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.

2006 has been an extremely busy year for the Committee with agreement of twelve statements. These cover diverse topics such as potential effects of exposure to incapacitant sprays, a topical insect repellent, cyanogenic glycosides in bitter apricot kernels, uranium in water, disinfection by-products in prepared salads, biotoxins in shellfish and various contaminants in fish and other foods. Also included are opinions on the report of the Royal Commission of Environmental Pollution on crop spraying and the health of residents and bystanders, and of a revision by the World Health Organization of the toxic equivalency factors to be used for dioxins and dioxin-like compounds in future evaluations.

The Committee held a scientific workshop on the development and function in adulthood of the human male reproductive system – potential chemical induced effects. A total of about 100 scientists and other interested individuals heard excellent presentations by internationally renowned speakers and discussed the implications for the Committee's risk assessments. A report of the workshop is included in this report. The Committee also had brief discussions or commenced evaluations on a number of other issues, including a major evaluation of cabin air environment, ill-health in aircraft crews and the possible relationship to smoke/fume events in aircraft. The Committee's two working groups continued their reviews: on the long term health effects of the Lowermoor incident and on variability and uncertainty in toxicology. The finalised reports are due to be published in 2007.

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

Professor I A Hughes (Chair)
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COT evaluations

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

1.1 At the request of the Home Office Science Development Branch (HOSDB) the COT discussed during 2005 the potential effects of exposure to both 2-chlorobenzylidene malonitrile (CS) and pelargonic acid vanillylamide (PAVA). CS and PAVA are dispersant incapacitant sprays used by routine patrol officers in police forces in England and Wales. The HOSDB had reported that as the use of PAVA increases there was a possibility that use of both incapacitants on the same individual could occur.

1.2 The COT concluded that co-exposure to CS and PAVA is likely to result in, at most, additive effects on skin, eyes and respiratory tract in most individuals, although in some individuals a lower response might occur as a result of desensitisation.

1.3 The COT made recommendations for recording of incidents and to consider surveillance for potential skin sensitisation among police officers.

1.4 The COT statement is at the end of this report.

Cyanogenic glycosides in apricot kernels

1.5 The Food Standards Agency became aware that bitter apricot kernels were being marketed as a health food in the UK. The kernels contained high levels of amygdalin, a cyanogenic glycoside. The COT was asked to consider whether there were sufficient data to establish a maximum upper level for the safe intake of cyanide or cyanogenic substances.

1.6 The COT reviewed the available data on cyanide and cyanogenic glycosides. The data were limited but it was noted that severe acute toxicity in adults was associated with consumption of approximately 30 kernels. There were insufficient data on chronic toxicity to establish a Tolerable Daily Intake (TDI).

1.7 The range of reported acute lethal doses in humans was 0.5 to 3.5 mg/kg bw. A 100 fold uncertainty factor (10 to account for inter-individual variability and 10 to extrapolate from an effect level to a no effect level, taking into account the steep dose-response relationship) was applied to the lowest lethal dose suggesting a nominal acute reference dose (ARfD) of 5 μg/kg bw which would be unlikely to cause acute effects.

1.8 Based on the analytical data, consumption of 1 kernel per day would result in a cyanide intake of 0.5 mg/day (equivalent to 8 μg/kg bw for a 60 kg adult) which is in the region of this nominal ARfD and would be unlikely to be of concern. The COT noted that this level of intake represents a threshold, above which increasing intake becomes increasingly hazardous.

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

Development and function in adulthood of the male reproductive system: potential chemical-induced effects

1.10 In 2004 the Committee issued a statement on adverse trends in the development of the male reproductive system focussing on the hypothesis that these effects were due to exposure to endocrine disrupting chemicals at critical developmental windows. One recommendation was for the evidence for adverse trends in human male reproductive health to be reviewed before considering possible causes and mechanisms. In response to this recommendation the COT held an open meeting in February 2006 to discuss the issue of potential chemical-induced effects on the development and function in adulthood of the human male reproductive system.

1.11 Presentations considered a range of topics, including cross-sectional and case-control studies of sperm quality and congenital malformations, the TDS hypothesis, potential chemical causes of reported effects, including cumulative effects of *in utero* exposure to anti-androgens and alternative hypotheses to that of endocrine disruption.

1.12 The COT noted that new epidemiology studies reported since the COT issued its statement on adverse trends in development of the male reproductive system provide further evidence that male reproductive health is declining in some populations. However, causal associations in humans have not been established.

1.13 With regards to plausible mechanisms, the COT agreed that the hypothesised causative role of exposure to anti-androgenic chemicals, supported by the data being produced in animal models, was more plausible than that postulated for environmental estrogenic chemicals. Even though a clear link between experimental data and epidemiology is still missing, the COT considered that the new data continue to emphasise the importance of this area of research, the need to actively investigate causation and for risk assessment to incorporate consideration of potential for combination effects.

1.14 The COT meeting report detailing information from the presentations and subsequent COT discussions is included at the end of this report.

N,N-Diethyl-m-toluamide (DEET) – Update of toxicology literature

1.15 The COT previously assessed the safety of the topical insect repellent *N,N*-diethyl-m-toluamide (DEET) in 2003 and at that time made a recommendation that the literature on DEET should be regularly reviewed. New information was obtained though an extensive literature search and by contacting HSE who are currently participating in a regulatory review under the Biocides Product Directive (BPD).

1.16 An update on the toxicology literature was provided to the COT in 2006. During their assessment, the COT looked at neurotoxicity studies, combined use of sunscreen and DEET, results from post-market monitoring in the UK and USA and further epidemiology/intervention studies. The outcome of this discussion was generally reassuring. However the neurotoxicity studies were found to have potential methodological problems and the results were difficult to interpret. Therefore the COT recommended that repeat studies be carried out to clarify these issues. Members requested further information on the toxicokinetics of DEET and sunscreen to provide further reassurance on the safety of their combined use.

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

Disinfectants and disinfection by-products in prepared salads

1.18 Wash aids, such as those employed by salad manufacturers, were identified as a potential future topic in the COT horizon scanning paper of February 2005, due to the concern about the potential generation of by-products on or in foods as a result of the use of chlorine-based disinfectants. At that time Members agreed that before any risk assessment could be undertaken there was a need for information on the nature and levels of the disinfection by-products that were formed. Reaction of chlorine-based disinfectants with organic matter in water can result in the formation of a number of by-products, including trihalomethanes, haloacetic acids, haloacetonitriles, haloketones, chloral hydrate and chloropicrin. The presence of bromide can lead to brominated and mixed chlorinated/brominated compounds. Similar by-products may be produced in or on foods treated with chlorine-based disinfectant wash-aids.

1.19 In June 2006 the Food Standards Agency received the results of a study conducted on behalf of the Fresh Prepared Salads Producer Group, investigating the occurrence and formation of disinfectants and disinfection by-products in prepared salads. The study involved analysing a range of prepared salads, purchased from various retail outlets for the presence of specific disinfectants and disinfection by-products.

1.20 In all samples tested, calculated ingestions for each compound (based on salad consumption data) were at least several orders of magnitude lower than tolerable daily intakes set by the World Health Organization. The COT concluded that the study results did not indicate any cause for concern with respect to the use of chlorine washes; and in the light of decreasing use of chlorination processes agreed that there was no need for the generation of additional data to confirm the results of this commercial study.

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

Mycotoxins in cheese

1.22 In 2006, the Food and Veterinary Office (FVO) of the European Commission audited the UK's implementation of new food hygiene legislation in the dairy sector. During the audit the FVO raised questions about cheese recovery operations, which included removal of mould from cheese which had become contaminated with mould not present as part of the production process or integral to the final product.

1.23 Since it is considered possible that the moulds may produce mycotoxins, the Food Standards Agency drafted provisional guidance to assist the UK dairy industry and local authorities in ensuring that cheese recovery is carried out safely. The guidance was intended to be precautionary in nature pending European level consideration of the public health and legislation issues.

1.24 The COT was asked for its advice on risk assessment of mycotoxins in cheese and whether the provisional guidance raised any concerns for the safety of consumers.

1.25 The available data on occurrence of mycotoxins in hard cheese are limited. The majority of studies involved experimental inoculation of mould into cheeses incubated at temperatures greater than refrigerator temperature, and the relevance of these data was unclear. In one published study on UK cheeses, conducted in 1979, there was a lack of information provided regarding limits of detection and a small number of samples was analysed. Therefore there was considerable uncertainty regarding the range of mycotoxins that may be present on naturally contaminated cheeses in the UK.

1.26 The COT considered that the available data were not sufficient to draw conclusions on the likelihood of mycotoxins being present in mould contaminated hard cheese, and therefore it was not possible to conduct a risk assessment for mycotoxins in recovered cheese. It was noted that there would be a need for more robust data on toxicogenic strains, levels of mycotoxins present in cheeses available in the UK and the ways in which recovered mouldy cheese is used before a risk assessment could be undertaken.

1.27 As there were concerns regarding the lack of evidence to underpin the draft provisional guidance, the COT concluded that the safety of consumers could not be assured. The COT agreed that exposure to genotoxic mycotoxins from mouldy cheese should be as low as reasonably practicable (ALARP).

1.28 The Committee was informed that the guidance will be further changed in line with comments received following the European level discussions.

Organic chlorinated and brominated contaminants in shellfish, farmed and wild fish

1.29 In February 2006 the Food Standards Agency completed two surveys that analysed 47 species of farmed and wild fish and shellfish consumed in the UK to determine the concentrations of a number of organic contaminants:

- Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs); and

- Brominated flame retardants (BFRs), i.e. polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA) as well as polybrominated dibenzo-*p*-dioxins (PBDDs) and polybrominated dibenzofurans (PBDFs) which occur as contaminants in brominated organic chemicals.

1.30 Although the COT had previously evaluated the chlorinated dioxin-like compounds, PBDEs, HBCD and TBBPA, data on the concentrations of PBDDs, PBDFs and PBBs in fish consumed in the UK had not been available for consideration previously.

1.31 In anticipation of these survey results, the COT had been asked in 2005 to advise on the approach to risk assessment for the brominated compounds. The COT considered that applying the toxic equivalency factors (TEFs) derived for the chlorinated dioxins, furans and dioxin-like PCBs to the brominated dioxin-like compounds would be more protective than presuming independence of action. The COT also concluded that, for the purpose of evaluating the data on dietary exposure, the total toxic equivalents (TEQs) for the brominated dioxin-like contaminants should be combined with the TEQs for the chlorinated dioxins, and in this way provide a measure of the total concentration of chemicals with dioxin-like properties. (2005 Annual Report, paragraphs 1.1-1.4).

1.32 In 2006, the Committee was invited to consider the results of the surveys and to advise on whether they formed a basis for the Food Standards Agency to amend its advice on fish consumption.

1.33 The COT considered that the concentrations of PBDEs, HBCD and TBBPA detected in the surveys did not raise toxicological concerns. In addition, for the majority of UK consumers, who do not eat fish frequently, the concentrations of dioxin-like compounds detected in the surveys were not a concern for health. The Committee concluded that the new survey data did not indicate a need for a change in the Food Standards Agency's current advice on consumption of oily fish.

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

Tolerable Daily Intake for perfluorooctanoic acid

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

1.36 The Food Standards Agency commissioned research to determine the concentrations of PFOA in the 2004 Total Diet Study (TDS) samples. The COT was invited to assess the toxicology of PFOA in order to advise on any health implications arising from the results of the survey.

1.37 The COM and COC had concluded in 2005 that, overall, PFOA was not mutagenic and that a threshold approach to establishing a TDI was appropriate (2005 Annual Report, paragraphs 2.37-2.41 and 3.21-3.23).

1.38 The COT considered that a dose level of 0.3 mg/kg bw/day was a suitable point of departure expected to be without adverse effect on the basis of a number of endpoints of PFOA toxicity. An uncertainty factor of 100 was applied to allow for inter- and intra-species variation in order to establish a TDI of 3 μg/kg bw/day.

1.39 The Committee noted the results of the Food Standards Agency analysis of composite food group samples that estimated high level adult dietary intakes of PFOA were lower than the recommended TDI. The COT considered that the estimated intakes were not of concern regarding human health.

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

Tolerable Daily Intake for perfluorooctane sulfonate

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

1.42 PFOS has the potential to enter the food chain and could have a negative impact on human. The Food Standards Agency commissioned analysis of the 2004 Total Diet Study (TDS) samples for PFOS and the Committee was invited to consider the toxicology of PFOS and the results of the analysis.

1.43 The COM and COC had concluded in 2005 that, overall, PFOS was not mutagenic and that a threshold approach to establishing a TDI was appropriate (2005 Annual Report, paragraphs 2.35-2.36 and 3.17-3.20)

1.44 Considering the complete toxicological database, a 26-week cynomolgus monkey study provided the lowest NOAEL of 0.03 mg kg bw/day for decreased serum T3 levels. The Committee applied an uncertainty factor of 100 to allow for inter- and intra-species variation to the NOAEL and provisionally proposed a Tolerable Daily Intake (TDI) of 0.3 µg/kg bw/day. The COT considered that this TDI would be adequate to protect against the range of identified effects.

1.45 The Committee noted the results of the Food Standards Agency analysis of composite food group samples that indicated that some groups of consumers may exceed the recommended TDI. However, the COT considered that as there were considerable uncertainties in the dietary intake estimates the potential exceedances did not indicate immediate toxicological concern.

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

Risk assessment and monitoring of Paralytic Shellfish Poisoning (PSP) toxins in support of public health

1.47 A number of marine phytoplankton produce biotoxins that can be bioconcentrated in shellfish. Consumption of shellfish contaminated with sufficiently high levels of these toxins can result in human illness.

1.48 The COT first considered the risk assessment and monitoring of marine biotoxins associated with Paralytic Shellfish Poisoning (PSP) in December 2005, and discussions continued into 2006. PSP is a neurotoxic syndrome with symptoms including tingling and numbness of extremities, respiratory distress and muscular paralysis leading to death by asphyxiation. The predominant toxin responsible for PSP is saxitoxin (STX), but at least 20 other related compounds have also been identified.

1.49 The COT agreed that an acute reference dose for PSP toxins of 0.7 µg STX equivalents (eq)/kg bw, identified from human PSP case reports, was appropriate for protection of public health. On the basis of the estimated portion size for high-level shellfish consumption in the UK (250g), a maximum PSP toxin concentration of 20 µg STX eq/100g shellfish meat would be considered to be without appreciable health risk.

1.50 In considering methods of detection of PSP toxins in shellfish, it was concluded that high performance liquid chromatography (HPLC) was currently the only method adequate for detection of PSP toxins at the concentration considered to be necessary for protection of public health. The COT concluded that HPLC should be used for quantification of PSP toxins, subject to appropriate quality control measures and method validation in the testing laboratories.

1.51 At the current regulatory limit of 80 µg STX eq/100g shellfish meat, the COT concluded that an immunoassay known as the Jellett Rapid Test could be used to screen out samples containing approximately ≤40 µg STX eq/100g shellfish meat, and to identify samples containing approximately ≥40 µg STX eq/100g shellfish meat for quantitative testing, subject to adequate quality control measures.

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

Risk assessment of marine biotoxins of the okadaic acid, pectenotoxin, azaspiracid and yessotoxin groups in support of public health

1.53 The okadaic acid (OA) group of marine biotoxins are known to cause Diarrhetic Shellfish Poisoning (DSP), characterised by symptoms of nausea, vomiting, diarrhoea and abdominal pain. Cases of human illness have also been reported following consumption of shellfish contaminated with azaspiracids (AZAs), and symptoms of AZA poisoning are similar to those of DSP. There are no known cases of human illness associated with pectenotoxins (PTXs) or yessotoxins (YTXs), but adverse effects have been reported in experimental animals.

1.54 The COT reviewed the human epidemiology and animal toxicology data for the OA, PTX, AZA and YTX groups and advised on an appropriate acute reference dose for each biotoxin group.

1.55 In addition, the COT identified the maximum concentration of each biotoxin group within shellfish that would be considered to be without appreciable health risk, on the basis of the estimated portion size for high-level shellfish consumption in the UK (250g).

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

Royal Commission on Environmental Pollution (RCEP): crop spraying and the health of residents and bystanders

1.57 The RCEP report on crop spraying and the health of residents and bystanders was published in September 2005. The RCEP had been asked by the DEFRA minister the Rt Hon Alun Michael to examine the evidence on which DEFRA's policy of not requiring mandatory buffer zones, strips of farm land on which pesticides may not be sprayed in order to reduce exposure to bystanders, was based and the reasons for people's concerns. The Advisory Committee on Pesticides (ACP) had advised ministers that the current regulatory system was adequate and that buffer zones were not required to protect the health of residents and bystanders. The remit the RCEP set itself was to "examine the scientific evidence on which DEFRA has based its decision on bystander exposure and its policy on access to information on crop spraying. The Commission will also consider wider issues related to the handling and communication of risk and uncertainty, as well as public involvement, values and perceptions in this context."

1.58 The COT and COC were asked by DEFRA and the ACP to comment on the RCEP report. The COT and COC agreed their remit was restricted to a review of the content of the RCEP report as written. This involved using members' expertise and the evidence presented in the RCEP report to consider whether the conclusions and recommendations reached in respect of health related topics were appropriate, to form our own conclusions on these topics, and to consider whether any further work should be undertaken by the COT, COC or COM, or by the ACP, with respect to bystander pesticide risk assessment. The Committees were not on this occasion asked to undertake an independent review of pesticide safety and use.

1.59 The remit of the Committees referred to the scientific aspects of the RCEP report in relation to health and did not include wider aspects outlined by the RCEP in its report.

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

Uranium in water used to reconstitute infant formula

1.61 The World Health Organization (WHO) established a TDI for uranium of 0.6 μg/kg body weight (bw) per day and a guideline value for the maximum concentration of uranium in drinking water of 15 μg/L. To assist the Food Standards Agency in developing advice on the suitability of using natural mineral water and other bottled waters to reconstitute infant formula, the Committee was asked to comment on the potential health implications for infants consuming formula milk made up with water containing uranium at this guideline level.

1.62 The WHO TDI and guideline values for uranium were based on the results of a 3 month study in which rats were given drinking water containing uranium at a range of concentrations. The COT noted that there were some limitations in the design and interpretation of the study but considered that the values derived from it would be expected to be protective of public health.

1.63 The COT noted that infants up to six months of age consuming formula reconstituted with water containing uranium at the WHO guideline value of 15μg/L could exceed the WHO TDI by about 4-fold. It is possible that uranium absorption is higher in young infants, and the implications of a modest exceedance of the TDI are uncertain.

1.64 The database on uranium toxicity is incomplete, however, on the basis of the available evidence, the COT concluded that this potential exposure of formula fed infants did not raise specific concerns for health.

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

Vitamins and minerals – European Commission Document on establishing maximum and minimum levels in dietary supplements and fortified foods

1.66 European legislation covering the regulation of dietary supplements is currently being enacted. As part of this process, maximum levels will be established for vitamins and minerals in food supplements and fortified foods. The European Commission has not yet proposed any maximum levels for individual vitamins and minerals but have published a discussion paper which posed questions on how the setting of maximum levels might be achieved. The preliminary Food Standards Agency view was based on the conclusions of the 2003 report of the Expert Group on Vitamins and Minerals (EVM) but the Food Standards Agency were also seeking the views of stakeholders on the discussion paper. The COT was asked to consider the paper and comment.

1.67 The COT endorsed the approach and conclusions of the EVM report, in particular with respect to the use of guidance levels where there were insufficient data to set a tolerable upper level. Guidance levels represented a level of intake that would not be expected to result in adverse effects and allowed further explanation of why a TDI could not be set either because there were no data available or because there was no toxicological concern anticipated based on the available data.

2005 WHO Toxic Equivalency Factors for dioxins and dioxin-like compounds

1.68 Dioxins and dioxin-like compounds are persistent organic pollutants that are resistant to metabolism and subject to bioaccumulation. Most, if not all, of their toxic and biological effects are mediated by the aryl hydrocarbon receptor (AhR). Many different congeners are released into the environment by industrial activity and, since these chemicals share a common mechanism, risk assessment should reflect the mixture rather than the isolated chemical. The WHO has, on a number of occasions, convened Expert Panels to discuss and refine toxic equivalency factor (TEF) values for the various dioxins and dioxin-like congeners. The COT was invited to review the recommendations of the WHO Expert Panel meeting in 2005.

1.69 Members noted that the revised Relative Effect Potency (REP) database, upon which the TEF re-evaluation was based, provided a better analysis of the studies, and that the use of half order of magnitude increments on a logarithmic scale improved the description of the TEF values.

