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.

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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 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	Potentiation	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 bioinfomatic 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 products[1].

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 acid[2]. Neat malic acid caused severe ocular irritation in rabbits and was moderately irritating to rabbit skin[3]. 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% solution[4]

10. Skin irritation has been reported in humans at 3% citric acid[5]. Some studies have reported individual differences in the perception of a stinging sensation when 10% lactic acid solution was applied to the skin[6].

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 challenge[7]. 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 pH[8]. Tooth erosion is common in the UK: 50% of 5 year olds had evidence of tooth erosion in 1993[10]. 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 acids[9]. 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 beat[14].

15. Mice and rats dosed with a single administration of a 25% malic acid solution showed signs of toxicity including ataxia, prostration and convulsions[15]. 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 cyanosis[16].

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 dosing[17].

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 salts[18]. 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.

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 cancer[11], and possible increases in hypospadias[12,13] and cryptorchidism[14]. Cryptorchidism is the most common developmental abnormality occurring in 2.2- 3.8% boys at birth[15] 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 British[3]. For instance using time to pregnancy as an indicator, Britain is reported to have lower fertility than Finland[22]. 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 endpoints[3], 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 Agency[26], the EU Weybridge workshop[27], the Royal Society[28], an expert panel of the International Programme on Chemical Safety (IPCS)[29], and an International Symposium convened by IUPAC/SCOPE[30].

5. Most initial reports of possible adverse effects of EDCs focused on effects in wildlife. The IPCS[29] 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 that 2000μg/kg/day during early pregnancy; however no effect was reported at lower doses[32].

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), respectively[31]. 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 oestrogens[31]. 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 receptors[31].

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 disease[3]. 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 system[33]. However, as dietary exposure to dioxins has been decreasing for over 20 years[34] 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 offspring[39]. 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-17β[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 estrogens[40], 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 compounds[41]. 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 fish[42].

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 pollutant[43]. 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 disrupters 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 laboratories[49]. 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 assay[53]. 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 2003[54]. 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 cryptorchidism[21], 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 Assessment[29] 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 review[55] 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 cryptorchidism[56].

23. Recently the role of insulin-like factor-3 (INSL3), which acts to retain the gonad in the inguinal region, has been highlighted in mice[57] 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 reported[58].

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 quality[63] 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 weight[66] low birth weight (possibly due to intrauterine retardation)[59] height in young adulthood[67], 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 occur[72].

28. A recent study identified an association between male subfertility, and the chromosomal region 11p15[69]. 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 IPCS[29], 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).

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.

COT statement 2004/04

August 2004

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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 1999[1]. In May 2001 we were asked to consider the findings in a draft report of the first phase of a Government-funded epidemiological study[5] 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 published[15]. 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 conclusion[2]. COC also in 1999 reviewed additional relevant epidemiological studies of cancer published since the 1992 evaluation, and issued a revised statement[3]. 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 regions[4]. 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 abortion[6].

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 California[6], 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 effects[15].

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 byproducts[21-23], recent studies in laboratory animals[24-30], and recent reviews[31-33].

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.

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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.

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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October 2004

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Tetrabisphenol 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 available[3]. 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 LD_{50} 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 research[4], 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/day[5]. 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 TG426[7]. 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 rats[8], 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 F_2 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 F_0 males at 100 and 1000 mg/kg and in F_0 females at 1000 mg/kg. Levels of T4 were reduced in F_1 males and females at 100 and 1000 mg/kg. Levels of T3 were reduced in F_0 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 months[3]. 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 system[9].

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 resorptions[10]. 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 models[11-15].

COT evaluation

25. The reported effects of TBBPA on thyroid hormones differed between studies. As TBBPA was reported to inhibit the binding of ^{125}I-T3 ($1x10^{-10}$ M) to the thyroid hormone receptor in the range $1x10^{-6}$ to $1x10^{-4}$ M[15], 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 study[8], 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 60[16], 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.

COT statement 2004/02

July 2004

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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 samples[1]. 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 Cambridge[2]. 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, hepox (cis), hepox (trans), oxychlordane, chlordane (trans), chlordane (cis) and p,p-TDE 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/day[5], 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 UK[6].

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 μg/kg bw/day, which is used as the TDI for HCB[5]. 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 μg/kg bw/ day in various countries[5]. Thus, the maximal intake of HCB estimated from the SUREmilk project (0.485 μg/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 μg/kg bw/day, predominantly from the diet [5].

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 μg/kg, would result in an intake of 0.087 μg/kg bw/day in an infant of less than 2 months, which is below the PTDI of 0.1 μg/kg bw/day.

