2,3-DCP was sought from the COC at the June 2004 meeting in the light of the updated advice on mutagenicity from COM.
19. Although there are no carcinogenicity studies available for 2,3DCP, the World Health Organisation's International Agency for Research on Cancer (IARC) evaluated the brominated analogue, 2,3 dibromopropanol (2,3 DBP) and considered that "there is sufficient evidence in experimental animals for the carcinogenicity of 2,3 dibromopropan-1-ol". In addition skin application of 2,3 DBP produced multisite tumours in both rats and mice.³⁴ However, the Committee considered this information in 2001 and agreed that no conclusions could be drawn from the studies on 2,3 dibromo propan-1-ol in respect of the carcinogenicity of 2,3 DCP.
20. Thus no conclusions regarding the carcinogenicity of 2,3-DCP can be reached on the available information on this compound.

Conclusions

21. The Committee concluded

1,3-DCP: The COC concurs with its previous advice that 1,3-DCP should be regarded as a genotoxic carcinogen. It is not possible to exclude a genotoxic mechanism for the induction of the tumours of rat tongue seen in a long-term drinking water study with 1,3-DCP. The Committee recommended that further investigations regarding the mechanism of 1,3-DCP carcinogenicity in the rat tongue should include information on contact-irritancy, cell proliferation and formation of adducts in tongue tissue using ³²P-postlabelling in animals treated with suitably high doses of 1,3-DCP.

2,3-DCP: The available evidence is consistent with the conclusion that 2,3-DCP does not possess genotoxic activity *in-vivo*. There are no appropriate carcinogenicity bioassays of 2,3-DCP available. No conclusions regarding carcinogenicity of 2,3-DCP can be reached.

COC/04/S2 June 2004

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Olfactory neuroblastoma: evidence for an elevated incidence among dentists and dental nurses?

Introduction

1. Olfactory neuroblastoma (ONB. The alternative name is esthesioneuroepithelioma) is estimated to comprise approximately 3% of nasal neoplasms excluding benign polyps. The incidence in N. America/Western Europe is estimated to be approximately 0.15/million/year. There is no evidence for a sex difference in incidence. It occurs in all ages (but is rare below 10 y and over 70 y)¹. It has been reported to have bimodal incidence, with peaks in the 2nd -3rd decade and later in the 6th and 7th decades of life². It has also been estimated there have only been 950 cases cited in the scientific literature from 1924, when ONB was first cited in the literature, up to 1997³. Thus the available evidence suggests that ONB is a very rare tumour.
2. ONB is described as a neuroectodermal neoplasm showing predominantly neural features⁴. The most common symptoms in patients presenting with ONB are nasal obstruction (93%), epistaxis (55%) and rhinorrhea (30%). Other symptoms such as headache and anosmia occur at an incidence of below 10%². Diagnoses is based on clinical presentation, CT/MRI* screening and histology with the need for a battery of immunohistochemical stains to differentiate from other closely related head and neck cancers^{5,6,7}.

Published information: Association of ONB with occupation or chemical exposure

3. There is no published evidence to associate ONB with any particular occupation or chemical exposure. The only published case-report of ONB where an occupational exposure aetiology has been suggested refers to a woodworker exposed to wood dust for 25 years⁸. The Committee was aware that adenocarcinoma of the nasal cavities and paranasal sinuses is clearly associated with exposure to hard wood dust. The published evidence on wood dust had been thoroughly reviewed by the World Health Organisation's International Agency for Research on Cancer (IARC) in 1995 and no evidence for an association between exposure to wood dust (hard or softwoods) and ONB had been documented⁹. The Committee agreed that it was highly improbable that the researchers investigating wood workers would have misdiagnosed ONB as adenocarcinoma of the sinuses.

Association of ONB with dentists/dental nurses

Presentation from Professor Valerie Lund (Institute of Laryngology and Otolaryngology)

4. Professor Lund presented details of four individuals with ONB, two of whom had worked as dentists, and two who had been employed as dental nurses. Members heard that two pathologists had independently verified the diagnoses. Full details of these case reports have been submitted to a peer reviewed journal.