1.70 The COT agreed with the scientific rationale for the re-evaluated TEF values, although concurred with the WHO view that this should be thought of as an 'interim' methodology. They considered that the TEF re-evaluation did not raise any additional concerns regarding exposure to dioxins and dioxin-like compounds that had not been highlighted in previous COT evaluations.

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

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

Committee Procedures and Working Groups

Balance of expertise on the Committee

1.72 In advance of a number of Members completing the current terms of appointment, the COT was invited to comment on the appropriate balance of expertise.

1.73 It was agreed that the following types of specialist expertise are required by the Committee for some or all of its evaluations:

Analytical techniques	Biochemistry
Bioinformatics	Cell biology
Clinical practice	Endocrinology
Epidemiology	Immunology
Mechanistic toxicology	Molecular biology
Neurotoxicology	Nutrition
Paediatrics	Pharmacokinetics
Pharmacology	Probabilistic modelling
Reproductive toxicology	Respiratory toxicology
Risk assessment	Statistical aspects of experimental design
Statistics	Toxicological pathology
Xenobiotic metabolism	

1.74 It would not be necessary to have an individual member for each listed expertise as some people would have a combination of the required skills. However, it was noted that two opinions on toxicopathology would be helpful and respiratory toxicology could be important. Additional key experts could also be invited to attend meetings for specific topics to supplement missing knowledge.

Good Practice Agreement for Scientific Advisory Committees

1.75 The Food Standards Agency had drafted a Best Practice Agreement for Scientific Advisory Committees. This aimed to set out the processes which the Committees use in drawing up their advice and would help to provide the Food Standards Agency Board with assurance that the science has been properly gathered and assessed. COT members provided comments on the draft. Following consultation with the nine Scientific Advisory Committees that advise the Food Standards Agency, this was adopted as a Good Practice Agreement, and is included in Annex 4 of this report.

1.76 The principles contained in this Good Practice Agreement will be reconsidered by each committee annually as part of the preparation of its Annual report and will be reviewed in the light of experience.

Horizon scanning

1.77 At the February 2006 meeting, members were provided with information on planned and possible discussion items for the year, and invited to comment on emerging issues that might also need to be addressed.

1.78 It was suggested that the relevance of new approaches to risk assessment developed by the WHO/IPCS should be considered. In addition, it would be useful to consider risk communication strategies be discussed and to hold a one day meeting on risk analysis with COM and COC and possibly other scientific advisory committees.

Open Meetings – a review of procedures

1.79 COT meetings have been held in open session since 2003, and it was considered timely to review the procedures for open meetings in the light of the experience gained.

1.80 Members confirmed that in the interests of openness it is important to allow interested parties opportunity to observe committee discussions, but it was also important to ensure that their presence does not inhibit the COT evaluation. It was noted that if observers required clarification of any points made in the discussion, this could be submitted to the secretariat in writing after the meeting and raised at the subsequent meeting if needed. Therefore it was not essential to allow observers the opportunity to comment or ask questions within the meeting.

1.81 Interested parties could submit information prior to meetings. If the submitted information was considered important for the discussion, then they could be invited to comment at the meeting.

1.82 The procedures were revised and agreed by COT, COM and COC before publishing on the committee website at http://www.food.gov.uk/science/ouradvisors/toxicity/cotmeets/arrangementcotopenmeetings/cotopenmeetingprocedures.

Performance evaluation for committee members

1.83 The COT noted that a formal performance evaluation is likely to be required for Scientific Advisory Committee members in the future. It was agreed that this would require clear objectives and benefit for Members. It could not be comparable to employment appraisal systems, and a "self-assessment" might be more appropriate. A proposal would be developed further for introduction in 2007.

Workshop on Social science insights for risk assessment

1.84 In September 2005, following a request from the Food Standards Agency, the Royal Society organised a workshop exploring social science insights for risk assessment. The workshop examined two case studies, the transmission of bovine spongiform encephalopathy and the consumption of fish. The latter was based on the joint Scientific Advisory Committee on Nutrition and COT review of benefits and risks of fish consumption. The COT Chairman was one of five chairs of advisory committees invited to take part in the workshop, alongside four social scientists with expertise in the psychology and sociology of risk.

1.85 During the meeting, five principles were identified which may enable more effective risk assessment, and related management and communication processes:

- Consult stakeholders and the public (where appropriate) on the framing of questions to be put to expert scientific advisory committees
- Develop a cyclical and iterative process to inform risk assessment, management and communication
- Acknowledge assumptions and uncertainty in risk assessment
- Broaden public and stakeholder engagement at the different stages of the process, particularly on issues of controversy or high uncertainty
- Be clear about your audiences and communicate the things that matter to them

1.86 In March 2006, the COT considered a report of this meeting, which was due to be published shortly. It was felt that, in general, the Committee met all five of the principles to a reasonable extent. The inclusion of two non-specialist representatives was seen as being effective in increasing the Committee's awareness of public concerns.

1.87 The role of the COT is to provide specialist scientific advice, whereas communication of this advice is primarily a matter for risk managers and communicators. Similarly, the process of framing questions to be put to the Committee, and any consultation with stakeholders and the public that this might entail, was viewed as being the responsibility of the Secretariat, rather than the Committee itself. Expanding the COT's role to include these responsibilities could result in it becoming less effective in its central role.

1.88 Social science approaches were seen as being most useful in the processes of risk management and communication, where decisions can be influenced by factors additional to the risk assessment

Lowermoor Subgroup

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

- to advise on whether the chemicals involved in the incident caused, or were likely to cause, delayed or persistent harm to health.
- to advise whether the existing programme of monitoring and research into the health effects of the incident should be augmented and, if so, to make recommendations.

1.90 A draft report was issued for public consultation in 2005, and the subgroup met during 2006 to revise the draft. The final report is expected to be published early in 2007.

Working Group on Variability and Uncertainty

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

1.92 A draft report was issued for consultation in 2006, and the working group met during subsequently to revise the draft. The final report is expected to be published mid 2007.

Ongoing work

Nanomaterial toxicology

1.93 In December 2005 the COT, COC and COM published a joint statement on nanomaterial toxicology. In the concluding remarks the COT indicated additional information on medical applications of nanoparticles might be important to their discussions and might be potentially relevant with regard to information on structure activity relationships. Following discussions between the secretariat and Medicines and Healthcare products Regulatory Agency (MHRA), the MHRA produced a review of information on the toxicology of nanoparticles used in healthcare. This was discussed by the COT during 2006 and a draft COT addendum to the joint statement based on these discussions will be agreed and published early in 2007.

Nickel leaching from kettle elements into boiled water

1.94 The COT has discussed nickel leaching from kettle elements on a number of occasions in 2003. Previously in 2003, Members concluded that further studies would be beneficial in order to more accurately replicate domestic kettle usage patterns for consumers. In 2006, the Scottish Executive commissioned further research. The preliminary results were largely negative and the COT was asked to comment on the implications of the data.

1.95 The COT Members expressed concern that the study did not reflect domestic kettle usage patterns and could not draw further conclusions on the health implications of boiling water in kettles with exposed nickel-plated elements. A further study has been commissioned, focussing on re-boiling of water in kettles with nickel-plated elements, and will be discussed again early in 2007.

Cabin air environment, ill-health in aircraft crews and the possible relationship to smoke/fume events in aircraft

1.96 The Department for Transport (DfT) has asked the COT to undertake an independent scientific review of data submitted by the British Airline Pilots Association (BALPA). BALPA submitted data relating to organophosphates (OPs), the cabin air environment, ill-health in aircraft crews and the possible relationship to smoke/fume events in aircraft.

1.97 This was discussed at the meetings in July and December 2006 and two primary objectives were outlined:

- Firstly, to evaluate the BALPA submission and, based on the data submitted by BALPA and that sourced by the secretariat, assess the risk of exposure of aircraft crews to OPs and oil/hydraulic fluid pyrolysis products in cabin air and determine whether there is a case for a relationship between exposure and the ill-health in aircraft crews.

- Secondly, to provide the DfT with appropriate advice on any further research required to evaluate this subject.

1.98 The COT identified a number of topics for further consideration and the discussion will continue in 2007.

Reformulation of PAVA (Nonivamide) as an incapacitant spray

1.99 The COT has considered the use of PAVA as an incapacitant spray in 2002, 2004 and 2005. In October 2006, the Committee was asked to comment on a reformulation of this product and discussions are ongoing.

Statements of the COT

Statement on combined exposure to 2-chlorobenzylidene malonitrile (cs) and pava (nonivamide) sprays

Introduction

1. The Committee has been asked by the Home Office Science Development Branch (HOSDB) for advice on the potential effects of exposure to both 2-chlorobenzylidene malonitrile (CS) and pelargonic acid vanillylamide (PAVA). CS and PAVA are dispersant incapacitant sprays used by routine patrol officers in police forces in England and Wales The HOSDB have reported that as the use of PAVA increases there is a clear possibility that use of both incapacitants on the same individual would occur. For example, cross border use by British Transport Police who use PAVA attending an incident in an area where the local police force uses CS spray. A further scenario would be use of one incapacitant in the field and a different incapacitant in the prison/detention cell area. There might also be operational reasons for use of more than one incapacitant in the field. However, the HOSDB has reported that individual officers would not be issued with more than one type of incapacitant. In addition, there is clear guidance that if officers found that a particular incapacitant does not work, there is no recourse to using a second type of incapacitant.[1,2]

CS (2-chlorobenzylidene)

2. CS is a peripheral sensory irritant[3]. It interacts locally with receptors on sensory nerves in the skin, eyes and other mucous membranes causing severe pain and irritation. Typical signs and symptoms during exposure include eye discomfort, excessive lacrimation, blepharospasm, burning sensation in the nose and throat, rhinorrhea, salivation, constricting sensation in exposed skin etc. The full effects arise within 20-30 seconds but some kind of effect is often seen immediately. Recovery is gradual and can begin within 15 minutes of being sprayed, with the disappearance of most effects within an hour later[1]. However, some individuals have taken up to 12-14 hours to recover completely. CS always has some effect even if not totally incapacitating. As CS affects the breathing as well as sight it tends to slow down and stop individuals much more quickly than PAVA, as they begin to panic when they think they cannot breathe. As CS affects a range of senses it can become disorientating. The HOSDB has reported that the short-term effects have led to the use of CS sprays by all but three police forces in England and Wales as a chemical incapacitant.[1] Such sprays consist of 5% CS in methyl isobutyl ketone (MIBK) with nitrogen as a propellant.[3]

PAVA (Nonivamide)

3. PAVA is a structural analogue of capsaicin, the active ingredient of natural pepper.[1] It is a potent sensory stimulant. It is also used as a food flavour (1 to 10 ppm in baked foods, meat products and soups; 57.9 to 93.1 ppm in chewing gum) and in human medicine (the rubifaciant, Nonivamide). PAVA primarily affects the eyes causing closure and severe pain and this is its principal mode of action. The pain to the eyes is reported to be greater than that caused by CS.[1] The police guidance on the use of incapacitant sprays issued by the Association of Chief Police Officers advises that PAVA must enter the eyes for it to work effectively and the effects are normally instantaneous if this happens.[2] However, there have been occasions where there has been a delay between spraying and the effects taking place, or no effects at all. PAVA remains effective, with the eyes closed and extremely painful, for a longer time than CS before

any recovery begins.[1] Once recovery starts, it is a rapid process[1] but people have been reported to be lacrimating for hours afterwards. Exposure to fresh moving air will normally result in a significant recovery from the effects within 15-20 minutes.[2] The pain worsens the first time the eyes are re-opened and then gradually subsides each subsequent time they are opened. PAVA spray consists of a 0.3% solution of PAVA in 50% aqueous ethanol with nitrogen as propellant

Trends in use

4. The HOSDB has reported that there are approximately 1500 CS discharges in England and Wales each year. PAVA spray is used by a number of forces including Sussex and Northamptonshire police forces. There are no data available on the number of PAVA discharges per year. There are a number of police forces who are in the process of considering a change to or adoption of PAVA. The HOSDB have reported that to date there is no information to suggest that both CS and PAVA had been used on the same individual, but as the use of PAVA increases there is a clear possibility that use of both incapacitants on the same individual would occur. The decision of when to use an incapacitant spray is left to the judgement of individual officers using the Officer Safety Model.[1,2]

Overview of previous COT consideration of CS and PAVA

5. The COT published statements reviewing the toxicity data on CS in 1999[3] and on PAVA in 2002[4] and 2004[5]. The overall conclusions reached on CS and PAVA are reproduced below

CS

6. In May 1999 a statement was issued by the Committees on Toxicity (COT), Mutagenicity (COM) and Carcinogenicity of Chemicals (COC) in Food, Consumer Products and the Environment regarding the use of CS spray as a chemical incapacitant. A copy of the full statement can be found at http://archive.food.gov.uk/dept_health/archive/cot/csgas.htm

    i. The Committee noted that there are considerable data available to assess the toxicity of CS itself, and to a lesser extent, the solvent MIBK itself. CS is a potent sensory irritant, particularly to the skin and eyes. It is rapidly hydrolysed and therefore tissue exposure to CS itself is transient. Experience of use indicates that it is a skin irritant and thee are some reports of skin sensitisation occurring.

    ii. There are no concerns relating to the mutagenicity, carcinogenicity or teratogenicity of CS itself.

    iii. The toxicity of the solvent MIBK used in the spray is characterised by the transient local effects and central nervous system effects, particularly headache and nausea, resulting from exposures of about 100 ppm and above of teratogenicity in developmental toxicity studies. There is no information from carcinogenicity or multigeneration reproductive toxicity studies.

    iv. Little toxicological information was available on the formulated spray. A 7% (w/v) solution of CS in MIBK produced severe irritant effects in rabbit eyes followed by recovery in 8 days. The spray has skin irritant properties and can cause dermatitis.

v. The Committee had concerns regarding exposure to CS spray in susceptible groups. Individuals with asthma or chronic pulmonary obstructive disease whose condition could be aggravated by the irritant effects of CS spray on the respiratory tract. Individuals with hypertension or other cardiovascular disease whose condition may be affected by the transient effects of CS spray in increasing blood pressure. It was not possible, on the basis of the available data, to comment on whether individuals being treated with neuroleptic drugs are more likely to be sensitive to the effects of CS spray.

vi. The Committee noted that adherence to the operational guidelines for the use of CS spray was of particular importance since at the time of exposure it would be exceedingly unlikely that the medical status of those exposed would be known. It was concluded that particular care needs to be taken to follow the recommended aftercare guidelines for all persons exposed to CS.

vii. The Committee considered that further information needs to be obtained on the effects of CS spray in humans. In this regard, it was noted that systematic studies in volunteers to investigate the toxicity of CS spray may present insurmountable difficulties. The Committee recommended that follow-up studies be carried out on people treated for the immediate effects of CS spray to obtain data on whether delayed effects occur. It was recommended that information should also be collected in these studies relating to the previous medical history of the individuals involved, particularly with regard to respiratory or cardiovascular disease, or treatment with neuroleptic drugs.

PAVA

7. The full COT statements from the evaluations undertaken in 2002 and 2004 can be found at http://www.advisorybodies.doh.gov.uk/cotnonfood/pava.htm and http://www.advisorybodies.doh.gov.uk/cotnonfood/pava04.htm . The overall conclusions are reproduced below.

i. The COT recognised that exposures would be low and for a short period. The Committee stated that it was impossible to calculate exposure with any accuracy but noted 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 of the order of 0.04 mg/kg bw.

ii. Animal model data and experience in use do not give rise to concerns regarding long-term harm to the skin and eyes arising from irritant effects. No conclusions can be drawn from the one available animal study to investigate skin sensitisation but experience in use, including in human medicines for topical application, indicates that PAVA is not a skin sensitising agent.

iii. There are no concerns regarding the mutagenicity of PAVA. PAVA gave a positive result in one of the three *in vitro* mutagenicity tests carried out indicating that it could have mutagenic potential and negative results from an unscheduled DNA synthesis study and a bone marrow micronucleus test.

iv. There are no concerns regarding developmental toxicity. PAVA 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, about 4 orders of magnitude above the expected exposure level arising form the use of the spray.

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 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. The Committee recommended continuation of the monitoring of experience-in-use.

CS/PAVA Sprays – Potential Interaction

8. The COT approach to the consideration of combined toxicological action of a mixture of CS and PAVA is based on the concepts described in the COT Report on Risk Assessment of Mixtures of Pesticides and Similar Substances.[6] A key aspect of the approach to the assessment of the combined risk involves consideration of the mode-of action of critical toxicological effects. Table 1 (appended at the end of this statement) summarises the potential interaction between CS and PAVA. The most evident area for potential interaction relates to effects at the site of contact, e.g. skin, eyes and respiratory tract. Some more detailed information on potential site of contact effects and their modes of action is given below.

CS Spray – Site of Contact Effects

9. CS is an SN_2 alkylating agent and reacts readily with nucleophilic sites.[7,8] Prime targets at the site of action include sulphydryl-containing enzymes such as lactic dehydrogenase. The findings of Cucinell et al suggest that lactic dehydrogenase is inhibited by CS, which was partially reversed by the addition of excess glutathione. Based on these results it has been suggested that alkylation of nucleophilic sites, including SH containing enzymes, is the underlying biochemical lesion responsible for CS-induced toxicity. CS reacts rapidly with the thiol groups of dihydrolipoic acid, the disulphydryl form of lipoic acid which is a coenzyme in the pyruvate decarboxylase system.[9] Alteration in dihydrolipoic acid biochemistry can lead to decreased acetyl CoA levels, resulting in cellular injury. CS has the ability to generate bradykinin *in vitro*[10] and *in vivo* in humans[7] and it has been suggested that the irritant and painful effect of CS may be due to bradykinin release.[9]

10. A recent report details a number of instances in which six police officers and a doorman developed a range of unpredictable long-term cutaneous reactions following both single and multiple exposures to CS spray over several months or years.[11] The six cases detailed in the report are out of out of the estimated several thousand officers who have used CS spray operationally over the last decade. The skin reactions consisted of contact allergy, leukoderma, initiation or exacerbation of seborrhoeic dermatitis and aggravation of rosacea. The skin reactions required long-term changes in working practice for the exposed individuals

PAVA Spray – Site of Contact Effects

11. Nonivamide, or synthetic capsaicin, has long been used as a topical application for the treatment of painful conditions of the muscles, joints and bones. Repeated or prolonged topical application of low concentrations or systemic administration of a single high dose can cause long lasting selective desensitisation.[12] Nonivamide binds to membrane receptors and selectively interacts with polymodal nociceptive neurones .[13] After binding, the membrane depolarises subsequent to the opening of a cation non-selective ion channel. As a result, the neurotransmitter substance P and other neurotransmitters are released from the nerve endings causing a sensation of burning pain and hyperalgesia. Prolonged and repeated administration of nonivamide causes desensitisation and inactivation of the sensory neurones to thermal, chemical and mechanical stimuli in a dose-dependent manner. Systemic nonivamide produces antinociception by binding to vanilloid receptors on afferent nerve endings in the spinal cord. Prolonged inactivation of sensory neurotransmitter release blocks spinal neurotransmission.

Studies of co-exposure to CS and PAVA

12. There are no studies of co-exposure to CS and PAVA. However, Foster and Weston (1986)[14] used a blister base testing approach in volunteers to assess pain response for CS and PAVA. They reported that PAVA induced more pain than CS. An inflammatory flare was often also noted with PAVA. The study of interaction used a desensitising protocol followed by a challenge by a different sensory irritant. The authors reported that when PAVA was used first it provided a generic desensitisation to challenge by other sensory irritants. When CS was used in the desensitising protocol there was a pain response from a subsequent PAVA challenge equivalent to that seen in control exposures.[14]

COT consideration of potential interaction between CS and PAVA.

13. Members were aware that concerns had been raised regarding possible sensitive subpopulations following exposure to incapacitants during the previous considerations of CS and PAVA. There was some evidence from volunteer trials that PAVA may exacerbate bronchospasm in asthmatics.[4,5] However no equivalent studies in asthmatic volunteers exposed to CS were available. The COT had noted in 1999 that CS might aggravate bronchial asthma in some individuals. There was thus some uncertainty regarding the potential effects of co-exposure in this subpopulation.

14. Members considered potential interaction between CS and PAVA might occur in relation to site of contact effects. The only available study where co-exposure had occurred related to a desensitisation protocol using a human skin blister base approach.[14] There was evidence that desensitisation with PAVA gave rise to no pain response upon challenge with CS. However desensitisation with CS did not have any effect on the pain response to PAVA. Overall members felt that the potential effects of co-exposure or sequential exposure to CS and PAVA would give rise to at most an additive effect, although there was a possibility that desensitisation to contact effects might occur.