15. In 1977 JMPR recommended an ADI of 0.1 μg/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 JMPR[8], 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 aldrin[9].

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 (1.72μg/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 DDE[10].

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 Committee[11]. The non-soya data are used because it is assumed that breastfed infants will not be fed on soya formula.

20. Cadimum, 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 PTWI[12], 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/day[13]. 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 μg/kg. Estimated intakes from breast milk ranged from 6.2 μg/kg bw/day below 2 months of age to 1.4 μg/kg bw/day at 8-10 months. The total estimated intakes of nickel ranged from 6.2 to 9.0 μg/kg bw/day. This is up to 80% above the TDI of 5 μg/kg bw/day set by WHO[14]. 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 nickel[11].

24. Arsenic was above the limit of quantitation in 7% of 91 samples, with a maximum concentration of 4.0 μg/kg. Estimated intakes from breast milk ranged from 0.64 μg/kg bw/day below 2 months of age to 0.15 μg/kg bw/day at 8-10 months. The total estimated intakes ranged from 0. 65 to 2.05 μg/kg bw/day, which are all within the JECFA PTWI[15], equivalent to 2.14 μg/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 Practicable[16]. 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 μg/kg. The estimated intakes from breast milk ranged from 0.96 μg/kg bw/day below 2 months of age to 0.22 μg/kg bw/day at 8-10 months. The total estimated intakes ranged from 1.5 to 19 μg/kg bw/day. All estimated intakes are considerably below the EVM guidance value[13], which is equivalent to 220 μg/kg bw/day.

26. Lead was above the limit of quantitation in 7% of 114 samples, with a maximum concentration of 2.6 μg/kg. The estimated intakes from breast milk ranged from 0.42 μg/kg bw/day below 2 months of age to 0.1 μg/kg bw/day at 8-10 months. The total estimated intakes ranged from 0.44 to 0.69 μg/kg bw/day. All estimated intakes are below the JECFA PTWI[17], which is equivalent to 3.6 μg/kg bw/day.

27. Copper was above the limit of quantitation in all 114 samples, with a maximum concentration of 896 μg/kg. The estimated intakes from breast milk ranged from 143 μg/kg bw/day below 2 months of age to 33 μg/kg bw/day at 8-10 months. The total estimated intakes ranged from 109 to 184 μg/kg bw/day. All estimated intakes are below the JECFA PTWI[18], which is equivalent to 500 μg/kg bw/day, but total estimated intakes for the younger age groups marginally exceeded the EVM Safe Upper Level[13], which is equivalent to 160 μg/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 μg/kg. The estimated intakes from breast milk ranged from 1508 μg/kg bw/day below 2 months of age to 349 μg/kg bw/day at 8-10 months. The total estimated intakes ranged from 1411 to 2264 μg/kg bw/day. All estimated intakes exceed the JECFA PTWI[19], which is equivalent to 1000 μg/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 μg/kg. The estimated intakes from breast milk ranged from 4.5 μg/kg bw/day below 2 months of age to 1.0 μg/kg bw/day at 8-10 months. The total estimated intakes ranged from 3.1 to 5.8 μg/kg bw/day All estimated intakes are below the EVM Safe Upper Level[13], which is equivalent to 7.5 μg/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 foods[21]. 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 years[22]. 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/day[4]. 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 PCBs[23]. 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 PCBs[24]. 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 months[25]. 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-milk[26]. 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 infant[22]. 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 fed[23]. 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-feeding[27].

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.

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.

COT statement 2004/03

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 food[1]. 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, kyneuric acid. It is also the precursor of NAD and NADP and can replace niacin[2].

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 acids[3]. 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/day[5].

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 x 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 syndrome[6]. Case reports also described arthralgia, swelling of the extremities, rash, fever, cough, interstitial lung disease, arrythmias, 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 patients[7]. 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 not[8].

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 patients[7].

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 relationship[7]. 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 days[6].

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 used[11].

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 EMS[17]. 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)-tryptophan[17]. One of the contaminants remains unidentified.

Toxicological studies of tryptophan and its implicated contaminants

14. L-tryptophan has low oral toxicity[2]. The lowest reported LD_{50} 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 days[18]. In a rat carcinogenicity bioassay conducted by the US National Cancer Institute no evidence of carcinogenicity was found[2].

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 blood[19]. 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 controls[20]. 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 not[21]. 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 controls[22]. 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 animals[23]. 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 analysis[24]. 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-ß-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 effect[27].

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 eosinophils[28]. 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 EBT[29].