* Computerised Topography/Magnetic Resonance Imaging

COC discussion

5. Members reviewed the available information and considered the data on the case-series held by the Institute of Laryngology and Otolaryngology in the context of information identifiable through the Office for National Statistics (England and Wales) for the past 10 years. No dentists or dental nurses had been identified in the limited review of ONS data. Members acknowledged that details of occupation were underreported to ONS. The Committee felt that there was no evidence of referral bias of dentists/dental nurses to the Institute. The Committee agreed that the finding of 4 dentists/dental nurses with ONB out of a series of 52 cases of ONB referred to the Institute over a period of 23 years was likely to be a statistically significant association.
6. The Committee considered available information on potential chemical exposures of dentists/dental nurses (e.g. to metallic mercury, oil of cloves (principle ingredient eugenol) and methymethacrylate) (a copy of the covering paper can be found on <http://www.advisorybodies.doh.gov.uk/pdfs/cc0337.pdf>). It was agreed that there was no evidence to associate exposure to these chemicals with ONB in dentists/dental workers.
7. The Committee noted a report of cytogenetic damage in nasal tissue from dental technicians.¹⁰ It was agreed that dental technicians were a separate and distinct group from dentists/dental nurses with regard to chemical exposures. Thus data from dental technicians was not helpful in identifying relevant chemical exposures of dentists/dental nurses.
8. The Committee considered that the first priority for further work would be to consider additional epidemiological investigations to confirm the finding reported by the Institute of Laryngology and Otolaryngology. This might include evaluation of case-reports of ONB from other countries or detailed evaluation of information held by centres of excellence (for head and neck tumours) and pathology departments from the U.K., Europe and elsewhere.

COC Conclusion

9. The Committee concluded that the finding of 4 dentists/dental nurses with ONB by the Institute of Laryngology and otolaryngology was likely to be a statistically significant association. Additional epidemiological data are needed to substantiate this observation. No definite conclusions on the potential association between dentists/dental nurses and olfactory neuroblastoma can be reached at this point in time.

COC/04/S1 April 2004

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Prostate cancer

Introduction

1. Prostate cancer is the most common cancer in men in the UK, with over 24,700 new cases a year (2000 data). Prostate cancer is the second largest cause of death from cancer in the U.K. There were 9,900 deaths reported in 2002 accounting for around 13% of cancer deaths in men. Around 70% of these deaths are in men aged over 70 years. The mortality rate for prostate cancer peaked in the early 1990s and has now fallen to 25 per 100,000 population at risk. The lifetime risk for being diagnosed with prostate cancer is 1 in 14. The cancer develops from cells within the prostate gland. The majority of prostate cancers are slow growing and many men are unaware that they have this cancer. However, a small number of prostate cancers grow more quickly and may spread to other parts of the body. Cancer Research UK reported a 57% increase in prostate cancer incidence in Great Britain between 1991 and 2000¹⁻³.
2. The Committee was asked to review the available epidemiological and other research to identify if there were any potential chemical exposures which might be associated with prostate cancer.
3. The Committee initially considered the evidence for an increasing incidence of prostate cancer in order to elucidate if there were diagnostic and registration changes which might be important in interpreting the documented increased incidence of prostate cancer. The second phase of the review concerned an evaluation of the epidemiological and other data regarding occupational groups (such as farmers/farm workers) and chemical exposures which have been proposed as being associated with prostate cancer.
4. The Committee considered an overview drafted by the DH Toxicology Unit (<http://www.advisorybodies.doh.gov.uk/pdfs/annex1cc0335.pdf>) and evaluated additional commissioned papers published studies. The DH Toxicology Unit overview and additional commissioned papers list the full set of references considered during the COC discussions. The Committee considered the evidence regarding chemical causation of prostate cancer at its November 2003 and April 2004 meetings. The additional discussion papers on prostate cancer from the DH Toxicology Unit on the evidence regarding a possible association between PAH exposure (<http://www.advisorybodies.doh.gov.uk/pdfs/Cc0412.pdf>) and occupation in the rubber industry (<http://www.advisorybodies.doh.gov.uk/pdfs/Cc0413.pdf>) were reviewed at the June 2004 meeting.

Trends in Prostate cancer in U.K.

5. Age-adjusted rates for prostate cancer increased in all age groups in England and Wales during the 1970s and 1980s. A marked increase occurred after this, rates peaked in 1994 and subsequently decreased in some, but not all, age groups⁴. The Committee agreed that increased use of trans-urethral resection of the prostate (TURP) for benign prostatic hyperplasia accounted in part for increased reporting of prostate cancer during the 1970-80s. The adoption of Prostate-Specific Antigen (PSA) testing during the 1990s partly explained the subsequent increased reporting of prostate cancer. Similar patterns were observed in Scotland, where the increases in incidence were closely correlated with rates of TURP (up to 1988) and, subsequently, PSA testing (1989-1996)⁵.
6. The Committee reviewed a Small Area Health Statistics Unit (SAHSU) study on geographical variation in the incidence of prostate cancer in the U.K. at the June 2002 meeting⁶. The Committee agreed that the study showed no evidence for significant geographical variation in prostate cancer in the U.K. This would suggest there were no major environmental factors affecting incidence of prostate cancer in the U.K.
7. The Committee agreed that the impact of screening for prostate cancer using Prostate Specific Antigen (PSA) on recording of incidence and to a lesser degree mortality data severely complicated the interpretation of epidemiological studies and time trends in incidence in particular.