15. The committee was aware that there had been a request for follow-up of individuals sprayed with CS, but no data had been forthcoming in view of the lack of compliance by individuals sprayed with CS with requests for clinical follow-up. The Committee explored possibilities for investigating possible adverse interactions. One suggestion was that it might be possible to review a summary of data from custody records for relevant information on effects in individuals who had been sprayed with CS and/or PAVA. Members suggested that police forces should flag all incidents where a police surgeon had been called to attend an incident or police station and that a summary of the number of such incidents (relating to CS or PAVA or combined exposure) should be made available. If possible information on whether individuals experienced breathing difficulties should be recorded. The Committee also noted the evidence from case reports of allergic sensitisation in police officers exposed to CS for a possible enhancement of skin effects in individuals with rosacea. Although the available evidence came from only a few individuals, in the context of the number of officers exposed or who have used CS sprays, it was felt that further surveillance for potential skin sensitisation among police officers was needed.

COT conclusions

16. Co-exposure to CS and PAVA is likely to result in, at most, additive effects on skin, eyes and respiratory tract in most individuals, although in some individuals a lower response might occur as a result of desensitisation.

17. The COT recommended that police forces should flag all incidents where a police surgeon had been called to attend an incident or police station and that a summary of the number of such incidents (relating to CS or PAVA or combined exposure) should be made available, together with any available on whether exposed individuals experienced breathing difficulties.

18. The COT agreed that the Association of Chief Police Officers (ACPO) should be asked to consider surveillance for potential skin sensitisation among police officers.

January 2006
COT/2006/04

Table 1. Summary of potential for toxicological interaction of CS and PAVA

Toxicological end point	MIBK	CS	PAVA (50% in ethanol)	Potential for interaction
Metabolism	Metabolised & cleared predominantly as metabolites (enzyme inducer)	Rapid in seconds	Some absorption across skin in 50% ethanol. Extensive hydrolysis in liver/skin	Unlikely following single co-exposure
Acute Toxicity (systemic effects)	Low acute toxicity	Low acute toxicity	Moderate acute oral (Capsaicin)	Unlikely following single co-exposure
Skin Irritancy	Low skin irritancy (defattening)	Sensory irritating with prompt recovery. Mild skin irritant	Mild skin irritant up to 3 days in rabbit	Potential for interaction at sensory receptors possible. Effects might be altered by solvents.
Eye Irritancy	Low eye irritancy	Severe eye irritant in MIBK (effects dependent on solvent)	Significant eye irritant (reversible)	Potential for increased severity of effect likely.
Skin sensitivity	No evidence from available studies.	Evidence from human exposure of skin sensitivity	LLN assay considered inadequate. No evidence of skin sensitisation from medicinal use	Unlikely following single co-exposure
Mutagenicity	No evidence of mutagenicity from available studies	*In vitro* mutagen and aneugen. Negative *in vivo* mutagen	Positive evidence from an *in vitro* chromosome aberration assay. Negative in two *in vivo* mutagenicity assays.	Unlikely following single co-exposure
Carcinogenicity	No data available	No evidence of carcinogenicity including sites of contact (these data were used to assist the mutagenicity evaluation)	No data available.	Unlikely following single co-exposure
Repeat dose systemic target organs	Liver, kidney (rat)	None identified	None identified	Unlikely following single co-exposure
Reproduction	No evidence of adverse effects	No study available	No study available	Unlikely following single co-exposure, but no data on PAVA available.
Teratogenicity	No evidence of teratogenicity	No evidence of teratogenicity	No study available	Unlikely following single co-exposure, but no data on PAVA available
Human data	Localised irritation and CNS depression at >100 ppm. Odour threshold 0.4 ppm, irritancy threshold 2 ppm	0.5-1mg/m^3 involuntary closure of eyes (blepharospasm), burning in mouth, nasal irritation, tightness in chest. Skin irritation, contact sensitisation reported. Sever pain in contact with eyes	Application in accordance with specified use resulted in bronchospasm in some asthmatics.	Potential for interaction of local site effects on eyes, skin and respiratory system.

References

1. Home Office Scientific Development Branch. (2004) Comparison of CS and PAVA: operational and toxicological aspects. Publication No 88/04 2004.

2. Association of Chief Police Officers of England, Wales and Northern Ireland.(2005) Incapacitant sprays – notes for guidance on police use. April 2005.

3. Committees on Toxicity, Mutagenicity, and Carcinogenicity of chemicals in food, consumer products, and the environment. (1999). Statement on 2-chlorobenzylidene malonitrile (CS) and CS spray. COT/1999/06

4. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. (2002) Statement on the use of PAVA (nonivamide) as an incapacitant spray. COT/02/2 – April 2002.

5. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. (2004) Statement on the use of PAVA (nonivamide) as an incapacitant spray. COT/04/6 – November 2004.

6. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. Risk assessment of mixtures of pesticides and similar substances. September 2002 (http://www.food.gov.uk/science/ouradvisors/toxicity/COTwg/wigramp/wigrampfinalreport)

7. Cucinell SA, Swenttzel KC and Biskup R et al. (1971) Biochemical interactions and metabolic fate of riot control agents. Fed Proc 30 86-91.

8. Ballantyne B and Swanston DW. (1978) The comparative acute mammalian toxicity of 1-chloro-acetophenone (CN) and 2-chlorobenzylidene malononitrile (CS). Arch. Toxicol. 40 75-95.

9. Olajos EJ and Salem H.(2001) Riot control agents: pharmacology, toxicology biochemistry and chemistry. J Appl Toxcicol 21 355-391.

10. Blain P (2003). Tear gases and irritant incapacitants. Toxicol Rev 22 (2) 103-110.

11. Watson K and Rycroft R (2005). Unintended cutaneous reactions to CS spray. Contact Derm 53 9-13.

12. Govindarajan VS and Sathyanarayana MN. Capsicum – production, technology, chemistry and quality. (1991) Part V. Impact on physiology, pharmacology, nutrition and metabolism; structure, pungency, pain and desensitisation sequences. Crit Rev Food Sci & Nut 29 (6) 435-474

13. Ebner F (1999). Expert report on the pharmacological-toxicological documentation of nonivamide (ABC pain plaster sensitive). October 1999.

14. Foster RW and Weston KM (1986). Chemical irritant analgesia assessed using the human blister base. Pain 25 269-278.

Statement on cyanogenic glycosides in bitter apricot kernels

Background

1. Bitter apricot kernels have recently been marketed as a health food in the UK. They contain high levels of amygdalin, a cyanogenic glycoside. The Committee were asked to consider whether there were sufficient data to establish a maximum upper level for safe intake of cyanide or cyanogenic substances.

2. In the 1970s and 1980s, amygdalin (also known as laetrile or, though not a recognised vitamin, as vitamin B17) extracted from bitter apricot kernels was sold as a treatment for cancer. The treatment was never proven and was associated with significant toxicity. Sale of these extracts was restricted under the terms of "The Medicines (Cyanogenetic Substances) Order 1984".

3. The Medicines and Healthcare products Regulatory Agency (MHRA) has advised that the kernels would be considered foods regardless of the cyanide content, unless presented as medicines by claiming to treat, cure or prevent a medical condition.

Cyanogenic glycosides in foods

4. As well as bitter apricot kernels, low levels of cyanide are also present in almonds, sweet apricot kernels and in the stones of other fruits such as cherries and consequently cyanide is present in some foods [1]. The maximum level of cyanide that can be present as a result of using such foods as flavourings is regulated under the terms of The Flavourings in Food Regulations 1992 (as amended). Otherwise the cyanide content of food is not specifically regulated except under the terms of the Food Safety Act 1990 which makes it an offence to sell or possess for sale food which is injurious to health.

5. Analytical data indicate that the bitter apricot kernels currently on sale have a mean cyanide (CN) content of 1450 mg/kg, approximately 0.5 mg CN/kernel. Data on the range of values for individual kernels are not available The value of 1450 mg/kg is consistent with data from the literature[2] which reports cyanide contents of <0.05, 1-2 and >2000 mg/kg for low, medium and high amygdalin containing apricot kernels respectively.

6. A number of other cyanogenic glycosides are found in foods, including linamarin (cassava, lima beans), prunasin (ferns) and sambunigrin (elderberries)[1].

Reviews by other regulatory agencies

7. The database on cyanide toxicity is limited particularly with respect to chronic intake.

8. As a result of the occurrence of cyanide in food originating from flavouring substances, the Council of Europe[3] reviewed cyanide toxicity and established a Tolerable Daily Intake (TDI). The TDI was based on data from a case-control study [4] which considered the effects of chronic intake of inadequately processed cassava, thought to be linked to the neurological condition konzo. In this study konzo was associated with a cyanide intake of 0.19-0.37 mg/kg body weight (bw) per day. The Tylleskär *et al* (1992) study[4] is considered in more detail in paragraph 19. An uncertainty factor of 10 was applied for inter-individual variation, resulting in a TDI of 20 μg/kg bw/day. An additional factor was not applied to extrapolate a lowest observed adverse effect level (LOAEL) to a no observed adverse effect level (NOAEL) since the condition was thought to be exacerbated by other dietary deficiencies such as of sulphate which would not be relevant to other populations. It was noted that the aetiology of konzo is not fully understood.

9. Safe intakes of cyanide from drinking water were considered by the World Health Organisation[5]. A TDI was established using data from a study in pigs fed 1.2 mg CN/kg bw/day for 6 months resulting in changes in behaviour and serum biochemistry[6]. This was used to establish a TDI of 12 μg/kg bw/day. An additional uncertainty factor was not applied to extrapolate from a LOAEL to a NOAEL since there were doubts about the biological significance of the observed changes.

10. In contrast, EFSA[1] concluded that there were insufficient chronic data to establish a TDI for cyanide but concluded that the current high level intake of 3-6 μg CN/kg bw/day from foods (notably certain types of marzipan) was not of concern.

Absorption and metabolism of cyanide.

11. Amygdalin (D-mandelonitrile-β-D-glucoside-6 -β-glucoside) (see fig 1, below) degrades to hydrogen cyanide, two molecules of glucose and benzaldehyde. Amygdalin hydrolysis is catalysed by the enzyme emulsin, a β-glucosidase also found in apricot kernels. Since β-glucosidase enzymes do not occur intracellularly in humans, swallowing of whole apricot kernels may not release much cyanide[7], however, chewing or grinding increases toxicity by releasing emulsin from lysosomes. The enzymatic breakdown of amygdalin occurs most rapidly in alkaline conditions. The β-glucosidase may be deactivated in the acid environment of the stomach but can then be partially reactivated in the alkaline environment of the gut[8]. Cyanogenic glycosides can also be hydrolysed by gut flora.

12. After oral administration, hydrogen cyanide is readily absorbed and rapidly distributed within the body.

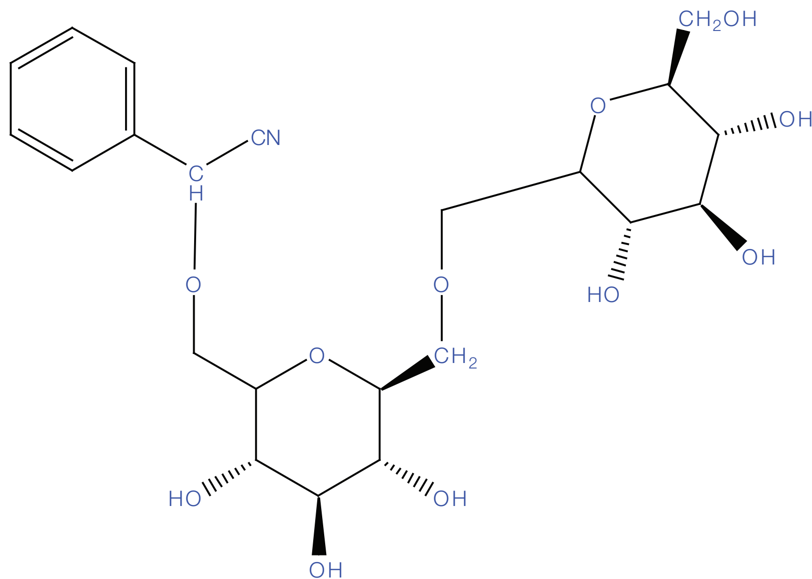

Fig 1. Structure of amygdalin

Toxicity of cyanide and cyanogenic glycosides

Acute toxicity in humans

13. Cyanide has high acute toxicity with a very steep and absorption rate-dependent dose-response curve[9]. The lethal dose of cyanide in humans is in the range 0.5 to 3.5 mg/kg bw[1]. Signs and symptoms of acute toxicity include headache, dizziness, mental confusion, stupor, cyanosis with twitching and convulsions, followed by terminal coma.

14. There are case reports of toxicity (including fatalities) resulting from the consumption of laetrile or amygdalin in a concentrated form, but also of toxicity resulting from the consumption of apricot kernels. Cyanide toxicity was also observed in an uncontrolled clinical trial of amygdalin[10].

15. Suchard *et al* (1998)[7] reported that a 41 year old female was found in a comatose and hypothermic state following the consumption of approximately 30 bitter apricot kernels. The patient responded to antidotal treatment and subsequently recovered. The authors noted that 5 other cases of poisoning had been reported in the US from consumption of bitter apricot kernels for their amygdalin content. In an earlier case reported by Rubino and Davidoff (1979)[11], an adult female was hospitalised following the consumption of 20-40 kernels.

16. There are case reports of poisonings in children consuming kernels from wild apricots[12]. The doses involved are unclear but the children were thought to have eaten more than 10 kernels. Similar cases have been reported in Gaza both from the wild apricot kernels and where the kernels were made into sweets without proper processing[13].

17. In a case reported by Bromley *et al* (2005)[14] an adult female presented at an emergency room feeling dizzy and unwell, having consumed 6 x 500 mg amygdalin tablets 30 minutes earlier. The toxicity was more significant than would be expected for the dose consumed. The authors concluded that the 3 g of vitamin C also consumed may have enhanced the toxicity of the amygdalin by promoting the release of cyanide from the molecule and decreasing stores of the amino acid cysteine which is involved in the detoxification of cyanide.

Chronic toxicity in humans

18. Several conditions have been observed in cassava eating populations which have been attributed to chronic cyanide intake. These include malnutrition, diabetes, congenital malformations, neurological disorders and myelopathy[1]. Goitre is thought to have occurred where cyanogenic glycosides are present in the diet at levels greater than 10- 50 mg/kg food.

19. Konzo is a distinct form of tropical myelopathy characterised by abrupt onset of spastic paraparesis (slight paralysis of the lower limbs). Epidemics occur where processing times for cassava are reduced[4]. A number of epidemiology studies have considered konzo (see[1,8]). In a konzo-affected population in former Zaire, the condition was associated with a cassava flour intake greater than 0.5 kg/day equivalent to an intake of 0.19 to 0.37 mg cyanide/kg bw/day[4]. Urinary thiocyanate levels (reflecting cyanide intake) were comparable in cases and controls but whole blood cyanide levels were elevated in 3/3 cases compared to 2/23 controls, suggesting that sustained high blood cyanide maintained by sulphur deficiency was associated with Konzo.

Cyanide toxicity in animals

20. Exposure to cyanide was reported to produce dose-related increasing ambivalence and slower response time to stimuli in pigs given oral doses of up to 1.2 mg/kg bw/day cyanide for 6 months[6]. Since behaviours demanding low energy were more affected it was suggested that an effect on glucose metabolism could be involved. There was a dose-dependent increase in fasting blood glucose which was evident after 12 weeks, and became statistically significant following 18 weeks of treatment. In the top dose group, blood glucose was increased by up to 60%. Serum thyroxine and, notably, triiodothyronine were reduced at all doses, but markedly at the top dose only.

21. Rats were given drinking water containing up to 300 mg/L sodium cyanide for 13 weeks (NTP, 1993- discussed[1]) (equivalent to approximately 12.5 mg/kg bw/day cyanide). No significant changes were apparent in haematology, clinical chemistry or urinary parameters. There were no treatment-related gross or histopathological changes in the rats. Slight changes were observed in the testes and spermatozoa of treated males. Comparable results were obtained from a 13 week study in mice. Testicular effects have also been observed in dogs fed a cassava or rice plus cyanide diet[15]. The data from these studies suggest that humans are more sensitive to cyanide toxicity since the lethal dose in humans is 0.5–3.5 mg/kg bw.

22. There are no data available from chronic or reproductive toxicity studies.

Exposure assessment

23. The kernels currently available contain approximately 0.5 mg cyanide/kernel (information on variation between individual kernels is not available). Consumers are advised to eat 5 kernels in an hour, but no more than 10 in a day. This represents a cyanide intake of 2.5 mg in an hour, with a maximum of 5 mg in a day, equivalent to 42 μg/kg bw (1 hour) or 83 μg/kg bw/day. The latter figure is 4 and 8 fold higher than the TDIs set by the Council of Europe and WHO respectively.

24. Whilst the retailer of these kernels recommended that this intake should not be exceeded, other information is readily available from the internet which advises that those suffering from cancer should gradually increase their consumption to 5 kernels/hour, 6 to 10 times a day. This would represent a maximum intake of 15-25 mg cyanide/day (equivalent to 250-417 μg/kg bw).

Discussion and Conclusions

25. The database for the toxicity of cyanide and cyanogenic glycosides in humans is incomplete. The acute lethal dose for cyanide is in the range 0.5 to 3.5 mg/kg bw. Case reports suggest severe toxicity arising from the consumption of approximately 30 bitter apricot kernels in adults, fewer in children. The cyanide concentration of the kernels is known to be variable and is not included in published reports, making precise comparisons difficult.

26. There are also relatively few data on the effects of chronic cyanide intake in humans. Konzo is a neurological condition associated with cyanide intake from improperly processed cassava. These data were used by the Council of Europe to establish a TDI of 20 μg/kg bw/day for cyanide.

27. The available evidence on konzo indicates that there are many confounding factors, and whilst cyanide intake may contribute it is likely to be one of a number of possible causal factors specific to a high cassava diet.

28. There is no available evidence in adequately nourished humans to show that chronic intake of cyanogenic glycosides causes a cumulative hazard above that of repeated acute toxicity. However, data from animal studies suggest that adverse effects may result from chronic exposure to cyanides and cyanogenic glycosides. Data on biochemical and behavioural changes in pigs were used by the WHO to derive a TDI of 12 μg/kg bw/day, which is comparable to that established by the CoE.

29. Overall, the Committee concluded the limited chronic data available were not sufficient to propose a TDI.

30. The range for the acute lethal dose in humans is 0.5 to 3.5 mg/kg bw. A 100 fold uncertainty factor (10 to account for inter-individual variability and 10 to extrapolate from an effect level to a no effect level, taking into account the steep dose-response relationship) could be applied to the lowest lethal dose. This would indicate that a dose of 5 μg/kg bw would be unlikely to cause acute effects, ie. a nominal acute reference dose (ARfD). This is comparable to the TDIs of 12 and 20 μg/kg bw/day established by WHO and CoE respectively.

31. Taking the available evidence together, consumption of 1 kernel per day would result in a cyanide intake of 0.5-mg/day (equivalent to 8 μg/kg bw for a 60 kg adult) which is in the region of this nominal ARfD and the TDIs proposed by others and would be unlikely to be of concern. This level of intake represents a threshold above which, increasing intake becomes increasingly hazardous.

32. The consumption of 10 kernels/day recommended with the sampled product would represent an intake of 5 mgs cyanide (equivalent to 83 5 μg/kg bw). This is one sixth of the lowest lethal dose and would cause a consumer to exceed the TDIs set by CoE and WHO for cyanide by 4-8 times and the nominal ARfD established as above, by 8-16 times. Such intake would therefore be hazardous. In addition, readily available information recommends far higher intakes of the kernels, which could be severely toxic, or, lethal in some people. Given the background to the product, exceedance of the dose recommended on the packaging seems probable.

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December 2006

References

1. EFSA (2004). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on Hydrocyanic Acid in Flavourings and other Food Ingredients with Flavouring Properties. Question no EFSA-Q-2003-0145. EFSA Journal (2004) 105.

2. Holzbecher, M.D., Moss, M.A., Ellenberger, H.A. (1984). The Cyanide Content of Laetrile Preparations, Apricot, Peach and Apple seeds. Clinical Toxicology, 22, 341-347.

3. CoE (2000) Council of Europe Committee of Experts on Flavouring Substances 46th meeting-RD 4.13/1-46 data sheet on HCN.