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 tissue[30]. However, in a subchronic toxicity study of 3-(phenylamino)-L-alanine in rats, no EMS-like or other abnormalities were found[31]. 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 recently[17] 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 metabolism[33]. 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-tryptophan[13].

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 count[13]. 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 epidemic[34]. 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 supplements[6]. 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-ß-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 EMS[41]. It has been suggested that Optimax may not have been the only source of L-tryptophan this case had been exposed to[42]. 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-tryptophan[42]. 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 monograph[42]. 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 Republic[44]. 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 recommended[45].

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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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 assay[2], 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 issue[5-8]. 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* mutagen[9]. 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 assay[2]. 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 gestation[4]. 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 spray[5]. 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 considered[6-8]. 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

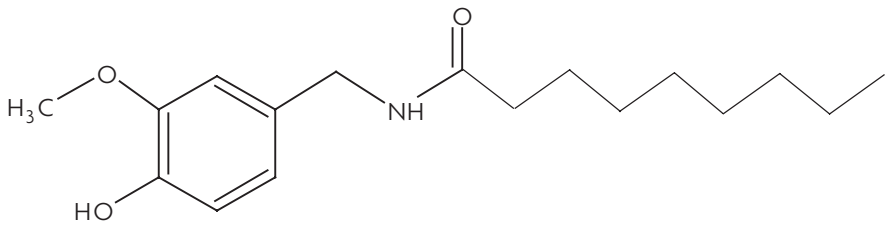
Use of PAVA (Nonivamide) as an incapacitant spray

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 rubifaciant). 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 dressing[3]. 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 vehicle[3]. 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 skin[2]. There is some evidence for aliphatic hydroxylation, as also occurs with capsaicin[3].

Experimental studies in animals

7. Only very limited data are available on PAVA itself; it has a comparable toxicity, as measured by the LD_{50} 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 LD_{50} values being reported as 119 and 97 mg/kg in male and female mice and 161 and 148 in male and female rats[6]. 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 drawn[7].

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 exposure[8]. 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 method[9]. 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 rat[10]. 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 fertility[11]. 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 (FEV_1, 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 FEV_1 heart rate and blood pressure (bp) in the normal subjects at the in-use concentration (1% reduction in mean FEV_1, 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 FEV_1 3%, increase in heart rate 5% and increase in systolic bp 5%), although a transient but clinically significant reduction in FEV_1 ($>$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 LD_{50} 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 Council[1] 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 Conference[2] and a number of peer-reviewed publications in scientific journals[3-9]. 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 reviewed[6-9]. 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 chemical[2].

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 $tk^{+/-}$ cells[3]. 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 arrays[4]. 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 excluded[4] . 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 tests[6] .

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 hepatocarcinogens[7] .

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[7] .

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[8] . 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 site[9] .

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 data[10] . 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 challenge[11]. 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 future[12].

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.

a) We *recognise* the rapid development in toxicogenomic methods (transcriptomics, proteomics and metabonomics) 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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9. Presentation by Dr D Lovell to COT 7 September 2004. (http://www.food.gov.uk/science/ouradvisors/toxicity/cotmeets/cot_2004/cot070904/cotagenda070904)

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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 1989[1], 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 skin[2].

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 carcinogenicity[3]. The COT therefore concluded that there are currently no relevant tolerable intake guidelines or references 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.

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 μg/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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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 Transco	Shares		

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 Transco	Shares	Elsevier	Editor-in-chief, Food and Chemical Toxicology
	BT Group	Shares		
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
			Inamed	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	Scilucent 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		
	Woolwich	Shares	Alpro (Belgium)	Partner in EC Funded project
			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
			Yakult UK	Funded Research
			Nicobrand	CAST PhD studentship
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		
	Unilever	Consultancy – advice on design of an epidemiological survey relating to dermatitis	American Chemistry Council	Contract to IEH for systematic review and meta – analysis

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
			Etiologics	Company Contract
			Ferring	Company Contract
	Eurochlor	Consortium Client	Grupovita	Company Contract
	European Council for Plasticisers and Intermediates	Consortium Client	Guerbet	Company Contract
			Ionix	Company Contract
			Nestle	Company Contract
	Halogenated Solvents Industry Association	Consortium Client	Neurosearch	Company Contract
			Oncosense	Company Contract
			Serono	Company Contract
	AstraZeneca	Research Collaboration	Stiefel	Company Contract
	GlaxoSmithKline	Research Collaboration	Strakan	Company Contract
			Yamanouchi	Company Contract
	NovoNordisk	Research Collaboration		
	Pfizer	Research Collaboration		
	Wyeth	Research Collaboration		
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