Risk factors associated with prostate cancer

8. There are a number of suspected risk factors for prostate cancer. The most important two are age and family history. Clinical prostate cancer is rare in men under 40 years of age⁷. The risk of prostate cancer increases by 2-3 fold in men who have a first-degree relative with the disease⁸. It is estimated that high penetrance familial prostate cancer accounts for 5-10% of all cases and a higher proportion of cases identified before the age of 55 y^{9,10}.
9. A number of other risk factors have been suggested including vasectomy^{12,16}, sexual activity^{12,16}, viral exposure¹⁷ and physical activity¹⁸. However, the evidence for these factors is uncertain and no conclusions can be drawn. There is some limited evidence that smoking is associated with prostate cancer^{11,12,16} but no convincing evidence regarding alcohol consumption¹⁹.
10. The evidence for an association between diet and risk of prostate cancer was reviewed fully by the Working Group on Diet and Cancer of the Committee on Medical Aspects of Food and Nutrition Policy in 1998¹⁵. The Working Group concluded that the limited data were weakly consistent with the hypothesis that higher total fat intakes are associated with higher risks of prostate cancer. The Working Group also concluded that the limited evidence was moderately consistent that higher vegetable consumption, especially raw and salad vegetables, is associated with a lower risk of prostate cancer. The evidence for an association between consumption of fruit and risk of prostate cancer was

inconsistent. There were insufficient data on intakes of soya products to reach a conclusion on the association of soya products with risk of prostate cancer. Advice on nutritional factors is the responsibility of the Scientific Advisory Committee on Nutrition (<http://www.sacn.gov.uk/>).

11. Other potential risk factors include ethnicity^{11,12}, a number of genetic polymorphisms (for example genes involved in androgen metabolism and signalling pathways)¹², and endocrine factors (such as low testosterone levels, and elevated IGF-1 levels)^{11,13,14}. These observations have led to the suggestion that androgenicity may influence prostate cancer risk.

Chemical exposures associated with prostate cancer

Cadmium

12. Occupational exposure to cadmium was associated with an increased incidence of lung cancer and was considered by the World Health Organisation's International Agency for Research on Cancer (IARC) to be carcinogenic to humans (i.e. Group 1)²⁰. Chronic dietary administration of 50 ppm cadmium in the diet to rats has been reported to be associated with proliferative responses of the prostate (e.g. hyperplasia and adenoma)²¹. The finding of increased incidence of prostate cancer in rats given a single subcutaneous injection of cadmium²² was considered by members to be dependent on functioning of the testes and androgen production. Subcutaneous administration of higher cadmium doses, which induced testicular toxicity and thus reduced androgen production, resulted in no evidence for prostate cancer. It was noted that prostate cancer had been induced following direct injection of cadmium into the prostate of rats but members considered that this route of administration was of limited relevance²³.
13. The Committee considered one published paper²⁴ which reported evidence that low concentrations of cadmium chloride could interact with androgen receptor *in vitro* and could also produce an androgenic response (e.g. increased prostate weight) *in vivo* in rats given relatively low intraperitoneal doses. This suggests a plausible mechanism by which cadmium might be associated with prostate tumours in rats.
14. However the evidence from occupational studies regarding prostate cancer showed no association in the majority of studies including relatively large cohort studies. Thus overall there was no evidence to associate occupational exposure to cadmium with cancer of the prostate. The Committee was aware of a relatively old study of residual exposure which reported 40 years of mortality follow-up of the residents of Shipham, Somerset, England where there were high soil-levels of cadmium. No evidence for an excess mortality from prostate cancer was found, though this was only based on 2 cases²⁵. The possibility that cadmium might induce androgen imbalance and thus might potentially be associated with prostate cancer should be monitored any relevant information considered in the future.

Pesticides and endocrine disrupting chemicals.

15. There is evidence from a variety of *in-vitro* studies that a number of pesticides (such as certain organochlorine insecticides (o,p'DDT, p,p'DDT, β -hexachlorocyclohexane (β -HCH) and the fungicide chlorothalonil) can produce androgenic effects^{26,27}. However it has been noted that some of these pesticides can also induce anti-androgenic effects (e.g. vinclozolin)²⁷.
16. The Committee agreed that any potential androgenic effect *in vivo* following environmental exposure to pesticides and other endocrine disrupting chemicals was likely to be minimal. The Committee noted that there was some epidemiological evidence suggesting a weak association between herbicide exposure and prostate cancer²⁷⁻²⁹. The Committee considered the evidence for an association between farmers, farm workers and pesticide applicators and increased risk of prostate cancer. These studies are considered below in paragraphs 24-30.
17. The Committee was aware that the development of appropriate biomarker approaches to the determination of exposure might aid in the evaluation of exposure to pesticides. There was however, comparatively limited information available where biomonitoring data had been used to evaluate exposure in epidemiological studies of prostate cancer. The only retrieved publication was in abstract form³⁰.