4. Tylleskär, T., Banea, M., Bikangi, N., Cooke, R.D., Poulter, N.H., Rosling, H. (1992). Cassava Cyanogens and Konzo, an Upper Mototneuron Disease Found in Africa. The Lancet, 339, 208-211.

5. WHO (2003). Cyanide in Drinking water. Background Document for development of WHO Guidelines for drinking water quality. WHO/SDE/WSH/03.04/05.

6. Jackson, L.C. (1988) Behavioural Effects of Chronic Sublethal Dietary Cyanide in an Animal Model: Implications for Humans Consuming Cassava (Mnihot esculenta). Human Biology, 60, 597-614.

7. Suchard, J.R., Wallace, K.L. Gerkin, R.D. (1989) Acute Cyanide Toxicity Caused by Apricot Kernel Ingestion. Annals of Emergency Medicine, 32:6, 742-744.

8. JECFA (1993) Joint Expert Committee on Food Additives. Cyanogenic glycosides. WHO Food Additives Series 30.

9. CICAD 61, (2004). Concise International Chemical Assessment Document (CICAD) 61, Hydrogen Cyanide and Cyanides: Human Health Aspects.

10. Moertel, C. G., Fleming, T.R., Rubin, J., Kvols, L.K., Sarna, G., Koch, R., Currie, V.E., Young, C.W., Jones. S.E., Davignon, J.P. (1982) A Clinical Trial of Amygdalin (Laetrile) in the Treatment of Cancer. NEJM, 306, 201-206.

11. Rubino, M.J., Davidoff, F. (1979). Cyanide Poisoning from Apricot Seeds, Journal of the American Medical Association, 241, 359.

12. Sayre, J.W., Kaymakcalavu, S. (1964) Hazards to Health. Cyanide Poisoning from Apricot Seeds among Children in Central Turkey. NEJM, 270, 1113- 1114.

13. Lasch, E.E., El Shawa, R. (1981). Multiple Cases of Cyanide Poisoning by Apricot Kernels in Children from Gaza. Pediatrics, 68, 5-7.

14. Bromley, J., Hughes, B.G.M.., Leong, D.C.S., Buckley, N.A. (2005) Life-Threatening Interaction Between Complementary Medicines: Cyanide Toxicity Following Ingestion of Amygdalin and Vitamin C. Annals of Pharmacotherapy, 39, 1566-1569.

15. Kamalu, B.P. (1993). Pathological Changes in Growing Dogs Fed on a Balanced Cassava (*Manihot esculenta* Crantz) diet. British Journal of Nutrition, 69, 921-934.

Update to statement on the review of toxicology literature on the use of topical insect repellent N,N-diethyl-m-toluamide (DEET)

Introduction

1. The COT previously assessed the safety of DEET in 2003 and at that time made a recommendation that the literature on DEET should be regularly reviewed. New information was obtained though an extensive literature search and by contacting HSE who are currently participating in a regulatory review under the Biocides Product Directive (BPD).

2. During their assessment, members looked at neurotoxicity studies, combined use of sunscreen and DEET, results from post-market monitoring in the UK and USA and further epidemiology/intervention studies. The outcome of this discussion was generally reassuring. However the neurotoxicity studies were found to have potential methodological problems and the results were difficult to interpret. Therefore the Committee recommended that repeat studies be carried out to clarify these issues. Members requested further information on the toxicokinetics of DEET and sunscreen to provide further reassurance on the safety of their combined use.

Background

3. The COT was asked by the Department of Health to review the available toxicology data on the insect repellent N,N-diethyl-m-toluamide, commonly known as DEET, as part of the strategy being developed by the Chief Medical Officer for England, Professor Sir Liam Donaldson on combating the potential for West Nile Virus (WNV) infection (see paragraph 4). The COT agreed a statement on DEET in 2002 and also agreed to keep DEET under review (http://www.advisorybodies.doh.gov.uk/pdfs/deetstatement.pdf). This update to the statement incorporates toxicology information published since 2002, information on biomonitoring of DEET in the UK and all other available data on DEET that has been made available since the original statement was published.

4. Insect repellents are used to prevent nuisance bites from mosquitoes (as well as ticks, biting flies and mites) and may aid in lowering disease transmission from these pests e.g. malaria and West Nile Virus (WNV). N,N-Diethyl-m-toluamide is the most widely used and best studied insect repellent currently available to the general public. DEET has been used world-wide for 40 years. It has been reported to give the best duration of protection and broad-spectrum effectiveness of topically applied insect repellents and is recommended by the United States Centre for Disease Control in helping to prevent infection with WNV.

5. DEET is marketed in the United Kingdom in a variety of formulations and concentrations including aerosol and pump-spray products intended for application to skin as well as for treating clothing. Liquid, cream, lotion and stick products enable direct skin application. The concentration in these products varies according to formulation type, between 10-95%. There are no data on the pattern of usage in the UK but a wide range of products is freely available over the counter or via the internet.

6. The Department of Health (DH) has published a strategy for combating the possibility of WNV infection. The strategy is intended to provide advice to the general public and to Environmental Health Departments. http://www.dh.gov.uk/assetRoot/04/08/33/33/04083333.pdf

Summary of Recommendations made in the DEET statement in 2002

- Information on exposure should be made publicly available
- Additional animal studies are required to verify the neuropathological effects seen in repeat dosing dermal studies of DEET in rats
- The Department of Health should undertake further monitoring for reports of adverse effects associated with exposure to DEET
- Consideration should be given to undertaking epidemiological studies
- Industry should seek to attain a consistent approach to labelling through voluntary action

Rational for Update Review

7. The objective of this review is to provide an update on the request for additional data requested by the COT in 2002. In this context information from adverse health surveillance schemes has been collated and reviewed. Additional toxicological information from the published literature has been reviewed, in particular a number of absorption studies on DEET following concurrent application of DEET and sunscreen. In addition comments on the risk assessment submitted by the DEET Joint Venture Group (DJV) as part of the regulatory review of DEET under the Biocides Product Directive (BPD) were sought from the COT. Exposure assessment was considered in the 2002 review and is only briefly referred to in this updated statement.

Regulatory control of insect repellents

8. At the present time there is no requirement for DEET-based insect repellents for topical application to human skin to be authorised under a regulatory scheme within the U.K. Topically applied insect repellents are regulated under the Biocides Products Directive (BPD)(98/8/EC introduced 14th May 2000) enacted in U.K legislation by the Biocide Products Regulations 2001 (which came into force on 6thApril 2001). Topically applied insect repellents for human skin are not considered as pesticides or as medicines. There are 23 categories of biocide product listed under 98/8/EC. Insect repellents are included in category 19: (Repellents and Attractants). A centralised review scheme for existing biocides products was set up by the European Union. Members were informed that DEET is currently being considered as part of this review scheme under the Biocide Products Directive. It is only once the review has been completed that individual products containing DEET will require authorisation in the UK. The Committee was also made aware that it would be possible that the COT updated statement could be forwarded to the rapporteur Member State (Sweden). The U.K Competent Authority is the HSE (Biocides and Pesticides Unit).

9. The available products would also have to conform to labelling requirements as established by the Chemicals (Hazards Information and Packaging for Supply) Regulations 2002 (CHIP) which enact EU Directives on Dangerous Substances and Preparations [76/548/EEC]. The COT was also informed that the EU review would provide information on usage and would also allow for consistent labelling to be applied to DEET products.

Summary of Additional Toxicology Information received since 2002

Metabolism studies in animals and humans

10. A number of publications regarding the transdermal absorption of DEET following concurrent application with sunscreen preparations are available. Generous and frequent application of sunscreens is recommended to minimize skin damage due to sun exposure. On the other hand, repellents are recommended for application on an 'as needed' basis. Concurrent application of commercially available repellent and sunscreen products resulted in significant percutaneous permeation of the repellent DEET and the sunscreen oxybenzone across mouse or piglet skin, *in vitro* (Gu et al., 2005; Gu et al., 2004 and Ross et al., 2004) and in an *in vivo* animal study (Kasichayanula et al., 2005).

11. Data from Gu et al. (2005) indicated that to minimize the transdermal absorption of active ingredients arising from the concurrent application of repellent and sunscreen products, sunscreens should be applied first to saturate the skin surface. Physically mixing these products prior to, or during application was not recommended as this could increase transdermal penetration of the active ingredients. These studies demonstrated that the permeability of DEET across mouse or piglet skin, *in vitro,* lead to increased DEET penetration but this was dependent on formulation type, application amount and the application sequence. In an *in vivo* animal study in nine week old piglets a slight enhancement of percutaneous penetration and systemic absorption of DEET and oxybenzone was observed when repellent and sunscreen preparations were used concurrently (Kasichayanula et al., 2005). Measurement of skin penetration rate and extent of a topical preparation was performed by tape stripping (Kasichayanula et al., 2005).

12. COT members considered the absorption of DEET when used concurrently with sunscreen. The committee was reassured by the *in vivo* study in pigs, which had shown only a slight enhancement in the absorption of DEET on concurrent application with sunscreen compared with DEET applied alone (Kasichayanula et al., 2005). Members agreed that if there was any effect of sunscreen on the absorption of DEET, this could reduce the DEET margin of safety following co-exposure with sunscreen. The committee concluded, in view of the differences between the *in vitro* and *in vivo* absorption studies, additional studies to investigate the effect of sunscreen on DEET absorption in human volunteers would be helpful to provide reassurance with regard to the risk assessment based on data from pigs.

Toxicology Studies in animals

Subchronic Neurotoxicity

13. The Committee considered additional neurotoxicity studies from Abou-Donia and colleagues (Abdel-Rahman et al., 2002, 2004a 2004b). These studies added to papers from this group, already reviewed by the Committee in 2002. These papers suggested that DEET applied dermally at 40 mg/kg/day for periods of 28-60 days can result in adverse effects on sensorimotor performance and histopathological changes in the CNS. The majority of these studies investigated the combined effect of DEET, permethrin and pyridostigmine bromide on sensorimotor and neuropathological effects on the brain.

14. Abdel-Rahman et al. (2004a) investigated the neurological effects induced by DEET, malathion and permethrin alone or in combination in adult rats. Groups of 10 male Sprague-Dawley rats received dermal doses of DEET at 40 mg/kg bw/day for 7 days a week for 30 days (in 70% ethanol). Animals treated with DEET (40 mg/kg bw/day) exhibited significant sensorimotor impairment compared to controls, which was reflected in inclined plane performance, forepaw grip time, beam-walk scores, and beam walk time when assessed after 30 days of daily exposure. Treatment with DEET alone at 40 mg/kg bw/day did not cause any significant changes in plasma BChE activity compared to control. However, treatment with DEET caused a significant increase in AChE activity in the cortex and the cerebellum of the brain. The authors found no change in AChE activity in the brainstem following treatment with DEET and reported significant reduction in the density of healthy or surviving neurons in the dentate gyrus, the CA1 and CA3 subfields of the hippocampal formation, the midbrain, the brainstem and cerebellum. The authors contended that a significant number of degenerating neurons were documented in these brain regions.

15. Abdel-Rahman et al. (2002) and a follow-up study in 2004b, investigated the effects of a combined exposure to restraint stress and low dose of pyridostigmine bromide (PB, 1.3 mg/kg bw/day, orally), permethrin (0.13 mg/kg bw/day, dermally) and DEET (40 mg/kg bw/day, dermally) in adult male rats, exposed daily for 28 days. Exposure to chemicals and stress produced blood brain barrier disruption and neuronal cell death in the cingulate cortex, dentate gyrus, thalamus and hypothalamus. Other regions of the brain such as the cerebellum, the cerebral cortex and the hippocampus demonstrated some neuronal cell death but did not exhibit blood brain barrier disruption. There was also decreased AChE activity in the forebrain, midbrain, brainstem and cerebellum and decreased m2-AChR binding in the midbrain and cerebellum. In contrast, in animals exposed to stress or chemicals alone, the above indices were mostly comparable to those of animals exposed to vehicles alone. The authors concluded that combined exposure to stress and low doses of the chemicals pyridostigmine bromide, permethrin and DEET leads to significant brain injury.

16. Inconsistent outcomes between studies were observed during neurobehavioural testing depending on the duration of treatment with DEET. Abdel Rahman et al. (2004a) reported that animals treated with DEET (40 mg/kg bw/day) exhibited significant sensorimotor impairment compared to controls, which was reflected in inclined plane performance, forepaw grip time, beam-walk scores, and beam walk time when assessed after 30 days of daily exposure. Abou-Donia *et al*. (2001a) reported significant effects on beam-walking, beam-walking time and grip strength when DEET was tested at 4, 40 and 400 mg/kg bw/day DEET for 60 days. These changes were not reproduced in another study, carried out by the same group, in which a dose of 40 mg/kg bw/day was administered for 45 days (Abou-Donia *et al*. 2001b). As discussed at COT previously, the results from a study by Schoenig et al. (1993) differ from these findings. Schoenig et al. (1993) observed neurobehavioural changes due to DEET but only at a higher dose, when rats were administered undiluted DEET at dose levels of 50, 200, or 500 mg/kg bw/day by gavage. The two measures of neurotoxicity evaluated by Schoenig et al. were functional observational battery (FOB) and motor activity measurements.

17. Different results were also observed for the effects of DEET on acetylcholinesterase (AChE) activity in the different brain regions in the available studies (Abdel-Rahman et al., 2004 and Abou-Donia et al., 2001b). This might have been due to differences in the duration of treatment with DEET with regard to effects on AChE. In a 30 day study (Abdel-Rahman et al., 2004a), treatment with DEET caused a significant increase in AchE activity in the cortex of the brain but had little or no effect on activity in the midbrain, brainstem, cerebellum in rats. However, in a 45 day study (Abou-Donia et al., 2001b) treatment with DEET caused a significant increase in brainstem AChE activity but had little or no effect on AChE activity in the cortex, midbrain or cerebellum in rats.

18. Members commented that the neuronal effects attributed to DEET in some of the studies might be due to artefacts such as the “dark cell” artefact caused by incorrect handling of the brain tissue after the death of the animal. Members expressed concern that the reported eosinophilic degeneration of neurons might reflect a basophilic post mortem change. However if there were significant microglial and astrocytic reaction to neuronal damage, it was more likely that the observed lesions occurred in-life.

19. Members agreed that Professor Abou-Donia should be asked to comment on the neuronal effects caused by DEET reported in these studies by his group. In the absence of reply, the Committee agreed that it was not possible to draw definitive conclusions on the evidence reported by Abou-Donia and colleagues and that there was a need for independent verification of these subchronic dermal neurotoxicity studies in rats using the dermal route of administration to evaluate the significance of the published findings for human health. The Committee reaffirmed its opinion reached in 2002 that there were considerable uncertainties regarding the studies published by Abou-Donia and colleagues. The Committee concluded that, in view of the potential methodological problems with these studies and difficulties in assessing the reported neuropathological and neurobehavioural effects, additional repeat studies to verify the results obtained represented the most appropriate course of action to take. Overall, it was not considered appropriate to use the data from these studies for risk assessment. This was consistent with the conclusions reached in 2002. The committee were aware of pre-publication experimental results of microglial reactions in the same tissues that showed neuronal cell death by Professor Abou-Donia but commented that no weight could be attributed to this information until it was available in a peer reviewed publication.

Toxicology Evidence from Human Case reports in the UK

20. In order to follow up the recommendation to undertake further monitoring for reports on adverse effects associated with exposure to DEET, the DH Toxicology Unit obtained data on any reports concerning DEET from the Hospital Accident Surveillance Scheme, the Hospital Episode Statistics and information from the National Poisons Information Service Centres from 1st Jan 2002 to 31st July 2005. Data were also obtained from the Royal Society for the Prevention of Accidents (ROSPA) from 1993 to 2001. In total there were reports of 35 individuals exposed to DEET and evidence to demonstrate potential for localised effects (skin/eye irritation). There were no reports of severe CNS toxicity in children (23 reports of minor adverse effects in children). The Committee was reassured that the effects were relatively minor and did not include any cases with overt neurotoxicity. The small number of cases when compared to the estimated high usage of DEET was also reassuring, but it was agreed that there were no precise data for the U.K. in this regard. It was noted that definitive data on exposure would be included in the review being undertaken under the Biocides Products Directive (98/8/EC).

21. Following a request from the COT secretariat, the DJV submitted a poster presentation on post-market biomonitoring data on DEET from the US. The National Registry of Human Exposure to DEET (DEET registry) was operated from 1995 to 2001. It was devised to better understand the role of DEET in more serious medical events. The DEET registry was a voluntary effort by 14 companies that either produce DEET and/or market formulated consumer insect repellents. The presentation indicated that there were over 5 billion applications of DEET during the 7 year span of the Registry and the authors found the overall risk from DEET of clinically significant adverse events to be very low.

Epidemiology Studies

22. When the first review of DEET by COT was undertaken in 2002, the COT commented that no published epidemiological studies of DEET exposure and adverse effects were available. Clinical investigation studies from McGready et al., (2001) and Menon and Brown (2002) have since become available in the literature.

23. McGready et al. (2001) undertook a study investigating the safety of DEET applied daily during the second and third trimesters of pregnancy in a group of Thai women as part of a double-blind, randomized, therapeutic trial of insect repellents for the prevention of malaria in pregnancy. The study received approval from the Ethical Review Committee of the Faculty of Tropical Medicine of Mahidol University, the Central Scientific Ethical Committee of Denmark, and the Karen Refugee Committee. Subjects were randomly allocated to receive a daily target dose of either DEET and thanaka, a local cosmetic (1.7 g of DEET and 3.2 g of thanaka) or thanaka alone (3.2 g of thanaka) until delivery. Women were instructed to apply the treatment daily after the evening shower to the exposed areas of the arms and legs. Apart from the sensation of skin warming with application of DEET, no significant adverse effects for the mother or the fetus following daily use of DEET were observed. Survival, growth, and neurological development in infants followed from birth up to one year of age did not differ from infants whose mother received thanaka alone. Whilst the authors concluded that the results of their study indicate little risk of DEET accumulating in the foetus and that DEET (20%) is safe to use in later pregnancy, the committee did not agree with this conclusion. The committee concluded that the study did not provide any information on the accumulation of DEET in the foetus and showed only that the risk of any adverse outcome in pregnancy was low, under the conditions of the study.

24. Menon and Brown (2002) conducted a cross-sectional survey on the use patterns of repellents on children and the associated effects in Maryland campgrounds in 2002. The research protocol was approved by the University of Maryland Institutional Review Board, and all parents of participants gave informed consent. The study yielded 301 respondents (numbers of non-respondents not indicated). DEET was the active ingredient used by most families. In only two instances (one case of eye irritation through direct contact and one case of skin rash), were possible adverse reactions observed by the parent within 24 hours of application of a repellent. In both cases, the repellent contained DEET.

25. Members stated that the available human studies were difficult to interpret but felt reassured that no serious effects were observed in these studies following exposure to DEET.

Risk assessment based on animal studies

26. The Committee was aware that the DJV had proposed that risk assessment of DEET should be undertaken on the basis of a comparison of Area Under the Curve (AUC) of DEET between dermal application in humans at the 75th percentile exposure (1.5 g/day for males and 1.0 g/day for females for the European population) and the NOAELs from subchronic dermal toxicity studies conducted with rats and mini-pigs. This approach is different to the approach outlined previously by the DJV that risk assessment could be undertaken on the basis of peak blood levels (Schoenig and Osimitz, 2001). The Committee felt that in the absence of direct evidence to support the use of the AUC, it was prudent to use peak blood levels for the risk assessment of DEET since an end point of acute neurotoxicity had been demonstrated (in oral studies in rats and dogs). The NOAEL in dogs of an oral dose of 75 mg/kg bw was agreed by the Committee in 2002 to be appropriate for use in the risk assessment and this was concurred by the present Committee.

27. Conclusions from the COT risk assessment of DEET made in 2002 were that a risk assessment should be undertaken on the basis of a comparison of peak plasma levels of DEET between dermal application in humans at the 95th percentile exposure (i.e 3 g DEET/day in adult females and 4 g DEET/day in adult males) and the NOAELs for neurotoxicity in rats and dogs. Quantitative comparison of the peak plasma levels of DEET showed that levels were 33x higher in dogs and 16-34x higher in rats given oral doses compared to dermal administration to humans. Members noted that there was no good marker of effect to evaluate dose response for neurotoxicity but agreed that the approach of using peak plasma levels of DEET was pragmatic and acceptable. However, members noted that, although toxicokinetic data were available from the studies in sensitive animal species and for humans, an uncertainty factor was still required for interspecies variability to take into account potential differences in toxicodynamics. It was also noted that the number of human volunteers was small so an uncertainty factor would be required to take into account inter-human variation. The Committee felt it was not possible, based on the data at the time, to determine the appropriate Uncertainty Factor to use in risk assessment but that it was likely to be between 10 and 100.

28. The risk assessment of combined use of DEET and sunscreen (oxybenzone) was complicated. The DJV had proposed the use of AUC kinetic data from piglets and toxicological data from the micro-piglet to provide consistency of species. The kinetic AUC data from piglets was then compared to AUC data from DEET exposure alone for humans at maximum predicted use levels (no data are available for co-exposure of humans to DEET and oxybenzone). The kinetic data for humans was adjusted to take account of differences in US and UK body weights and likely maximum use. A margin of safety (MOS) assessment compared the AUC blood level for piglet dermal exposure at the NOAEL and human dermal exposure based on blood level data adjusted for all UK adults was presented by the DJV. The DJV noted that there were many assumptions and uncertainties in this approach but in their view the MOS values were acceptable (see Table 1).

Time (hours)	MOS (AUC)	
	DEET and oxybenzone	DEET alone
8	751	676
24	536	433

Table 1: Data from the DJV. Calculated Margins of Safety (MOS) for AUC blood level comparisons of piglet dermal exposure at a NOAEL and human dermal exposure based on blood level data adjusted for UK adults

29. The Committee commented that combined data on DEET and oxybenzone in animals might not be completely appropriate for humans and noted that there were no relevant data for combined exposure to sunscreen and oxybenzone available for humans.

COT Discussion

30. The COT was aware of data to update its 2002 review of DEET. This particularly related to post-market monitoring and risk assessment of combined use of DEET and sunscreen. The COT was reassured with regard to the data on the likely acute CNS effects in children and considered no further follow up of data was required.

31. With regard to the risk assessment of DEET, the Committee concluded that the most appropriate approach for DEET alone was a conservative one using peak blood levels. With regard to the use of DEET and sunscreen the available approach suggested by the DJV needed additional human data on the toxicokinetics of DEET following combined use with sunscreen and data on repeated exposure in humans. The COT agreed these data requests should be forwarded to the UK regulatory authorities (HSE) and the rapporteur for the BPD review when it became available.

Conclusions

The Committee agreed the following conclusions.

Regulatory control of insect repellents

32. The Committee was aware that DEET was currently being considered as part of a review scheme under the Biocide Products Directive and that it would be possible that the COT updated statement could be forwarded to the rapporteur Member State.

Animal toxicity data

33. Additional evidence for neurotoxicity and neuropathological lesions following repeated dermal application of DEET to rats at comparatively low dose levels have been published since the 2002 review. The Committee concluded in 2002 and again in 2006 that, in view of the potential methodological problems with these studies, and difficulties in assessing the results, additional repeat neuropathology studies were important in order to adequately assess the claimed effects. Members felt that industry should be asked to consider commissioning appropriate research. However the balance of evidence suggested that it was not appropriate to use the data from these studies for risk assessment until further clarification of the studies is obtained.

Risk Assessment

34. The Committee concluded that the most appropriate approach for risk assessment of DEET alone was a conservative one using peak plasma levels of DEET in experimental animals at the NOAEL and in humans at the 95th percentile of exposure and this is in agreement with the conclusions reached by the Committee in 2002. Further studies on the toxicokinetics following combined exposure to DEET and sunscreen in humans were considered desirable in order to confirm the risk assessment which had been submitted. The Committee requested that this information be made available to the appropriate regulatory agencies, once the studies have been completed.

Evidence in humans

35. The Committee was reassured by the results of post-market monitoring of DEET for reports of adverse effects associated with exposure to DEET, Human case reports, collated from information provided by the National Poisons Information Service Centres (NPIS), the Hospital Episode Statistics (HES) and the Hospital Accident Surveillance Scheme (HASS), indicated that the effects seen following exposure to DEET were relatively minor and did not include any cases with overt neurotoxicity. The available information from the US was also reassuring and suggested that any acute adverse effects following normal use were very rare.

36. Since the 2002 review, two epidemiological/intervention studies of DEET exposure have been published. The Committee agreed that these studies were difficult to interpret but felt reassured that no serious effects were observed in the subjects following exposure to DEET.

37. The Committee noted the ongoing regulatory review under the BPD and agreed that future consideration of DEET should be undertaken by the appropriate regulatory agencies.

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November 2006

References

1. Abdel-Rahman, A. Shetty, A.K. Abou-Donia, M.B. (2002). Disruption of the blood brain barrier and neuronal cell death in cingulated cortex, dentate gyrus, thalamus and hypothalamus in a rat model of gulf-war syndrome. Neurobiology of disease, 10, 306-326.

2. Abdel-Rahman, A. Dechkovskaia, A.M. Goldstein, L.B. Bullman, S.H. Khan, W. El-Masry, E.M. Abou-Donia, M.B. (2004a). Neurological deficits induced by malathion, DEET and permethrin alone or in combination in adult rats. J. Tox Environ. Health Part A 67, 331-357.

3. Abdel-Rahman, A. Abou-Donia, S. El-Masry, E. Shetty, A. Abou-Donia, M.B. (2004b). Stress and combined exposure to low dose of pyrdiostigmine bromide, DEET and permethrin produce neurochemical and neuropathological alterations in cerebral cortex, hippocampus and cerebellum. J. Tox. Environ. Health 67 (2), 163-192

4. Abou-Donia, M.B. Goldstein, L.B. Dechovskaia, A. Bullman, S. Jones, K.H. Herrick, E.A. Abdel-Rahman, A.A. Khan, W.A. (2001a). Effects of daily dermal application of DEET and epermethrin, alone and in combination, on sensorimotor performance, blood-brain barrier, and blood-testi barrier in rats. J. Tox. Environ. Health part A. 62:523-541.

5. Gu, X. Kasichayanula, S. Fediuk, D.J. Burczynski, F.J. (2004). In-vitro permeation of the insect repellent N,N-diethyl-m-toluamide (DEET) and the sunscreen oxybenzone. J Pharm Pharmacol. 56(5), 621-8.

6. Gu, X. Wang, T. Collins, D.M. Kasichayanula, S. Burczynski, F.J. (2005). In vitro evaluation of concurrent use of commercially available insect repellent and sunscreen preparations. Br J Dermatol. 152(6), 1263-7.

7. Kasichayanula, S. House, J.D. Wang, T. Gu, X. (2005). Simultaneous analysis of insect repellent DEET, sunscreen oxybenzone and five relevant metabolites by reversed-phase HPLC with UV detection: application to an in vivo study in a piglet model. J Chromatogr B Analyt Technol Biomed Life Sci. 822(1-2), 271-7.

8. McGready, R. Hamilton, K.A. Simpson, J.A. Cho, T. Luxemburger, C. Edwards, R. Looareesuwan, S. White, N.J. Nosten, F. Lindsay, S.W. (2001). Safety of the insect repellent N,N-diethyl-M-toluamide (DEET) in pregnancy. Am J Trop Med Hyg. 65(4), 285-9.

9. Menon, K.S. Brown, A.E. (2005). Exposure of children to Deet and other topically applied insect repellents. Am J Ind Med. 47(1), 91-7.

10. Ross, E.A. Savage, K.A. Utley, L.J. and Tebbett, I.R. (2004). Insect repellent [correction of repellant] interactions: sunscreens enhance DEET (N,N-diethyl-m-toluamide) absorption.Drug Metab Dispos. 32(8), 783-5.

11. Schoenig, G.P. and Osimitz, T. G. (2001) DEET. In: Krieger, R., ed., *Handbook of Pesticide Toxicology*, Vol. 2. Agents; San Diego, Academic Press, pp. 1439 – 1459.

12. Schoenig, G.P. Hartnagel, R.E., Schardein, J.L. & Vorhees, C.V. (1993) Neurotoxicity Evaluation of N,N-Diethyl-M-Toluamide (DEET) in Rats. *Fundamental and Applied Toxicology* **21**, 355-365.

Meeting report on the development and function in adulthood of the human male reproductive system – potential chemical-induced effects

Introduction

1. In August 2004, the Committee issued a statement on adverse trends in the development of the male reproductive system focussing on the hypothesis that these effects were due to exposure to endocrine disrupting chemicals at critical developmental windows[1]. At that time, although the evidence of endocrine disruption in wildlife was convincing, the Committee noted that extensive international reviews had not provided direct evidence that exposure to endocrine disrupting chemicals has adversely affected the human male reproductive system.

2. One of the Committee's recommendations was that a scientific meeting be held to review the evidence of adverse trends in male reproductive health, which in 2004 was conflicting, particularly with regards to sperm quality. Although not within the terms of reference of the COT, it was also considered important that the mechanisms involved in the formation of developmental abnormalities be investigated.

3. Male reproductive tract development is primarily driven by fetal testicular production of a number of hormones and signalling factors[2,3]. Disturbance of this complex process, either by genetic mutation or by pharmaceutical or environmental interference is hypothesised to result in disorders of male reproductive health, including low sperm counts, hypospadias, cryptorchidism and testicular cancer. These disorders are common in Western Europe, incidence may still be increasing[4-7], and evidence increasingly supports that all are interrelated symptoms of an underlying hypothesised pathology, namely a testicular dysgenesis syndrome (TDS)[8]. However, low sperm counts, hypospadias, cryptorchidism and testicular cancer may arise independently and there remains considerable uncertainty regarding the etiology of TDS[9].

4. In order to evaluate the evidence produced since this subject was last reviewed, in February 2006 the COT held a one-day workshop on development and function in adulthood of the male reproductive system. Presentations considered a range of topics, including cross-sectional and case-control studies of sperm quality and congenital malformations, the TDS hypothesis, potential chemical causes of reported effects, including cumulative effects of *in utero* exposure to anti-androgens and alternative hypotheses to that of endocrine disruption. Although potential chemical causes of cancer fall within the remit of the Committee on Carcinogenicity (COC) the inclusion of testicular cancer in the TDS hypothesis required its consideration by the COT. Information from the talks and subsequent discussions is summarised here. This statement is not a comprehensive review of the extensive scientific literature of relevance to this topic.

Evidence of a trend towards lower sperm quality and counts

5. In 2004, the COT considered that, given the conflicting reports of significantly declining sperm counts[10-17], the evidence was equivocal. It is likely that these differences were in part due to the fact that some studies suffered from subject selection bias. In addition, the Committee noted that measurement of the quality of human sperm (density, motility and morphology) was subject to a number of sources of uncertainty, e.g. semen analysis methodological differences.

6. In the International Study of Semen Quality in Partners of Pregnant Women, a coordinated cross-sectional study of men across four European cities (Turku, Edinburgh, Paris and Copenhagen)[18], significant geographical differences in semen quality were detected. This study also detected seasonal variations in sperm concentrations and total sperm counts highlighting the need for future prospective studies to factor this into their design. The study population consisted of male partners (aged 20–45) of pregnant women, inevitably not including infertile men and likely to under-represent sub-fertile men. However, for these four cities, the data may be considered as a reference point for future studies on time trends in semen quality.

7. The Study for Future Families (SFF) utilised a design consistent with the International Study of Semen Quality in Partners of Pregnant Women[18] and examined sperm quality and other reproductive parameters in fertile couples in four cities in the north, east, west and south-central USA[19,20]. Sperm concentration and motility were significantly lower in the Missouri cohort relative to the cohorts from New York, Minneapolis and Los Angeles. The study authors hypothesised that the Missouri cohort's proximity to intensive agriculture using agricultural pesticides may relate to the poor sperm quality characteristics and further conducted a nested case-control study within this cohort, measuring urinary concentrations of eight pesticide metabolites. Pesticide metabolite levels were elevated in cases compared with controls for the herbicides alachlor and atrazine and for the insecticide diazinon. The association suggested to the study authors that exposure to current-use pesticides may have contributed to the reduced sperm quality seen in fertile men.

8. The European Union-funded INUENDO Project (http://www.inuendo.dk) has recently published initial findings from a cross-sectional study in pregnant women and their partners in Poland, Ukraine and Greenland[21]. A cohort of Swedish fishermen and their spouses were included but recruited independently of current pregnancy. An association between lipid adjusted serum concentrations of the persistent organic pollutants (POPs) PCB-153 and *p,p'*-DDE and time to pregnancy, sperm motility and morphology was investigated. A geographical difference between cohorts in fecundability compatible with serum *p,p'*-DDE concentrations was identified. However, it was not possible for the study authors to control for residual confounding given the differences in sample population demographics.

9. Following on from the Partners of Pregnant Women study[18], a "historically prospective cohort study" of Scottish male reproductive health was commissioned by the Department of Health (with involvement of DEFRA and HSE). Two of the aims of this study are:

   - To obtain a 1999-2000 estimate of (i) exposures to various factors suspected to adversely affect sperm quality and (ii) sperm quality, and of any association between these.

   - To distinguish the effects of parental exposures (intra-uterine and perinatal effects mediated through maternal diet, smoking and potentially exposure to environmental chemicals) and direct effects (adult exposures and lifestyle such as smoking, scrotal heating and exposure to defined testicular toxicants), using a matched pairs design to study twin births.

   This study is yet to report its main findings, but it would appear that gaining and meeting the terms of the ethical approval for this study proved a significant obstacle, and impacted negatively on the achieved response rate. However, when complete, data comparison with historical data for Scottish males[18,22] should provide an indication of sperm and semen quality trends in this population.

Testicular cancer and congenital genital malformations (cryptorchidism and hypospadias)

10. An increasing trend in the incidence of testicular cancer has been shown in Northern and Western Europe[23-26], Canada[27,28], New Zealand[29], Australia[30] and the US[31,32]. This was recently confirmed for northern European countries in an investigation using cancer registry data, although this study also highlighted large geographical variations and an attenuated incidence trend in Sweden from the early 1990s[5]. In this, and other studies[23,27,31,32], the increasing incidence of testicular cancer closely correlated with year of birth, i.e. a birth cohort phenomenon.

11. Cryptorchidism (undescended testis) is the best characterised and numerically most important risk factor for testicular cancer[33-35], but the etiologic fraction (proportion of cases of testis cancer explained by cryptorchidism) is only around 10% and cannot explain the observed temporal trends. Previous investigations and studies have consistently shown an increased risk of testicular cancer among fathers and brothers of testicular cancer patients[36-38]. Potentially relevant gene loci have been identified by association studies[39], segregation analysis[40], linkage, and microsatellite analysis[41,42]. Recently, a population-based case-control study in Germany showed that testicular cancer aggregates in families[43]. However, the study authors noted that such studies on familial disease aggregation require careful interpretation in order to attribute disease accumulation to genes when lifestyle, environmental factors and sibship sharing gestational characteristics are shared by family members.

12. Cryptorchidism and hypospadias (abnormally placed urethral meatus) are common congenital abnormalities. However, determining whether reported increases in incidence of these abnormalities are real has been confounded by differences in diagnostic criteria. Cryptorchidism has an established association with hypospadias[44].

13. In general, registry data on hypospadias are not reliable and not comparable between countries, and often cryptorchidism is not listed in registries of malformations. This was highlighted by a retrospective study in the Netherlands[45] that, using a carefully structured diagnostic procedure, reported a 0.7% incidence of hypospadias whereas the registry data for the same region reported a 4- to 6-fold lower incidence.

14. A significant increase in the incidence of cryptorchidism in the UK from the late 1950s to late 1980s was determined following time-trend analysis of two well-standardised and clearly reported studies[46,47].

15. Notable differences in semen quality between Denmark and Finland, with Denmark having poorer reproductive health[18,48], led researchers to undertake a synchronised and standardised cohort study to investigate the prevalence of congenital cryptorchidism in Denmark and Finland[6]. Significantly, researchers used the examination technique and definition of cryptorchidism developed by Scorer[46] allowing direct comparison of results with previous studies in the UK[46,47] and Lithuania[49]. Boisen and colleagues[6] reported a marked increase in the birth prevalence of cryptorchidism in Danish boys with normal birthweight (1.8% in 1959-61 compared to 8.5% in 1997-01). In addition, prevalence was four-fold higher in Denmark than Finland, which corresponds to a high incidence of testicular cancer in Danish men and a prevalence in Finnish men that is amongst the lowest in Europe[23,24].

16. In terms of hypospadias, the joint prospective cohort study of Finnish and Danish boys (recruited 1997-1999 and 1997-2002, respectively)[6,7] also showed significant differences in prevalence of hypospadias between the two cohorts[7]. A 1% birth-rate of hypospadias in the Danish cohort was detected which compared to a significantly lower rate of hypospadias (0.27%) in the Finnish cohort study. The etiology of hypospadias remains unclear, although this study suggested associations between hypospadias and fetal growth impairment, and hypospadias and elevated serum FSH levels at 3 months of age.

17. In rodents, as perineal growth is dihydrotestosterone-dependent, anogenital distance (AGD) is a sensitive intermediate endpoint of anti-androgenic effects. Its measurement is included in the OECD Two-generation Reproductive Toxicity Test Guideline (TG 416). This measure of prenatal anti-androgen exposure has only recently been evaluated in human infants[50], and shown to be, as in rodents, sexually dimorphic and about twice as long in males as in females. A recently published study of AGD among human infants[51] reported shortened AGD and impaired testicular descent in boys whose mothers had elevated levels of prenatal phthalate exposure. The authors acknowledged that the reliability of AGD measurement in humans has not been established and the impact of shortened AGD at birth to male reproductive health in adulthood is unknown.

Testicular dysgenesis syndrome

18. The testicular dysgenesis syndrome hypothesis arose out of the findings that human male reproductive disorders in babies (cryptorchidism, hypospadias) or in young men (testis cancer, low sperm counts) are interrelated. This hypothesis proposes that maldevelopment (dysgenesis) of the fetal testis results in hormonal or other malfunctions of the testicular somatic cells, which in turn predispose to the disorders that comprise TDS.

19. A recent review[52] highlighted the evidence in support of the TDS, in particular for cryptorchidism, hypospadias and low sperm counts, identifying the points of vulnerability to endocrine disruption. For testicular cancer, although it is postulated that failure of normal differentiation of fetal germ cells and their subsequent conversion to pre-malignant carcinoma-*in-situ* (CIS) cells is involved, the mechanisms are not fully established.

20. Considerable research effort has sought to establish causal links between TDS and environmental chemicals with endocrine disrupting properties. However, it has also been proposed that epidemiological evidence supports a contribution of environmental and genetic components[53,54]. Within this proposal is the hypothesis that exposure to a toxic agent would lead to a mutation, genetic damage or epigenetic process that increases the risk of one or more endpoints of TDS[55]. Survival of the mutation in subsequent generations will be dependent on its effect on health and fertility, and behavioural factors such as family size. However, candidate chemicals that fit into this hypothesis have yet to be identified.

21. A TDS-like pattern of disorders can be induced in male rodents by exposure *in utero* to high doses of certain phthalate esters[56-62]. This shows some clear parallels with human TDS (cryptorchidism, hypospadias, low sperm counts/low fertility, areas of focal dysgenesis and Sertoli cell-only tubules in the testis) including the relationship of these disorders to malfunction of the somatic cells of the fetal testis, which is hypothesised to underlie human TDS. For example, using dibutyl phthalate (DBP) it has

been shown that *in utero* exposure induced focal dysgenesis in the rat testis by inducing aberrant migration/aggregation of Leydig cells in fetal life. This manifests in adult animals as focal dysgenetic areas, intratubular Leydig cells and focal occurrence of Sertoli cell-only tubules. However, so far, no animal model has been able to mimic all the symptoms of TDS, i.e. including testicular germ cell tumours (TGCTs), although CIS-like cells have been found in a spontaneous testicular neoplasm in a rabbit[63,64]. Although anti-androgens are able to reproduce the TDS-like changes in rodent testis, it remains to be established whether any associations between the symptoms of TDS in humans and exposure to external chemicals can be detected and are causal. The phthalate model of TDS in rodents offers the possibility to study the mechanisms that lead to TDS disorders. Although phthalates could theoretically contribute to TDS disorders in humans these studies cannot be regarded as providing evidence that phthalates cause TDS in humans.

Male reproductive system disorders and chemical exposure

22. Following the initial reports, in the early 1990s, of declining male reproductive capacity, Sharpe and Skakkebaek[65] redefined the 'estrogen hypothesis' originally proposed for testicular cancer[66-68], to implicate altered prenatal estrogen exposure in the increasing incidence of other male reproductive abnormalities.