Genotoxic chemicals.

18. A number of genotoxic chemicals including the polycyclic aromatic hydrocarbon benzo(a)pyrene have been found to induce prostate tumours in experimental animals (<http://www.advisorybodies.doh.gov.uk/pdfs/cc0335.pdf>). Co-administration of testosterone enhanced the tumorigenicity in experimental animals. Epidemiology studies investigating occupational exposure to polycyclic aromatic hydrocarbons have reported an association with prostate cancer in a number of studies^{31,32}.
19. A further discussion paper which presented a detailed review of the epidemiology studies of the potential association between occupational exposure to PAHs and increased risk of prostate cancer was provided by the DH Toxicology Unit. (<http://www.advisorybodies.doh.gov.uk/pdfs/Cc0412.pdf>)
20. The overall conclusion reached in the additional review prepared by the DH Toxicology Unit was that there are several occupational groups where relatively high exposures to PAHs could be anticipated, e.g. truck drivers, foundry workers, chimney sweeps and to a lesser extent fire-fighters. There have been a number of epidemiological investigations of the potential association between exposure to PAHs in these occupational groups and risk of prostate cancer. This has included several cohort studies in coke oven workers, fire-fighters and chimney sweeps. The adequacy of exposure data documented is very limited in most of the reports reviewed in the DH Toxicology Unit paper. Where exposure data

or information on the duration of occupational exposure were available, there was evidence from some studies of both greater exposure to PAHs and slightly higher risks of PC, but there was no compelling evidence for an increased risk of prostate cancer in any of the PAH exposed occupational groups studied.

21. The Committee agreed that overall the available studies do not provide evidence that is convincing for an association between occupations with exposure to PAHs and an increased risk of prostate cancer.

Vitamin supplements (zinc)

22. The Committee was asked to comment on the recent paper by Leitzmann M et al which investigated approximately 47,000 individuals as part of the U.S.A. based Health Professionals Follow-up study³³. Members considered that the study had been adequately undertaken by a well respected group using the Health Professionals cohort established in the U.S.A. in 1986. There was a statistically significant increase in relative risk for advanced prostate cancer in men consuming ≥ 100 mg/day supplemental zinc (RR = 2.29, 95% Confidence Interval (CI) 1.06-4.95). There was limited evidence for a dose-response relationship which was not statistically significant. There was a statistically significant association with taking supplemental zinc for ≥ 10 years (RR = 2.37, 95% CI 1.42-3.95). Members were uncertain as to whether individuals who consumed high amounts of supplemental zinc would be more likely to seek PSA testing. However it was noted that the authors had considered routine screening for PSA up to the year 2000 in their evaluation. Members felt that the arguments presented by Leitzmann and colleagues regarding the role of intracellular concentrations of zinc in prostate tissue were inconsistent with the existing information on zinc and it was not possible to derive a biologically plausible hypothesis from the information reported. It was noted that a number of comments regarding the approach to statistical analysis of the data, the small number of cancer patients who reported intakes of zinc over 100 mg/day and the limited evidence to support a biologically plausible hypothesis concerning dietary supplements containing zinc and increased risk of prostate cancer had been raised in published correspondence commenting on the paper by Leitzmann M et al³⁴⁻³⁷.
23. The Committee considered that the study results could not be dismissed. However, members heard that dietary supplements available in the U.K. each contained up to 50 mg of zinc. The Expert Group on Vitamins and Minerals had recently established a Safe Upper Level for dietary supplementation of 25 mg zinc/day based on evidence that consumption of 50 mg/day might reduce the absorption of copper across the gastrointestinal tract (http://www.foodstandards.gov.uk/multimedia/pdfs/evm_zinc.pdf)³⁸. Information from the EPIC-Norfolk cohort (for first 1860 individuals entering the cohort in 1993/4) reported that 5% of subjects were consuming zinc supplements (the intake from supplements was 4.9 (ffl 4.1 mg/d). Thus the number of individuals consuming more than 50 mg zinc/day from supplements was likely to be very small. The Committee agreed that it was not possible to identify sufficient numbers of individuals for study from the EPIC cohort, and that therefore that it was not feasible to undertake any further epidemiological investigation of dietary zinc intake and prostate cancer in the UK.

Occupations associated with prostate cancer.