23. It has been established that administration of diethylstilbestrol (DES; a potent synthetic estrogen) to pregnant women and rodents causes male reproductive tract malformations[69,70]. These effects were observed at pharmacologically active doses of DES. Such exposures may have little relevance to potential exposures to estrogens occurring in the environment, which are significantly less potent than DES and present only at low concentrations. As such, although evidence from animal studies shows that potent estrogens are capable of inducing the phenotype of TDS, the concentrations of less potent environmental estrogens required to induce such effects has brought into question whether estrogen exposure is an etiological factor in inducing TDS[71]. In fact, a recent review of published epidemiological studies of male reproductive disorders and prenatal indicators of estrogen exposure[72], found, with the exception of testicular cancer, no strong evidence to indicate that prenatal exposures to estrogens are linked to disturbed development of the male reproductive organs. However, some estrogenic chemicals (e.g. bisphenol A and nonylphenol) have been shown to also exhibit anti-androgenicity *in vitro*[73,74] and *in vivo*[75] and it may be their anti-androgenic properties that are of importance.

24. The original hypothesis proposed (i) suppression of follicle stimulating hormone (FSH) secretion and (ii) impaired Leydig cell development as plausible mechanisms via which estrogen exposure could induce these disorders[65]. However, new findings implicating (i) suppression of testosterone and insulin-like factor 3 production, and (ii) inhibition of androgen receptor expression point towards the male reproductive disorders being caused by chemicals exhibiting anti-androgenic rather than estrogenic properties[71]. Genetic disorders affecting normal androgen production and action in fetal life (e.g. complete androgen-insensitivity syndrome) provide support for this hypothesised role of anti-androgens.

Cumulative exposure to similarly acting anti-androgens

25. Anti-androgenic phthalate esters, such as dibutyl phthalate, induce cryptorchidism, hypospadias, impaired spermatogenesis, and reduce male fertility in rats[56-59,61]. Although these findings have been

supported by the recent development of a possible animal model for TDS[60], whether the level of environmental exposure to any single anti-androgen is sufficient to impact on human male reproductive health is questionable[76]. However, in practice, exposure is to multiple anti-androgenic chemicals, never to single agents, and this has motivated research into the question as to whether several anti-androgens are capable of acting together.

26. Researchers have begun to investigate the effects of binary mixtures of anti-androgens in order to establish whether cumulative effects are additive and predictable on the basis of knowledge of the dose-response relationships of the individual mixture components[77-79]. Endpoints that have been shown to be sensitive and relevant in rodents are anogenital distance, retained nipples, sex accessory organ weight and reproductive tract malformations. Results from these studies have largely indicated that joint effects are predictable and dose additive[77], even for two chemicals with apparently different mechanisms of action[78].

27. In addition, the COT was informed about ongoing *in vivo* studies with multi-component mixtures (with between three and seven chemicals) investigating whether joint effects occur when each individual mixture component is present at concentrations below that which induces a detectable effect (Hass, U. and Gray Jr, L.E.; personal communications). These studies have been designed to not only assess mixtures of chemicals shown to have common mechanisms of action but to also specifically investigate mixtures of chemicals which act via different mechanisms to induce the same toxicological effect. Interestingly, data on AGD in rats from experiments combining seven anti-androgens with various mechanisms of action indicate that joint effects are dose additive (simple similar action) rather than response additive (simple dissimilar action)[80]. If these findings are confirmed then this might indicate the need to review the current assumptions relating to risk assessment of mixtures of chemicals with dissimilar mechanisms of action.

COT Discussion

28. The new epidemiology studies reported since the COT issued its statement on adverse trends in development of the male reproductive system, including those presented at the COT one-day workshop, provide further evidence that male reproductive health is declining in some populations. However, causal associations in humans have not been established, and in fact, it should be noted these studies were not designed to provide such evidence. The report that anogenital distance (the most sensitive marker of anti-androgen action in studies in rodents) is shortened and testicular descent impaired in human male offspring of mothers with elevated prenatal phthalate exposure[51] was considered of interest. The Committee agrees with the study authors assertion that follow-up of this cohort into adulthood, as well as confirmation of these findings in a significantly larger cohort, is necessary before any conclusions of regulatory relevance can be drawn. The COT would further recommend analysis of possible confounding factors (in addition to ethnicity and diet) and whether phthalate exposure is a marker of mixed chemical exposures.

29. The COT noted that in Europe new legislation on ethical approval for human epidemiological studies was likely to make conducting studies on semen quality and congenital genital malformations unfeasible. Considering the importance of monitoring trends in these endpoints and the need to establish any causal associations, the Committee would encourage changes to legislation that would facilitate the undertaking of such studies in the future.

30. The high quality animal studies reported at the workshop were considered to be identifying plausible mechanisms of action. In particular, the Committee agreed that the hypothesised causative role of exposure to anti-androgenic chemicals, supported by the data being produced in animal models, was more plausible than that postulated for environmental estrogenic chemicals.

31. The Committee awaits the publication of results from the ongoing studies in rats of multi-component mixtures of similarly acting and mixtures of dissimilarly acting anti-androgens where individual chemicals are administered at doses more relevant to the human exposure situation than previously published high-dose studies. It remains to be seen whether the tools used in these studies will be useful for risk assessment of chemicals that cause a common effect. Initial indications from these studies on the effects of binary mixtures of chemicals with the same mode of action support the default assumption of dose additivity, previously recommended by the COT[81,82]. Studies of mixtures of dissimilarly acting anti-androgens will add significantly to understanding of their joint action and how to conduct risk assessments of such chemicals. The COT supports the continued research on characterising dose-response relationships for mixtures of anti-androgens. Analysis of these studies may require a more detailed understanding of the toxicokinetics of these chemicals.

32. Even though a clear link between experimental data and epidemiology is still missing, it was considered that the new data continue to emphasise the importance of this area of research, the need to actively investigate causation and for risk assessment to incorporate consideration of potential for combination effects.

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References

1 COT. (2004). Adverse trends in development of the male reproductive system – potential chemical causes. COT Statement 2004/04. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment.

2 Rouiller-Fabre, V., Levacher, C., Pairault, C., Racine, C., Moreau, E., Olaso, R., Livera, G., Migrenne, S., Delbes, G., Habert, R. (2003). Development of the foetal and neonatal testis. *Andrologia* 35: 79-83.

3 Brennan, J. and Capel, B. (2004). One tissue, two fates: molecular genetic events that underlie testis versus ovary development. *Nat Rev Genet* 5: 509-521.

4 Toppari, J., Larsen, J.C., Christiansen, P., Giwercman, A., Grandjean, P., Guillette, L.J., Jr., Jegou, B., Jensen, T.K., Jouannet, P., Keiding, N., Leffers, H., McLachlan, J.A., Meyer, O., Muller, J., Rajpert-De Meyts, E., Scheike, T., Sharpe, R., Sumpter, J., Skakkebaek, N.E. (1996). Male reproductive health and environmental xenoestrogens. *Environ Health Perspect* 104 Suppl 4: 741-803.

5 Richiardi, L., Bellocco, R., Adami, H.O., Torrang, A., Barlow, L., Hakulinen, T., Rahu, M., Stengrevics, A., Storm, H., Tretli, S., Kurtinaitis, J., Tyczynski, J.E., Akre, O. (2004). Testicular cancer incidence in eight northern European countries: Secular and recent trends. *Cancer Epidemiol Biomarkers Prev* 13: 2157-2166.

6 Boisen, K.A., Kaleva, M., Main, K.M., Virtanen, H.E., Haavisto, A.M., Schmidt, I.M., Chellakooty, M., Damgaard, I.N., Mau, C., Reunanen, M., Skakkebaek, N.E., Toppari, J. (2004). Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. *Lancet* 363: 1264-1269.

7 Boisen, K.A., Chellakooty, M., Schmidt, I.M., Kai, C.M., Damgaard, I.N., Suomi, A.M., Toppari, J., Skakkebaek, N.E., Main, K.M. (2005). Hypospadias in a cohort of 1072 Danish newborn boys: prevalence and relationship to placental weight, anthropometrical measurements at birth, and reproductive hormone levels at three months of age. *J Clin Endocrinol Metab* 90: 4041-4046.

8 Skakkebaek, N.E., Rajpert-De Meyts, E., Main, K.M. (2001). Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 16: 972-978.

9 Skakkebaek, N.E.(2004). Testicular dysgenesis syndrome: new epidemiological evidence. *Int J Androl* 27: 189-191.

10 Carlsen, E., Giwercman, A., Keiding, N., Skakkebaek, N.E. (1992). Evidence for decreasing quality of semen during past 50 years. *BMJ* 305: 609-613.

11 Auger, J., Kunstmann, J.M., Czyglik, F., Jouannet, P. (1995). Decline in semen quality among fertile men in Paris during the past 20 years. *N Engl J Med* 332: 281-285.

12 Fisch, H., Goluboff, E.T., Olson, J.H., Feldshuh, J., Broder, S.J., Barad, D.H. (1996). Semen analyses in 1,283 men from the United States over a 25-year period: no decline in quality. *Fertil Steril* 65: 1009-1014.

13 Irvine, D.S., Cawood, E.H., Richardson, D.W., MacDonald, E., Aitken, R.J. (1995). A survey of semen donation: phase II the view of the donors. *Hum Reprod* 10: 2752-2753.

14 Paulsen, C.A., Berman, N.G., Wang, C. (1996). Data from men in greater Seattle area reveals no downward trend in semen quality: further evidence that deterioration of semen quality is not geographically uniform. *Fertil Steril* 65: 1015-1020.

15 Van Waeleghem, K., De Clercq, N., Vermeulen, L., Schoonjans, F., Comhaire, F. (1996). Deterioration of sperm quality in young healthy Belgian men. *Hum Reprod* 11: 325-329.

16 Swan, S.H., Elkin, E.P., Fenster, L. (1997). Have sperm densities declined? A reanalysis of global trend data. *Environ Health Perspect* 105: 1228-1232.

17 Swan, S.H., Elkin, E.P., Fenster, L. (2000). The question of declining sperm density revisited: an analysis of 101 studies published 1934-1996. *Environ Health Perspect* 108: 961-966.

18 Jorgensen, N., Andersen, A.G., Eustache, F., Irvine, D.S., Suominen, J., Petersen, J.H., Andersen, A.N., Auger, J., Cawood, E.H., Horte, A., Jensen, T.K., Jouannet, P., Keiding, N., Vierula, M., Toppari, J., Skakkebaek, N.E. (2001). Regional differences in semen quality in Europe. *Hum Reprod* 16: 1012-1019.

19 Swan, S.H., Kruse, R.L., Liu, F., Barr, D.B., Drobnis, E.Z., Redmon, J.B., Wang, C., Brazil, C., Overstreet, J.W. (2003). Semen quality in relation to biomarkers of pesticide exposure. *Environ Health Perspect* 111: 1478-1484.

20 Swan, S.H.(2006). Semen quality in fertile US men in relation to geographical area and pesticide exposure. *Int J Androl* 29: 62-68.

21 Toft, G., Axmon, A., Giwercman, A., Thulstrup, A.M., Rignell-Hydbom, A., Pedersen, H.S., Ludwicki, J.K., Zvyezday, V., Zinchuk, A., Spano, M., Manicardi, G.C., Bonefeld-Jorgensen, E.C., Hagmar, L., Bonde, J.P. (2005). Fertility in four regions spanning large contrasts in serum levels of widespread persistent organochlorines: a cross-sectional study. *Environ Health* 4: 26-

22 Irvine, S., Cawood, E., Richardson, D., MacDonald, E., Aitken, J. (1996). Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. *BMJ* 312: 467-471.

23 Moller, H.(2001). Trends in incidence of testicular cancer and prostate cancer in Denmark. *Hum Reprod* 16: 1007-1011.

24 Adami, H.O., Bergstrom, R., Mohner, M., Zatonski, W., Storm, H., Ekbom, A., Tretli, S., Teppo, L., Ziegler, H., Rahu, M., . (1994). Testicular cancer in nine northern European countries. *Int J Cancer* 59: 33-38.

25 Boyle, P., Kaye, S.B., Robertson, A.G. (1987). Changes in testicular cancer in Scotland. *Eur J Cancer Clin Oncol* 23: 827-830.

26 Power, D.A., Brown, R.S., Brock, C.S., Payne, H.A., Majeed, A., Babb, P. (2001). Trends in testicular carcinoma in England and Wales, 1971-99. *BJU Int* 87: 361-365.

27 Liu, S., Semenciw, R., Waters, C., Wen, S.W., Mery, L.S., Mao, Y. (2000). Clues to the aetiological heterogeneity of testicular seminomas and non-seminomas: time trends and age-period-cohort effects. *Int J Epidemiol* 29: 826-831.

28 Weir, H.K., Marrett, L.D., Moravan, V. (1999). Trends in the incidence of testicular germ cell cancer in Ontario by histologic subgroup, 1964-1996. *CMAJ* 160: 201-205.

29 Pearce, N., Sheppard, R.A., Howard, J.K., Fraser, J., Lilley, B.M. (1987). Time trends and occupational differences in cancer of the testis in New Zealand. *Cancer* 59: 1677-1682.

30 Stone, J.M., Cruickshank, D.G., Sandeman, T.F., Matthews, J.P. (1991). Trebling of the incidence of testicular cancer in Victoria, Australia (1950-1985). *Cancer* 68: 211-219.

31 Zheng, T., Holford, T.R., Ma, Z., Ward, B.A., Flannery, J., Boyle, P. (1996). Continuing increase in incidence of germ-cell testis cancer in young adults: experience from Connecticut, USA, 1935-1992. *Int J Cancer* 65: 723-729.McGlynn, K.A., Devesa, S.S., Sigurdson, A.J., Brown, L.M., Tsao, L.,

32 Tarone, R.E. (2003). Trends in the incidence of testicular germ cell tumors in the United States. *Cancer* 97: 63-70.

33 Prener, A., Engholm, G., Jensen, O.M. (1996). Genital anomalies and risk for testicular cancer in Danish men. *Epidemiology* 7: 14-19.

34 Weir, H.K., Marrett, L.D., Kreiger, N., Darlington, G.A., Sugar, L. (2000). Pre-natal and peri-natal exposures and risk of testicular germ-cell cancer. *Int J Cancer* 87: 438-443.

35 Stang, A., Ahrens, W., Bromen, K., Baumgardt-Elms, C., Jahn, I., Stegmaier, C., Krege, S., Jockel, K.H. (2001). Undescended testis and the risk of testicular cancer: importance of source and classification of exposure information. *Int J Epidemiol* 30: 1050-1056.

36 Forman, D., Oliver, R.T., Brett, A.R., Marsh, S.G., Moses, J.H., Bodmer, J.G., Chilvers, C.E., Pike, M.C. (1992). Familial testicular cancer: a report of the UK family register, estimation of risk and an HLA class 1 sib-pair analysis. *Br J Cancer* 65: 255-262.

37 Dieckmann, K.P. and Pichlmeier, U. (1997). The prevalence of familial testicular cancer: an analysis of two patient populations and a review of the literature. *Cancer* 80: 1954-1960.

38 Dong, C., Lonnstedt, I., Hemminki, K. (2001). Familial testicular cancer and second primary cancers in testicular cancer patients by histological type. *Eur J Cancer* 37: 1878-1885.

39 Harries, L.W., Stubbins, M.J., Forman, D., Howard, G.C., Wolf, C.R. (1997). Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. *Carcinogenesis* 18: 641-644.

40 Heimdal, K., Olsson, H., Tretli, S., Fossa, S.D., Borresen, A.L., Bishop, D.T. (1997). A segregation analysis of testicular cancer based on Norwegian and Swedish families. *Br J Cancer* 75: 1084-1087.

41 Rapley, E.A., Crockford, G.P., Teare, D., Biggs, P., Seal, S., Barfoot, R., Edwards, S., Hamoudi, R., Heimdal, K., Fossa, S.D., Tucker, K., Donald, J., Collins, F., Friedlander, M., Hogg, D., Goss, P., Heidenreich, A., Ormiston, W., Daly, P.A., Forman, D., Oliver, T.D., Leahy, M., Huddart, R., Cooper, C.S., Bodmer, J.G., Easton, D.F., Stratton, M.R., Bishop, D.T. (2000). Localization to Xq27 of a susceptibility gene for testicular germ-cell tumours. *Nat Genet* 24: 197-200.

42 Rapley, E.A., Crockford, G.P., Easton, D.F., Stratton, M.R., Bishop, D.T. (2003). Localisation of susceptibility genes for familial testicular germ cell tumour. *APMIS* 111: 128-133.

43 Bromen, K., Stang, A., Baumgardt-Elms, C., Stegmaier, C., Ahrens, W., Metz, K.A., Jockel, K.H. (2004). Testicular, other genital, and breast cancers in first-degree relatives of testicular cancer patients and controls. *Cancer Epidemiol Biomarkers Prev* 13: 1316-1324.

44 Akre, O., Lipworth, L., Cnattingius, S., Sparen, P., Ekbom, A. (1999). Risk factor patterns for cryptorchidism and hypospadias. *Epidemiology* 10: 364-369.

45 Pierik, F.H., Burdorf, A., Nijman, J.M., de Muinck Keizer-Schrama SM, Juttmann, R.E., Weber, R.F. (2002). A high hypospadias rate in The Netherlands. *Hum Reprod* 17: 1112-1115.

46 Scorer, C.G.(1964). The descent of the testis. *Arch Dis Child* 39: 605-609.

47 John Radcliffe Hospital Cryptorchidism Study Group(1992). Cryptorchidism: a prospective study of 7500 consecutive male births, 1984-8. John Radcliffe Hospital Cryptorchidism Study Group. *Arch Dis Child* 67: 892-899.

48 Andersen, A.G., Jensen, T.K., Carlsen, E., Jorgensen, N., Andersson, A.M., Krarup, T., Keiding, N., Skakkebaek, N.E. (2000). High frequency of sub-optimal semen quality in an unselected population of young men. *Hum Reprod* 15: 366-372.

49 Preiksa, R.T., Zilaitiene, B., Matulevicius, V., Skakkebaek, N.E., Petersen, J.H., Jorgensen, N., Toppari, J. (2005). Higher than expected prevalence of congenital cryptorchidism in Lithuania: a study of 1204 boys at birth and 1 year follow-up. *Hum Reprod* 20: 1928-1932.

50 Salazar-Martinez, E., Romano-Riquer, P., Yanez-Marquez, E., Longnecker, M.P., Hernandez-Avila, M. (2004). Anogenital distance in human male and female newborns: a descriptive, cross-sectional study. *Environ Health* 3: 8

51 Swan, S.H., Main, K.M., Liu, F., Stewart, S.L., Kruse, R.L., Calafat, A.M., Mao, C.S., Redmon, J.B., Ternand, C.L., Sullivan, S., Teague, J.L. (2005). Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect* 113: 1056-1061.

52 Sharpe, R.M.(2006). Pathways of endocrine disruption during male sexual differentiation and masculinization. *Best Pract Res Clin Endocrinol Metab* 20: 91-110.

53 Hales, B.F. and Robaire, B. (2001). Paternal exposure to drugs and environmental chemicals: effects on progeny outcome. *J Androl* 22: 927-936.

54 Robaire, B. and Hales, B.F. (2003). Mechanisms of action of cyclophosphamide as a male-mediated developmental toxicant. *Adv Exp Med Biol* 518: 169-180.

55 Joffe, M.(2003). Infertility and environmental pollutants. *Br Med Bull* 68: 47-70.

56 Ema, M., Miyawaki, E., Kawashima, K. (1998). Further evaluation of developmental toxicity of di-n-butyl phthalate following administration during late pregnancy in rats. *Toxicol Lett* 98: 87-93.

57 Mylchreest, E., Cattley, R.C., Foster, P.M. (1998). Male reproductive tract malformations in rats following gestational and lactational exposure to Di(n-butyl) phthalate: an antiandrogenic mechanism? *Toxicol Sci* 43: 47-60.

58 Ema, M., Miyawaki, E., Kawashima, K. (2000). Critical period for adverse effects on development of reproductive system in male offspring of rats given di-n-butyl phthalate during late pregnancy. *Toxicol Lett* 111: 271-278.

59 Mylchreest, E., Wallace, D.G., Cattley, R.C., Foster, P.M. (2000). Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to Di(n-butyl) phthalate during late gestation. *Toxicol Sci* 55: 143-151.

60 Fisher, J.S., Macpherson, S., Marchetti, N., Sharpe, R.M. (2003). Human 'testicular dysgenesis syndrome': a possible model using *in utero* exposure of the rat to dibutyl phthalate. *Hum Reprod* 18: 1383-1394.

61 Barlow, N.J., McIntyre, B.S., Foster, P.M. (2004). Male reproductive tract lesions at 6, 12, and 18 months of age following *in utero* exposure to di(n-butyl) phthalate. *Toxicol Pathol* 32: 79-90.

62 Mahood, I.K., McKinnell, C., Walker, M., Hallmark, N., Scott, H., Fisher, J.S., Rivas, A., Hartung, S., Ivell, R., Mason, J.I., Sharpe, R.M. (2006). Cellular origins of testicular dysgenesis in rats exposed *in utero* to di(n-butyl) phthalate. *Int J Androl* 29: 148-154.

63 Veeramachaneni, D.N. and Vandewoude, S. (1999). Interstitial cell tumour and germ cell tumour with carcinoma in situ in rabbit testes. *Int J Androl* 22: 97-101.

64 Higuchi, T.T., Palmer, J.S., Gray, L.E., Jr., Veeramachaneni, D.N. (2003). Effects of dibutyl phthalate in male rabbits following *in utero*, adolescent, or postpubertal exposure. *Toxicol Sci* 72: 301-313.

65 Sharpe, R.M. and Skakkebaek, N.E. (1993). Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* 341: 1392-1395.

66 Henderson, B.E., Benton, B., Jing, J., Yu, M.C., Pike, M.C. (1979). Risk factors for cancer of the testis in young men. *Int J Cancer* 23: 598-602.

67 Depue, R.H., Pike, M.C., Henderson, B.E. (1983). Estrogen exposure during gestation and risk of testicular cancer. *J Natl Cancer Inst* 71: 1151-1155.

68 Depue, R.H.(1984). Maternal and gestational factors affecting the risk of cryptorchidism and inguinal hernia. *Int J Epidemiol* 13: 311-318.

69 Stillman, R.J.(1982). *in utero* exposure to diethylstilbestrol: adverse effects on the reproductive tract and reproductive performance and male and female offspring. *Am J Obstet Gynecol* 142: 905-921.

70 Kim, K.S., Torres, C.R., Jr., Yucel, S., Raimondo, K., Cunha, G.R., Baskin, L.S. (2004). Induction of hypospadias in a murine model by maternal exposure to synthetic estrogens. *Environ Res* 94: 267-275.

71 Sharpe, R.M.(2003). The 'oestrogen hypothesis'- where do we stand now? *Int J Androl* 26: 2-15.

72 Storgaard, L., Bonde, J.P., Olsen, J. (2006). Male reproductive disorders in humans and prenatal indicators of estrogen exposure. A review of published epidemiological studies. *Reprod Toxicol* 21: 4-15.

73 Sohoni, P. and Sumpter, J.P. (1998). Several environmental oestrogens are also anti-androgens. *J Endocrinol* 158: 327-339.

74 Lee, H.J., Chattopadhyay, S., Gong, E.Y., Ahn, R.S., Lee, K. (2003). Antiandrogenic effects of bisphenol A and nonylphenol on the function of androgen receptor. *Toxicol Sci* 75: 40-46.

75 Takahashi, O. and Oishi, S. (2001). Testicular toxicity of dietary 2,2-bis(4-hydroxyphenyl)propane (bisphenol A) in F344 rats. *Arch Toxicol* 75: 42-51.

76 Fisher, J.S.(2004). Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. *Reproduction* 127: 305-315.

77 Wolf, C.J., LeBlanc, G.A., Gray, L.E., Jr. (2004). Interactive effects of vinclozolin and testosterone propionate on pregnancy and sexual differentiation of the male and female SD rat. *Toxicol Sci* 78: 135-143.

78 Hotchkiss, A.K., Parks-Saldutti, L.G., Ostby, J.S., Lambright, C., Furr, J., Vandenbergh, J.G., Gray, L.E., Jr. (2004). A mixture of the "antiandrogens" linuron and butyl benzyl phthalate alters sexual differentiation of the male rat in a cumulative fashion. *Biol Reprod* 71: 1852-1861.

79 Jarfelt, K., Dalgaard, M., Hass, U., Borch, J., Jacobsen, H., Ladefoged, O. (2005). Antiandrogenic effects in male rats perinatally exposed to a mixture of di(2-ethylhexyl) phthalate and di(2-ethylhexyl) adipate. *Reprod Toxicol* 19: 505-515.

80 Gray, L.E., Jr., Wilson, V.S., Stoker, T., Lambright, C., Furr, J., Noriega, N., Howdeshell, K., Ankley, G.T., Guillette, L. (2006). Adverse effects of environmental antiandrogens and androgens on reproductive development in mammals. *Int J Androl* 29: 96-104.

81 COT. (2002). Risk Assessment of Mixtures of Pesticides and Similar Substances. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment.

82 COT. (2004). Annual Report 2004: Risk Assessment Strategies. 15-18.

Statement on a commercial survey investigating the occurrence of disinfectants and disinfection by-products in prepared salads

Introduction

1. Wash aids, such as those employed by salad manufacturers, were first discussed in February 2005 due to the concern about the potential generation of by-products on or in foods as a result of the use of chlorine-based disinfectant wash-aids. There is currently a lack of information in the scientific literature on the formation of such by-products.

2. In June 2006, the Food Standards Agency (FSA) received the results of a study conducted on behalf of the Fresh Prepared Salads Producer Group, investigating the occurrence and formation of disinfectants and disinfection by-products in prepared salads.

3. The Fresh Prepared Salads Producer Group study is not extensive but is the only available survey of the occurrence and formation of disinfection by-products in prepared salads. The COT was asked to consider the results of this study, in order to allow the FSA to formulate the appropriate consumer advice on the safety of wash aids and to consider whether further work is necessary.

Background

Chlorine wash aids

4. Chlorine washes can currently be used for non-organic fruit and vegetables in the UK provided they meet the legal definition of a processing aid, i.e. they should not perform a function in the final product and should leave no residues that present a health risk. It is the responsibility of producers to ensure food is not injurious to health. Because legislation on processing aids has not yet been harmonised in the European Union, national legislation applies and processing aids legally used in the UK may not be permitted in other countries and vice versa.

5. UK water supplies contain no more than 1 mg/L of free residual chlorine and typically contain less than 0.5 mg/L (WHO, 2003). Chlorine is added to water as either gaseous chlorine (Cl_2) or hypochlorite (OCl^-). Chlorine washes are typically employed post-harvest to remove debris and dirt; to reduce microbial contamination; and to retain optimal appearance once packaged (Baur *et al.*, 2005; Delaquis *et al.*, 2004; and Ong *et al.*, 1996). The procedure for salad washing varies around the world and between producers, but in the UK typical hypochlorite wash practices involve a 1 to 2 minute washing time with 15-20 mg/L free chlorine, as measured at the end of the wash system (personal communication, Bakkavor (Geest)). Following thus, the wash process generally incorporates a final rinse in chilled water with 2-4 mg/L free chlorine or in mains water, followed by a spin cycle to remove excess water.

6. Safety considerations for foods such as prepared salads most frequently focus on microbiological risks. However, concerns have occasionally been expressed about the potential generation of by-products on or in foods as a result of the use of chlorine-based disinfectants as wash aids.

Generation of disinfection by-products

7. Reaction of chlorine-based disinfectants with organic matter in water can result in the formation of a number of by-products, including trihalomethanes, haloacetic acids, haloacetonitriles, haloketones, chloral hydrate and chloropicrin. The presence of bromide can lead to brominated and mixed chlorinated/brominated compounds. Ozonation can lead to non-halogenated by-products, such as aldehydes (e.g. formaldehdye), ketoacids and carboxylic acids.

8. In November 2004 the COT reviewed evidence for associations between chlorinated disinfection by-products and adverse reproductive outcomes (statement available at: http://www.advisorybodies.doh.gov.uk/cotnonfood/chlorination.htm). The COT concluded that the data evaluated did not show a causal relationship between chlorinated drinking water and adverse pregnancy outcomes. However, the COT did recommend 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. This is the first time the COT has been asked for advice on other possible effects of disinfection by-products.

9. Similar by-products may be produced in or on foods treated with chlorine-based disinfectant wash-aids. There is no published research investigating the occurrence and formation of disinfectants and disinfection by-products in prepared salads. As such, there is generally a lack of information on exposure to disinfection by-products in pre-packed foods.

Future trends

10. The FSA has been informed that many salad manufacturers are now using water treated by other processes; and it is anticipated that within the next two years, all salad manufacturers will have moved away from chlorination wash processes (personal communication, Bakkavor (Geest)). Current alternative wash options include borehole/spring water (only relevant where producers have 'unlimited' access to water), peracetic acid and products based on extracts of citrus fruits.

Toxicology of chlorinated by-products

11. In 2000, IPCS reviewed the formation and risk characterisation for disinfection by-products in drinking water (IPCS, 2000). The evidence was either insufficient or inconclusive to support a link between bladder and colon cancer and long-term exposure to chlorinated drinking water, trihalomethanes or chloroform. In addition, they found no increased risk of cardiovascular disease or adverse pregnancy outcomes associated with chlorinated water (IPCS, 2000).

12. The International Agency for Research on Cancer (IARC) has evaluated a number of drinking water disinfectants and contaminants (IARC, 1991, 1999 and 2004). These include: chloramine, trichloroacetic acid and sodium chlorite (Group 3, *not classifiable as to their carcinogenicity to humans*); and dichloroacetic acid, potassium bromate and chloroform (Group 2B, *possibly carcinogenic to humans*).

13. The World Health Organization (WHO) has similarly evaluated a number of disinfectants and disinfection by-products in the 3rd edition of the Guidelines for Drinking Water Quality (2003). Tolerable daily intakes (μg/kg body weight/day) have been derived for: chlorite (30), chlorate (30), total chlorine (150), chloramine (94), chloroform (15), and trichloroacetic acid (32.5).

Fresh prepared salads producer group's study

14. Members of the Fresh Prepared Salads Producer Group, which include Bakkavor (Geest), Nature's Way Foods, Vitacress Salads, Florette and Kanes Foods, recently carried out a programme of testing to identify which, if any, by-products were present in prepared bagged salads and to re-confirm the safety of their products (personal communication, Bakkavor (Geest)). The programme of testing was managed by Bakkavor (Geest) and performed by ALcontrol Laboratories in early 2005. It represents a limited, initial study; and was neither influenced nor funded by the FSA.

15. The testing programme involved analysing a range of prepared salads, purchased from various retail outlets for the presence of specific disinfectants and disinfection by-products; i.e. various types of prepared salads from a number of manufacturers were tested. Although not a comprehensive study, the selection of salads was reported to be random and representative of the UK salad market. The range of potential disinfectants and by-products analysed for, included: chlorite, chlorate, bromate, free and total chlorine, chloramine, chloroform, total trihalomethanes, trichloroacetic acid (TCA) and dichloroacetic acid (DCA).

Test method

16. The test method was devised by ALcontrol Laboratories and involved steeping the salad in water, followed by testing of the leachate. The laboratory considered that the simple molecules being sought were most likely to be readily leachable from the surface of the leaves. In addition, they suggested that releasing the plant cellular material could lead to complex reactions with some of the analytes and possibly lead to loss of volatile analytes. In order to simulate consumer exposure, bagged salads were purchased off the shelf, refrigerated overnight and tested following addition of 300ml to every 100g bag and a one hour agitation. This 3:1 (water:leaf) ratio was established as the most suitable method, following a series of trial tests with different ratios. It should be noted that this was an empirical method, deemed to be the most suitable at the time, providing adequate detection limits whilst preventing break-up and break-down of the salad leaves.

Test results

17. The results are presented in Table 1 as μg chemical/kg lettuce salad. In the majority of samples tested, the levels detected were low enough to comply with drinking water standards. In one exception, the combined level of trichloroacetic acid and dichloroacetic acid (83.3 μg/kg) exceeded the 60 μg/L permitted by US drinking water regulations for total haloacetic acids (in this case, there are no UK Regulations). However, in all samples, all compounds analysed were within the WHO Guidelines for Drinking Water Quality, where available. In addition, for all samples, estimated ingestion of each compound, based on salad consumption data, was at least several orders of magnitude lower than tolerable daily intakes set by WHO (Table 2).

The table of results presented below has been provided by the study group for this statement. Various drinking water regulations have been included in the table for comparison.

Table 1: Results for disinfectants and disinfection by-products measured in 12 samples of prepared salads

	Chlorite [µg/kg]	Chlorate [µg/kg]	Bromate [µg/kg]	Free chlorine [µg/kg]	Total chlorine [µg/kg]	Chloramine [µg/kg]	Chloroform [µg/kg]	Total THMs [µg/kg]	TCA [µg/kg]	DCA [µg/kg]
UK Drinking Water Limit, 2000		700	10					100		
US Drinking Water Limit, 2004	1000		10		4000			80	60	
US max. limit for drinking water systems				4000						
WHO Guidelines for Drinking Water Quality, 1993 (2003 values in brackets)	200 (700)	n/a (700)	25 (10)		5000 (5000)	3000 (3000)	200 (300)		100 (200)	50 (50)
Sample 1	<200	<300	<6.0	700	700	<500	<10	<10	<2.4	<4.0
2	<200	<300	<6.0	700	700	<500	<10	<10	<2.4	<4.0
3	<200	<300	<6.0	500	500	<500	<10	<10	<2.4	<4.0
4	<200	<300	<6.0	<500	<500	<500	<10	<10	<2.4	12
5	<200	<300	<6.0	1000	1000	<500	<10	<10	15.6	<4.0
6	<200	<300	<6.0	<500	<500	<500	<10	<10	23.2	7
7	<200	<300	<6.0	<500	<500	<500	<10	<10	4.5	<4.0
8	<200	<300	<6.0	<500	<500	<500	<10	<10	2.7	<4.0
9	<200	<300	<6.0	800	900	100	16	16	51.3	32
10	<200	<300	<6.0	<500	<500	<500	<10	<10	5.9	<4.0
11				<300	<300	<300	<10	<10	<2	<2
12				<300	<300	<300	<10	<10	3.4	9.6
Samples exceeding limit	0	0	0	0	0	0	0	0	1	

The 'less than' (<) values represent limits of detection. These may vary between samples because this was a non-standard method that had never been run before and as such, these limits of detection were estimated.

When this study was conducted, the 2nd edition of the WHO Guidelines for Drinking Water Quality (1993) were in operation. However, the 3rd edition of the Guidelines (2003) has since been published; and hence both the 1993 and 2003 guideline values are provided in the table below (with the 2003 guideline values in brackets).

Table 2: Estimated intakes of each compound for average and extreme cases of salad consumption

Compound	WHO TDI (μg/kg bw/day)[1]	Maximum average concentration in salad (μg/kg salad)[2]	Amount ingested based on salad intake data (μg/kg bw/day)[3]	
			Average adult consumer	High level adult consumer
Chlorite	30	<200	0.0429	0.1611
Chlorate	30	<300	0.0644	0.2416
Bromate	–	<6	0.0013	0.0048
Total Chlorine	150	<575	0.1234	0.4631
Chloramine	94	<433	0.0930	0.3487
Chloroform	15	<10.5	0.0023	0.0085
Total Trihalomethanes	–	<10.5	0.0023	0.0085
Trichloroacetic acid	32.5	<9.85	0.0021	0.0079
Dichloroacetic acid	–	<7.55	0.0016	0.0061

[1] World Health Organization Tolerable Daily Intake (TDI), where available, expressed as μg compound per kg body weight per day (WHO, 2003).

[2] Although this value represents an average of the 10-12 samples tested, it is affected by a large number of non-detect values; and therefore is upper bound and represents an over-estimation.

[3] Calculated on a μg/kg body weight/day basis, assuming a 60 kg adult and salad consumption levels of 12.9g/day for an average adult consumer or 48.3g/day for a 97.5th percentile adult consumer. The high intake level was taken from the 1993 Vegetarian's survey as intakes for vegetarians are slightly higher than for the general population (MAFF, 1996). The average intake level was taken from the 2000-2001 NDNS survey (Henderson *et al.*, 2002).

COT Conclusions

18. Members noted that in a 150g bag of salad, there would be less chlorine and chlorination by-products than is permissible in a 250 ml glass of tap water.

19. Members agreed that the results from this study did not indicate any cause for concern with respect to the presence of chlorination by-products in prepared salads.

20. Given the current trend away from chlorination processes, Members agreed that there is no need for the generation of additional data to confirm the results of this commercial study. However, the new, alternative wash options will need to be kept under review in the future.

References

1. Baur S, Klaiber R, Wei H, Hammes WP and Carle R (2005). Effect of temperature and chlorination of pre-washing water on shelf-life and physiological properties of ready-to-use iceberg lettuce. *Innovative Food Science and Emerging Technologies*, **6**, 171-182.

2. Delaquis PJ, Fukumoto LR, Toivonen PMA and Cliff MA (2004). Implications of wash water chlorination and temperature for the microbial and sensory properties of fresh-cut iceberg lettuce. *Postharvest Biology and Technology*, **31**, 81-91.

3. Henderson L, Gregory J and Swan G (2002). National Diet and Nutrition Survey: adults aged 19-64 years. Volume 1: types and quantities of foods consumed. TSO.

4. IARC (1991). Chlorinated drinking-water; chlorination by-products; some other halogenated compounds; cobalt and cobalt compounds. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, **Volume 52**. International Agency for Research on Cancer, Lyon, France.

5. IARC (1999). Some chemicals that cause tumours of the kidney or urinary bladder in rodents, and some other substances. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, **Volume 52**. International Agency for Research on Cancer, Lyon, France.

6. IARC (2004). Some drinking-water disinfectants and contaminants, including arsenic related nitrosamines. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, **Volume 84**. International Agency for Research on Cancer, Lyon, France.

7. IPCS (2000). Disinfectants and disinfection by-products. Environmental Health Criteria International Programme on Chemical Safety **216**, WHO, Geneva.

8. MAFF (1996). Ministry of Agriculture, Fisheries and Food Research & Development and Surveillance Report: 181 (October 1996). Dietary Survey of Vegetarians: Final Technical Report.

9. Ong KC, Cash JN, Zabik MJ, Siddiq M and Jones AL (1996). Chlorine and ozone washes for pesticide removal from apples and processed apple sauce. *Food Chemistry*, **55**(2), 153-160.

10. WHO (2003). Guidelines for Drinking Water Quality, 3rd edition. **Volume 1** Recommendations. WHO, Geneva.

Statement on organic chlorinated and brominated contaminants in shellfish, farmed and wild fish

Introduction

1. The Food Standards Agency has recently completed two surveys that analysed 47 species of farmed and wild fish and shellfish consumed in the UK to determine the concentrations of a number of organic contaminants:

   i) Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs); and

   ii) Brominated flame retardants (BFRs), i.e. polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA) as well as polybrominated dibenzo-*p*-dioxins (PBDDs) and polybrominated dibenzofurans (PBDFs) which occur as contaminants in brominated organic chemicals.

2. The Committee was invited to consider the data and advise on whether they form a basis for the Food Standards Agency to amend its advice on fish consumption. The Agency's current advice on fish consumption is based upon the report of the Scientific Advisory Committee on Nutrition (SACN) and the COT review 'Advice on fish consumption: benefits and risk', published in 2004[1]. Data on the concentrations of PBDDs, PBDFs and PBBs in fish consumed in the UK have not been available for consideration previously.

Dioxins and dioxin-like organic contaminants

Polychlorinated dibenzo-p-dioxins, dibenzofurans and dioxin-like PCBs

3. Dioxins, a group of 75 PCDD and 135 PCDF congeners, are persistent organochlorine compounds that are widely dispersed environmental contaminants and accumulate in fatty foods. Dioxins can be formed as a result of thermal reactions and as trace contaminants in the synthesis of some chemicals and some industrial processes.

4. PCBs are persistent organochlorine chemicals that are no longer manufactured, but may be released to the environment during disposal of materials and obsolete electronic equipment. Twelve non-*ortho* or mono-*ortho* PCBs, of the 209 theoretically possible PCB congeners, exhibit similar biological activity to dioxins and are, therefore, referred to as dioxin-like PCBs.

5. Exposure of the general population to dioxins and dioxin-like PCBs is primarily from food[2,3]. The estimated exposures from the UK Total Diet Study samples for all age groups have declined substantially over the past 2 decades[3]. Based on occurrence and consumption in 2000/1, the most recent estimates of dietary exposure were in the region of 0.8 and 1.6 pg WHO-TEQ/kg bw/day for average and 97.5th percentile consumers [1].

Previous COT evaluations

6. In 2001, COT set a TDI of 2 pg WHO-TEQ/kg bw/day† to protect against the most sensitive effect of dioxins. This is considered to be impaired development of the fetal male reproductive system, caused by fetal exposure *in utero* and correlated with the maternal body burden of dioxins [3].