Farmers, farm workers and pesticide applicators

24. A number of studies have indicated a small excess of prostate cancer PC amongst farmers and farm-related workers and pesticide applicators, although other studies have failed to confirm this observation. Several reviews and meta-analyses of the epidemiological literature have been published. These generally described a slight excess of prostate cancer³⁹⁻⁴⁷.
25. The COC considered a systematic review on occupational related pesticide exposure and cancer by Van Maele-Fabry and Willems⁴⁷ in detail. This meta-analysis produced an overall relative risk of 1.13 (95% C.I. 1.04-1.22) for prostate cancer in workers exposed to pesticides in pesticide related occupations (from 11 cohort, 4 Proportional Mortality Ratio, and 7 case control studies). Members noted that for all studies (excluding proportional mortality ratio Studies) the relative risk was 1.09 (95% C. I. 1.00 – 1.19) and that the risk estimates were for all farming occupations and not just for pesticide applicators. North American studies tended to show higher prostate cancer risk than European studies. The Committee considered that pesticide exposure was likely to be lower in Europe. The separate risk ratio for pesticide applicators (Relative risk 1.64 95% C.I. 1.23 – 2.38) was greater than the overall risk ratio for all studies.
26. The Committee also considered the large retrospective cohort study reported by Morrison et al²⁹ in detail. This study comprised male farmer aged 45 or more at 1971 identified through the Canadian National Mortality Database during the period of June 1971 up to the end of 1987. A total of 1,148 prostate cancer deaths and over two million person years were observed. The analyses reported were based on a one third sample from this cohort who had completed the more extensive census questionnaire thus allowing for better classification of exposure. A relative risk of 2.23 (95% C.I. 1.30-3.84) for prostate cancer was reported for farmers (aged 45-69 y) who sprayed herbicides on to 250 or more acres ($p < 0.01$ test for trend). The Committee noted this analysis was based on younger farmers who were more likely to have applied the herbicides themselves. A subsequent analysis using data for mortality from 1981-1987 reported no evidence for a dose-related effect of herbicide use on prostate cancer²⁹. It was possible that changes in herbicides used by Canadian farmers may account for this finding.
27. The Committee agreed that these two studies provided some evidence for increased prostate cancer risk for farm workers most exposed to pesticides and with some evidence suggesting an association between increased risk of prostate cancer and exposure to pesticides and in particular herbicides.
28. The COC also noted two separate cohort studies of cancer incidence from the U.S.A. (a retrospective analysis of licensed pesticide applicators in Florida and a prospective analysis of pesticide applicators from Iowa/North Carolina)^{48,49}. The Florida study showed no association between prostate cancer and year of first licence (a proxy measure for duration of exposure). The Iowa/North Carolina study found no evidence for a trend with increasing exposure to herbicides, fumigants and fungicides but did report

a trend regarding organochlorine pesticides and older age. No trend was documented for individual organochlorine pesticides in this study. Overall these two studies provided some limited evidence for an association between pesticide exposure and prostate cancer but provided no convincing evidence regarding any specific pesticide exposures.

29. The Committee was aware of a review of prostate cancer undertaken by the Health and Safety Executive⁵⁰ published in 1998 which had concluded that the potential association between farmers/farm workers and prostate cancer merited further monitoring of the literature.
30. Overall the Committee agreed there was some evidence to suggest an association between farmers/farm workers, exposure to pesticides and increased risk of prostate cancer. The possibility of such an association could not be discounted and the published literature should continue to be monitored for further studies. Members commented on the need for improved measures of exposure to pesticides and in particular herbicides. It was considered that the potential association between herbicide use by farmers and farm workers should be kept under review.

Rubber workers

31. The further review undertaken by the DH Toxicology Unit at the request of the COC identified epidemiological studies undertaken at rubber manufacturing plants in the U.S.A., Europe and the U.K. as well as two studies where the location of the rubber manufacturing plant was not reported. (<http://www.advisorybodies.doh.gov.uk/pdfs/Cc0413.pdf>)
32. Overall there was no convincing evidence to associate employment in the rubber industry with prostate cancer. A number of the studies investigated the association between employment in particular jobs and prostate cancer. The suggestion for such an association came from studies in rubber manufacturing plants in the U.S.A. where the task of compounding and mixing was highlighted, but no definite conclusions could be drawn. On the basis of the limited available studies, there was no convincing evidence to associate prostate cancer with any particular chemical exposure at rubber plants.
33. The Committee concluded that the information from the available epidemiological studies are consistent with the view that overall, there is no evidence convincing of an increased risk of prostate cancer in rubber workers as a whole.

Other occupations

34. The Committee agreed that the evidence regarding other occupations and prostate cancer did not suggest any hypotheses that required further investigation.

COC conclusions

35. The Committee agreed the following overall conclusions;

- i) The increase in incidence of prostate cancer reported over the past 2-3 decades is largely accounted for by improved identification of cases due to increased numbers of individuals undergoing surgery for benign prostatic conditions and the use of Prostate Specific Antigen Screening.
- ii) The Committee concluded that there was some limited evidence to suggest an association between farmers/farm workers, exposure to pesticides and increased risk of prostate cancer. The possibility of such an association being causal could not be discounted and the published literature should continue to be monitored for further studies. Members commented on the need for improved measures of exposure to pesticides and in particular herbicides. It was considered that the potential association between herbicide use by farmers and farm workers should be kept under review.
- iii) The information from the available epidemiological studies are consistent with the view that overall, there is no convincing evidence of an increased risk of prostate cancer in rubber workers as a whole.
- iv) There is no convincing evidence to associate other occupations with prostate cancer.
- v) There is no convincing evidence to associate occupational exposure to cadmium with cancer of the prostate. The possibility that cadmium might induce androgen imbalance and thus might potentially be associated with prostate cancer should be monitored and relevant new information considered in the future.
- vi) The one available epidemiological study on dietary zinc supplementation and risk of prostate cancer dose found increased risk of prostate cancer at high levels of supplementation (>100 mg/day). Further epidemiology studies are unlikely to provide sufficient numbers of individuals regularly consuming high doses of supplements for a study to be undertaken in the U.K. The Committee agreed that it could not identify a biologically plausible rationale as to why zinc should be associated with prostate cancer.

COC/04/S6 December 2004.

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2004 membership of the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

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Dr D Benford BSc PhD Scientific Secretary – Food Standards Agency

Mr K Mistry Administrative Secretary

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

Ms F Pollitt MA Dip RCPATH

Declaration of interests during the period of this report

Member	Personal Interest		Non-personal Interest	
	Company	Interest	Company	Interest
Professor P G Blain (Chairman)	NONE	NONE	NONE	NONE
Dr C Allen	NONE	NONE	NONE	NONE
Professor A Boobis	Abbey National	Shareholder	Servier	Research support
	Barclays Bank	Shareholder		
	BG Group	Shareholder		
	BT Group	Shareholder		
	Centrica	Shareholder		
	Marks and Spencers	Shareholder		
	National Grid Transco	Shareholder		
	Scottish Power	Shareholder		
Dr P Carthew	Provalis	Share Holder	NONE	NONE
	Unilever	Salary		
Professor P B Farmer	Abbey National	Shareholder	American Chemistry Council CEFIC	Research Support
	Bradford & Bingley	Shareholder		
	Celltech	Shareholder		
	Foreign & Colonial	Shareholder		
	Friends Provident	Shareholder		
	Health Effects Institute	Research Committee Member		
	Torotrak	Shareholder		
Professor D Forman	Barclays	Shareholder		
	Friends Provident	Shareholder		
	HBOS	Shareholder		
	Woolwich	Shareholder		
Mrs R Glazebrook	Dr Foster Ltd	Salary	NONE	NONE
	BT Group	Shareholder		
	Lloyds TSB	Shareholder		
	National Grid	Shareholder		

Member	Personal Interest		Non-personal Interest	
	Company	Interest	Company	Interest
Professor D Harrison	Medical Solutions Quintiles Scottish Medicine	Shareholder Consultant Consultant	NONE	NONE
Ms D Howel	NONE	NONE	NONE	NONE
Dr S J Kennedy	Unilever	Shareholder	NONE	NONE
Professor D Phillips	Aviva Banco Santander BG Group Bradford & Bingley Centrica Lattice Group National Grid Takeda	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder	NONE	NONE
Dr R Roberts	AstraZeneca P & O	Salary Shareholder Shareholder	NONE	NONE
Professor D Shuker	NONE	NONE	NONE	NONE
Dr N Wallis	Pfizer	Salary Shareholder	NONE	NONE

ANNEX 1

TERMS OF REFERENCE

To advise at the request of:

Department of Health

Food Standards Agency

Department for the Environment, Food and Rural Affairs

Department of Transport, Local Government and the Regions

Department of Trade and Industry

Health and Safety Executive

Pesticide Safety Directorate

Veterinary Medicines Directorate

Medicines and Healthcare products Regulatory Agency

Home Office

Scottish Executive

National Assembly for Wales

Northern Ireland Executive

Other Government Departments and Agencies

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

ANNEX 2

CODE OF CONDUCT FOR MEMBERS OF ADVISORY COMMITTEES

Public service values

Members must at all times:

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

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

Standards in Public Life

All Committee members must:

- follow the Seven Principles of Public Life set out by the Committee on Standards in Public Life (see page 148);
- comply with this Code, and ensure they understand their duties, rights and responsibilities, and that they are familiar with the function and role of this Committee and any relevant statements of Government policy. If necessary members should consider undertaking relevant training to assist them in carrying out their role;
- not misuse information gained in the course of their public service for personal gain or for political purpose, nor seek to use the opportunity of public service to promote their private interests or those of connected persons, firms, businesses or other organisations; and
- not hold any paid or high profile unpaid posts in a political party, and not engage in specific political activities on matters directly affecting the work of this Committee. When engaging in other political activities, Committee members should be conscious of their public role and exercise proper discretion. These restrictions do not apply to MPs (in those cases where MPs are eligible to be appointed), to local councillors, or to Peers in relation to their conduct in the House of Lords.

Role of Committee members

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

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

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

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

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

The role of the Chairman

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

- ensuring that the Committee meets at appropriate intervals, and that the minutes of meetings and any reports to the FSA Board accurately record the decisions taken and, where appropriate, the views of individual members;

- representing the views of the Committee to the general public; and
- ensuring that new members are briefed on appointment (and their training needs considered), and providing an assessment of their performance, on request, when members are considered for re-appointment to the Committee or for appointment to the board of some other public body.

Handling conflicts of interests

The purpose of these provisions is to avoid any danger of Committee members being influenced, or appearing to be influenced, by their private interests in the exercise of their public duties. All members should declare any personal or business interest which may, or may be *perceived* (by a reasonable member of the public) to, influence their judgement. A guide to the types of interest that should be declared is at pages 276 to 277.

(i) Declaration of Interests to the Secretariat

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

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

(ii) Declaration of Interest and Participation at Meetings

Members of the Committee are required to declare any direct interests relating to salaried employment or consultancies, or those of close family members¹, 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.

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

Personal liability of Committee members

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

THE SEVEN PRINCIPLES OF PUBLIC LIFE

Selflessness

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

Integrity

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

Objectivity

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

Accountability

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

Openness

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

Honesty

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

Leadership

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

DIFFERENT TYPES OF INTEREST

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

Personal Interests

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

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

Non-Personal Interests

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

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

- iii) the commissioning of research or other work by, or advice from, staff who work in a unit for which the member is responsible.

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

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

DEFINITIONS

In this Code, 'the industry' means:

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

The Food Safety Act 1990

The Medicines Acts 1968 and 1971

The Food and Environmental Protection Act 1985

The Consumer Protection Act 1987

The Cosmetic (Safety) (Amendment) Regulations 1987

The Notification of New Substances Regulations 1982

- Trade associations representing companies involved with such products;
- Companies, partnerships or individuals who are directly concerned with research, development or marketing of a product which is being considered by the Committees on Toxicity, 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 Chief Medical Officer 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/COC/COM more open and to increase accountability. Proposals were published in "Quangos-Opening the Doors" (Cabinet Office, July 1998). As a result of these proposals, in 1999, the Committees adopted new principles of working in order to increase openness. relating to among other things, the handling of confidential data, the publication of meeting agendas, finalised minutes and agreed conclusions and statements. (These principles have been reproduced as an Annex to the Committees' Annual Reports since 1999).
3. In the following years, the Committees considered a number of options for greater openness of committee business. Relevant guidelines are contained in the Office of Science and Technology Code of Practice for Scientific Advisory Committees, the Government response to the report of the BSE enquiry (the Phillips Enquiry) and the Food Standards Agency Review of Scientific Committees which included a number of recommendation on openness.
4. As a result of these discussions the principles have been reviewed and revised to reflect the new procedures on openness as agreed by the Committees. The table below contains a summary of the agreed proposals for committee openness:

Issue	Comment
Meetings	All meetings held in open session subject to arrangements for handling confidential or sensitive information.
Agenda	Made publicly available via internet site prior to meeting.
Papers	Made publicly available via internet site prior to meeting. Papers may be edited to remove confidential information.
Minutes	Anonymised minutes to be made publicly available following the committee meeting. Subject to the temporary withholding of items subject to regulatory decisions or confidentiality. Minutes are available in draft prior to agreement by the Committee.
Conclusions/statements	Made publicly available as working papers prior to meetings and as finalised documents via internet site. Subject to temporary withholding of information that may be subject to regulatory decision or confidentiality.
Annual Report	The COT/COC/COM publish an Annual Report, which is available on request and can be viewed on the Committees' respective websites.
Reports on specific issues	Produced by the Committees and Working Groups. Available on request and can be viewed on the Committees' respective websites.

- Corporate information on the work of the Committees, their members (and their declared interests) and information on the Committees activities can also be found on the relevant on website.

Procedures for handling confidential information

Background

- COT/COM/COC quite often consider information that has been supplied in confidence. For the most part this comprises information that is commercially sensitive. For example, this could include product formulations/specifications, methods of manufacture, and reports of toxicological investigations and company evaluations and safety assessments.
- 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 adopted where possible a more open style of business and detailed working papers are published on the Internet before they have been finalised as final statements.

8. 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

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

Confidential data

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

Handling confidential data

- iv) The procedures by which the COT/COM/COC will handle confidential data and the public availability of papers, minutes, conclusions and statements where reference is made to such data will be discussed with the company prior to submission of papers to the Committee(s). 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 for holding Committee meetings in open session

10. The COT has been holding its meetings in open session since April 2003. The COC and COM followed in 2004. It has been recognised that holding meetings in open session needs to be managed to ensure that there is no adverse impact on the ability of the Committee to function effectively and to provide adequate security for members and officials.
11. It was therefore agreed that a protocol should be prepared that would address the advertising of meetings, application process, any restrictions on attendees, limitations on attendance and specify how attendees would be selected. The protocol would also set out the circumstances where discussion would not be possible in public and arrangements for discussions to be held in closed session.
12. The procedures adopted by the Committees for holding meetings in open session are detailed on their respective websites. The respective information for COT/COC/COM can be viewed at:

<http://www.food.gov.uk/science/ouradvisors/toxicity/cotmeets/arrangementcotopenmeetings/>

and

<http://www.advisorybodies.doh.gov.uk/foi/open.htm>

ANNEX 4

GLOSSARY OF TERMS

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

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

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

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

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

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

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

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

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

Allele: Alternative form of a gene.

Allergen: Substance capable of stimulating an allergic reaction.

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

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

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

Aneugenic: Inducing aneuploidy (qv).

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

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

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

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

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

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

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

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

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

- Strength – The stronger the association the more likely it is causal. The COC has previously noted that the relative risks of <3 need careful assessment for effects of bias or confounding.
- Consistency – The association has been consistently identified by studies using different approaches and is also seen in different populations with exposure to the chemical under consideration.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DNA (Deoxyribonucleic Acid): The carrier of genetic information for all living organisms except the group of RNA viruses. Each of the 46 chromosomes in normal human cells consists of 2 strands of DNA containing up to 100,000 nucleotides, specific sequences of which make up genes (qv). DNA itself is composed of two interwound chains of linked nucleotides (qv).

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

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

Dominant lethal assay: See dominant lethal mutation.

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

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

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

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

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

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

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

Erythrocyte: Red blood cell.

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

Exogenous: Arising outside the body.

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

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

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

Forestomach: (See glandular stomach).

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

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

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

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

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

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

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

Genetic polymorphism: a difference in DNA sequence among individuals, groups, or populations (e.g. a genetic polymorphism might give rise to blue eyes versus brown eyes, or straight hair versus curly hair).

Genetic polymorphisms may be the result of chance processes, or may have been induced by external agents (such as viruses or radiation). Changes in DNA sequence which have been confirmed to be caused by external agents are generally called “mutations” rather than “polymorphisms”.

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

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

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

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

Genomics: The study of genes and their function.

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

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

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

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

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

Hepatic: Pertaining to the liver

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

Hepatotoxic: Causing toxicity to the liver.

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

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

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

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

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

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

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

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

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

Intraperitoneal: Within the abdominal cavity.

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

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

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

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

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

Ligand: A molecule which binds to a receptor.

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

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

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

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

Malignancy: See 'tumour'.

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

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

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

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

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

Metabolite: Product formed by metabolism of a compound.

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

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

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

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

Micronucleus test: See Micronuclei.

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

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

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

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

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

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

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

Mycotoxin: Toxic compound produced by a fungus.

Neoplasm: See 'tumour'.

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

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)

³²P 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 ribosomes, where protein synthesis is completed.

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

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

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

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

Tumour (Synonym – neoplasm): A mass of abnormal, disorganised cells, arising from pre-existing tissue, which are characterised by excessive and uncoordinated proliferation and by abnormal differentiation.

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

- Tumours arising from epithelia (qv): benign – adenomas, papillomas; malignant – adenocarcinomas, papillary carcinomas.
- Tumours arising from connective tissues such as fat, cartilage or bone: benign – lipomas, chondromas, osteomas; malignant – fibrosarcomas, liposarcomas, chondrosarcomas, osteosarcomas.
- Tumours arising from lymphoid tissues are malignant and are called lymphomas (qv); they are often multifocal. Malignant proliferations of bone marrow cells are called leukaemias.

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

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

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

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

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

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

Xenobiotic: A chemical foreign to the biologic system.

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

ANNEX 5

Index to subjects and substances considered in previous Annual Reports of the Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

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ANNEX 6

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

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

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

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

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

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

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

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

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

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

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

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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).

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

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