7. SACN/COT[1] considered risks and benefits of consuming more oily fish than the recommended "at least two portions of fish per week, one of which should be oily." They recommended that in considering fish consumption the TDI of 2 pg WHO-TEQ/kg bw/day should be applied to women of reproductive age and girls, the most susceptible subgroup, by virtue of exposure of fetuses that they might bear. Other populations, particularly women past child-bearing age and men, are not at risk of the developmental effects and are likely to be less susceptible to dioxin toxicity. The most sensitive and relevant non-developmental effect was considered to be increased cancer risk. An alternative safety guideline level of 8 pg WHO-TEQ/kg bw/day was proposed for these groups to be used to indicate a long term average intake that would not be expected to be associated with an increase in cancer risk.

8. Together with the nutritional advice the guideline ranges for oily fish consumption were:

   - Women of reproductive age and girls should aim to consume within the range of one to two portions of oily fish a week, based on maintaining consumption of dioxins and dioxin-like PCBs below the tolerable daily intake (TDI) of 2 pg WHO-TEQ/kg bodyweight per day.

   - Women past reproductive age, boys and men should aim to consume within the range of one to four portions of oily fish a week, based on maintaining consumption of dioxins and dioxin-like PCBs below the guideline value of 8 pg WHO-TEQ/kg bodyweight per day.

9. In order to avoid providing over-complicated instructions that could be a deterrent to fish consumption as a whole, the general guidelines on fish consumption were based on an overview of the concentrations of contaminants previously detected in a range of commonly consumed fish[4,5,6].

Non-dioxin-like PCBs

10. A recent EFSA evaluation concluded that the simultaneous exposure to non-dioxin-like PCBs and dioxin-like compounds hampers the interpretation of the results of the toxicological and epidemiological studies. The data were insufficient to set tolerable intake levels and it was recommended that continued effort to lower the levels of non-dioxin-like PCBs in food is warranted (http://www.efsa.eu.int/science/contam/contam_opinions/1229_en.html).

† Toxicity Equivalency Factors (TEFs) allow concentrations of the less toxic dioxin-like compounds (16 PCDDs/PCDFs and 12 PCBs) to be expressed as a concentration equivalent to the most toxic dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). These toxicity-weighted concentrations are then summed to give a single value, which is expressed as a Toxic Equivalent (TEQ). The system of TEFs used in the UK and a number of other countries is that set by the World Health Organisation (WHO), and the resulting overall concentrations are referred to as WHO-TEQs.

Polybrominated dibenzo-p-dioxins, polybrominated dibenzofurans and dioxin-like polybrominated biphenyls

11. A group of substances that have been found as contaminants in brominated organic chemicals, in particular BFRs, are the PBDDs and PBDFs. PBDDs/PBDFs are structurally closely related to chlorinated dioxins and furans. They are not intentionally produced (except for scientific purposes) but, as with dioxins, are generated as undesired by-products in various processes. They can be formed by chemical, photochemical, or thermal reactions from precursors. In experimental animal models, exposure to PBDDs or PBDFs is reported to result in many of the effects typical for the chlorinated dioxins.

12. Theoretically, 75 PBDDs and 135 PBDF congeners are possible, and as with the chlorinated analogues the most toxic congeners are reported to be those substituted at the 2, 3, 7, and 8 positions[7]. In experimental animal models, PBDDs and PBDFs are reported as producing the classic effects demonstrated for the chlorinated dioxins and furans. These include lethality, wasting, thymic atrophy, teratogenesis, reproductive effects, chloracne, immunotoxicity, enzyme induction, decreases in T4 and vitamin A, and increased hepatic porphyrins. TCDD-like responses have also been measured *in vitro*, including enzyme induction, anti-estrogenic activity in human breast cancer cells, and transformation of mouse macrophages into tumour cells[7].

13. Additionally, limited toxicokinetic data for the brominated dioxins and furans indicate that the half-lives in rats are similar to those of their chlorinated analogues[8,9,10]. The vast majority of data are for the 2,3,7,8-tetrabrominated dioxin and furan, which are considered to be the most toxic. Golor and colleagues compared the kinetics of three pairs of corresponding polychlorinated and polybrominated dioxins and furans in Wistar rats monitored for ninety five days following a single dose (subcutaneous injection)[9]. Elimination rates from the liver and adipose tissue of both the 2,3,4,5,8-penta-chloro- and bromo-dibenzofuran congeners were similar, and the same was also the case for the 1,2,3,7,8-penta-chloro- and bromo-dibenzo-*p*-dioxin congeners. However, in the case of the 2,3,7,8-tetrahalogenated dibenzofurans, the chlorinated congener was rapidly eliminated from liver and adipose tissue in the rat, whereas the brominated congener was much more slowly eliminated from both tissues.

14. Both 2,3,7,8-TBDD and 2,3,7,8-TBDF are developmental toxicants in mice at subcutaneous and oral doses that do not produce maternal toxicity or fetal mortality. The LOAELs (in µg/kg bw) for hydronephrosis and cleft palate after a single oral dose to pregnant mice on gestation day 10 were, respectively, 3 and 48 for TBDD, 25 and 200 for TBDF, 400 and 2400 for 2,3,4,7,8-PeBDF and 500 and 3000-4000 for 1,2,3,7,8-PeBDF[11]. The dose-response curves for the induction of cleft palate by the four brominated compounds were parallel to that of TCDD, supporting a common mechanism of action involving the AhR. The results indicated that bromination decreased the teratogenic activity of TBDD relative to TCDD and of both PeBDFs relative to the chlorinated analogues. However, substitution of bromines for chlorines increased by two-fold the teratogenic potency of TBDF relative to TCDF.

15. PBDDs/PBDFs are believed to share a common mechanism of action with PCDDs/PCDFs, the first step of which involves binding to the aryl hydrocarbon receptor (AhR). A number of recent *in vitro* studies have used the ethoxyresorufin-O-deethylase (EROD) assay[12,13] or the chemical-activated luciferase gene expression (CALUX) assay[14,15] to assess activation of the AhR and estimating the relative potency of several PBDD/PBDFs. Results from these studies indicate that at the receptor level the activity of brominated dibenzo-*p*-dioxins, dibenzofurans and biphenyls are broadly comparable to their chlorinated congeners. The majority of PBDDs and PBDFs had comparable or lower relative potencies than the PCDD/PCDFs.

16. Polybrominated biphenyls (PBBs) are brominated hydrocarbons formerly used as additive flame retardants. As such these substances were added, rather than chemically bound, to plastics used in a variety of consumer products, such as computer monitors, television, textiles and plastic foams, and were able to leave the plastic and enter the environment. They are structurally similar compounds in which 2-10 bromine atoms are attached to the biphenyl molecular structure. In total, as with the structurally similar PCBs, 209 different PBB congeners are possible.

17. Individual PBB congeners vary in their pattern of toxicity. PBBs have been categorised on a similar structural basis as the PCBs, with category I comprising congeners lacking *ortho* substituents (coplanar PBBs). Coplanar PCBs are dioxin-like with regards to their toxicity and are included in the toxicity equivalency factor (TEF) concept. A number of PBB effects are dioxin-like and consistent with the AhR-mediated mechanism of action, including altered vitamin A homeostasis, thymic atrophy, dermal and ocular effects (e.g. chloracne and inflammation of eyelids), and body weight changes (wasting syndrome). This is determined by the magnitude of the response that is initiated by binding with the AhR. The binding affinity, in turn, is determined by the substitution pattern of the congener, many of the most toxic congeners resemble the structural configuration of 2,3,7,8-TCDD. The dioxin-like coplanar PBB-169 (3,3',4,4',5,5'-hexaBB) has been found to be the most toxic congener in several test systems[16]. However, this congener was present at low concentrations in commercial PBB mixtures and may not contribute significantly to the exposure profile for PBB congeners.

18. Category II comprises mono-*ortho* substituted derivatives and other PBBs, mainly those with two or more *ortho* bromines, are in category III. These congeners are not considered to have dioxin-like properties[17].

Preliminary advice from COT on combination of brominated dioxins, furans and biphenyls

19. In December 2005 COT discussed the key toxicological data for the PBDDs/PBDFs and dioxin-like PBBs. The limited data available supported the conclusion that these compounds share a common mechanism of action with their chlorinated analogues. Therefore, TEFs used in the assessment of chlorinated dioxins and dioxin-like PCBs, might have potential application to the assessment of PBDDs/PBDFs. In 1997, a WHO working group concluded that 'at present, insufficient environmental and toxicological data are available to establish a TEF value' for these compounds[18]. However, the WHO[7] report on PBDDs and PBDFs discusses the concept of using TEFs for the assessment of these chemicals and suggests that the preliminary use of the same TEF values for the brominated congeners as described for the chlorinated analogues appears to be justified.

20. On the basis of the available data COT concluded that TEFs developed for the chlorinated dioxins could be used as an indication of the dioxin-like activity of the PBDDs, PBDFs and dioxin-like PBBs. The TEQs for the brominated contaminants could be combined with the TEQs for the chlorinated dioxins to provide an indication of the total intake of chemicals with dioxin-like properties as this would be more protective of public health than to view the chemicals separately. However, the Committee highlighted that this was tentative advice. The uncertainties in the available data with regards to the comparative toxicokinetics in rodents and humans, and lack of chronic dosing studies with these compounds indicated the need for maintaining a watching brief. It was acknowledged that the use of TEFs assigned to chlorinated congeners for the brominated analogues was likely to be over-precautionary. Given the current state of the science this is a prudent science position as long as the uncertainties in the combined chlorinated and brominated TEQs are fully acknowledged. However, further data should be sought to support the use of the TEF concept for the brominated compounds.

Non-dioxin-like polybrominated biphenyls

21. PBB congeners that exhibit AhR-mediated responses constitute only a fraction of the components in commercial PBB mixtures. Therefore, it is presumed that congeners that act by other mechanisms (category II and III PBBs) also contribute to the toxicity of PBB mixtures. The mechanism(s) of toxicity for non-dioxin-like PBB congeners is less clearly elucidated, but also may involve receptors (e.g. the estrogen receptor), or the involvement of reactive intermediates (e.g., arene oxides) that can form potentially toxic covalently bound substrate-macromolecular adducts. The non-dioxin-like PBBs are considered to be less toxic that the coplanar PBB congeners.

22. WHO (1994) proposed a TDI for PBBs, based on a 2-year NTP carcinogenicity study that showed liver tumour formation in rats[19]. In the NTP study, the lowest dose of PBBs (FireMaster FF-1) tested that produced carcinogenic effects was 0.5 mg/kg bw/day. A dose of 0.15 mg/kg bw/day together with prenatal and perinatal exposure of the dam to 0.05 mg/kg bw/day did not result in any adverse effects, indicating a NOAEL of 0.15 mg/kg bw/day.

23. Assays for mutagenicity and genotoxicity have not shown positive effects with commercial mixtures or individual PBB congeners[20,21]. It was concluded that PBBs probably induce cancer by a non-genotoxic mechanism, and an uncertainty factor of 1000 was applied to the NOAEL to obtain a TDI of 0.15 µg/kg bw/day. However, analogy to the discussion above of non-dioxin-like PCBs indicates that the derivation of this proposed TDI may not be appropriate since simultaneous exposure to dioxin-like PBBs cannot be excluded.

Polybrominated diphenyl ethers

24. There are 209 individual PBDE congeners. Three commercial PBDE flame retardants have been available in the UK: pentabromodiphenyl ether (pentaBDE), octabromodiphenyl ether (octaBDE) and decabromodiphenyl ether (decaBDE). These commercial PBDEs are not pure products, but a mixture of various diphenyl ethers with varying degrees of bromination.

25. The European Union directive to restrict hazardous substances from electrical and electronic equipment will ban penta- and octaBDE from the production of electrical and electronic equipment from 1 July 2006. However, a voluntary ban on pentaBDE in Europe was formalised in July 2003.

COT statement 2003/04

26. The COT considered PBDEs in 2003 and issued a statement[22], in response to a survey of brominated flame retardants in brown trout and eels from the Skerne-Tees river system. The Committee noted that toxicity data are unavailable for many of the individual congeners. The concentrations of the individual congeners were therefore summed for comparison with the toxicity data on the commercial PBDE mixtures.

27. Studies on the commercial PBDEs indicate that pentaBDE is the most toxic. The COT therefore compared the estimated intakes of the sum of the measured PBDE congeners with the reported effect levels for pentaBDE. This was described as a precautionary approach, as some of the congeners are expected to be less toxic than pentaBDE.

28. Noting inadequacies in the toxicological database and the absence of identifiable no-effect levels, the COT felt it was not possible to determine a TDI. The Committee therefore decided to take a Margin of Exposure (MoE) approach and set a target MoE of 1000 for liver toxicity of pentaBDE. Above this MoE, risks to health would not be expected. The MoE was calculated by dividing the NOAEL for liver effects of pentaBDE in rats (450 μg/kg bw/day) by the estimated dietary exposure.

JECFA Evaluation – 64th Meeting, February 2005

29. JECFA published an opinion on PBDEs last year[23]. It noted that, although PBDEs are non-genotoxic substances, the available data on PBDEs were not adequate to allocate a provisional maximum tolerable daily intake (PMTDI) or provisional tolerable weekly intake (PTWI) because:

- PBDEs represent a complex group of related chemicals and the pattern of PBDE congeners in food is not clearly defined by a single commercial mixture

- Data are inadequate to establish a common mechanism of action that would allow a single congener to be used as a surrogate for total exposure or, alternatively, as the basis for establishing toxic equivalency factors

- There is no systematic database on toxicity including long-term studies on the main congeners present in the diet, using standardised testing protocols that could be used to define a NOEL for individual PBDEs of importance

- Several of the reported effects are biological outcomes for which the toxicological significance remains unclear

- Studies with purified PBDE congeners *in vitro* have shown a lack of Ah receptor activation; however, many of the adverse effects reported are similar to those found with dioxin-like contaminants, suggesting that some toxicity data may be confounded by the presence of traces of impurities that are potent Ah receptor agonists

30. It was noted that, for the most toxic PBDE congeners, adverse effects would be unlikely to occur in rodents at doses of less than approximately 100 μg/kg bw/day, and this figure was used as the basis for a MoE assessment. JECFA used dietary intake estimates of 0.004 μg/kg bw/day (for North American regions) and 0.1 μg/kg bw/day for breastfeeding infants. These would give MoEs of 25,000 and 1,000, respectively. These values were viewed as giving reassurance that intakes of PBDEs are not likely to be a significant health concern.

Hexabromocyclododecane (HBCD)

31. HBCD is a non-aromatic, brominated cyclic alkane used primarily as an additive flame retardant in materials such as styrene resins. The commercial product consists of three diastereoisomers α-, β-, and γ-HBCD. Although the technical HBCD typically consists primarily of γ-HBCD, the relative proportions of the isomers varies depending on product application.

32. Studies in laboratory animals have shown that, following oral administration, HBCD can be detected in adipose tissue, liver and muscle. Longer-term exposure shows HBCD has the potential to bioaccumulate. Following oral administration, the majority of HBCD was detected unchanged in the faeces, although it is unclear how much of this was unabsorbed material[24].

COT statement 2003/04

33. The COT also considered HBCD in 2003 in relation to levels in fish in the Skerne-Tees river system[22] using toxicological data from a draft EU risk assessment. The COT used a margin of exposure (MoE) approach in their risk assessment and set a target MoE of 3,000-10,000.

Tetrabromobisphenol A

34. Worldwide, TBBPA is the most widely used BFR and approximately 90% of TBBPA is used as a reactive intermediate in the manufacture of epoxy and polycarbonate resins. In this case it is covalently bound to the polymer and is unlikely to escape into the environment. The remaining 10% is used as an additive flame retardant, where it does not react chemically with the other components of the polymer and may therefore leach out of the matrix.

COT statement 2004/02

35. The COT considered TBBPA in 2004[25], primarily using data from the EU risk assessment. From the data available, the COT concluded that TBBPA did not raise specific toxicological concerns. In a 90-day study and a two-generation reproductive toxicity study, no clear adverse effects were observed at doses up to 1000 mg/kg bw/day. This dose was used as the basis for the TDI. An uncertainty factor of 100 was used to allow for inter- and intra- species variation and an additional factor of 10 was required because of the lack of chronic toxicity studies. The COT therefore recommended a TDI of 1 mg/kg bw/day.

Exposure data

36. Composite samples of 47 species of farmed and wild fish and shellfish consumed in the UK were analysed for 17 dioxins, 12 dioxin-like PCBs, 11 PBDDs/PBDFs, 3 dioxin-like PBBs, 7 non-dioxin-like PBBs, 17 PBDEs, HBCD and TBBPA. A total of 24 species of fresh wild fish, 7 of fresh farmed fish, 7 of fresh shellfish and 10 of canned or processed fish or shellfish were sampled between 2002 and 2004. Full details of the survey methodology are available in the Food Survey Information Sheets (FSIS) at http://www.food.gov.uk/science/surveillance/.

37. Estimates of total dietary exposure were derived from concentrations in samples from the 2003 and 2004 Total Diet Studies combined with consumption data from the 2000/1 National Diet and Nutrition Survey (NDNS)[26]. Single composite food group samples were formed by homogenising individual food groups (excluding beverages) from 24 locations. These composite samples were analysed for the same range of organic chlorinated and brominated contaminants as the fish survey.

Occurrence and consumption data

Polyhalogenated dioxins and dioxin-like polyhalogenated biphenyls

38. The concentrations of chlorinated dioxins and dioxin-like PCBs (ng WHO-TEQ/kg fresh weight) in composite fish and shellfish samples are presented in Tables 1 (oily fish) and 3 (non-oily fish). Time trend data for a limited number of species indicate that, for all but one species, concentrations of dioxins and dioxin-like PCBs are the same or have decreased since last surveyed[5,6]. All results refer to edible portions of the fish. The results from the composite samples show these to be a good representation of the range of results seen for the individual analyses.

39. Tables 1 and 3 also show estimates of average upper bound adult daily intake for 1-4 portions of fish per week taking into account intakes from the rest of the diet based on analysis of the 2001 TDS, the approach taken in the SACN/COT report[1]. The WHO-set TEFs for the chlorinated analogues have been used to give toxicity-weighted concentrations for the brominated dioxin-like congeners, these have been summed to give a single value expressed as a TEQ. As the TEFs have not been set by the WHO for brominated congeners the resulting overall concentrations are referred to simply as TEQs. These values are based on the total concentrations found in the composite samples and, based on published data, assume portion sizes of 140 g for most fresh fish, 70 g for fresh sardines/pilchards, whitebait, rollmops, most canned fish and all shellfish species, and 30g for fish paste, canned anchovy and surimi[26].

40. The concentrations of brominated dioxins and biphenyls found in sampled fish were on average lower than those of the chlorinated analogues. Total TEQ[†] concentrations (upper bound) for the PBDDs and PBDFs were in the range 0.02 – 0.26 ng TEQ/kg freshweight, and for the *non-ortho* PBBs were in the range 0 – 0.01 ng TEQ/kg freshweight. The total combined concentrations of polyhalogenated dioxins and dioxin-like polyhalogenated biphenyls (ng TEQ/kg freshweight) in composite fish and shellfish samples are presented in Tables 2 (oily fish) and 4 (non-oily fish).

† The WHO-set TEFs for the chlorinated analogues have been used to give toxicity-weighted concentrations for the brominated dioxin-like congeners, these have been summed to give a single value expressed as a TEQ. As the TEFs have not been set by the WHO for brominated congeners the resulting overall concentrations are referred to simply as TEQs.

41. It was estimated from the 2003 TDS that the average upper bound adult dietary intake of brominated dioxins and dioxin-like PBBs from the non-fish part of the diet is 0.4 pg TEQ/kg bw/day. These surveys analysed an incomplete brominated dioxin congener set (11 PBDDs/PBDFs and 3 dioxin-like PBBs), and the total upper bound intakes may be higher. However, comparison with the lower bound intake (0.08 pg TEQ/kg bw/day) demonstrates the uncertainty in these exposure estimates. Upper bound concentrations assume that all individual congeners that are present at concentrations below the reporting limit (limit of detection) are present at the reporting limit, and therefore could be an overestimate of the true concentrations. By contrast, lower bound concentrations assume that all individual congeners that are present at concentrations below the limit of detection are absent, and will therefore be an underestimate of the true concentrations. The true concentrations will lie somewhere between the upper and lower bounds.

42. Tables 2 and 4 also present estimated daily intakes of chlorinated and brominated dioxins and dioxin-like PCBs and PBBs from the whole diet (on the basis of analysis of the 2003 TDS samples) including one to four portions of oily or non-oily fish per week.

43. The combined chlorinated and brominated dioxins data (Table 2) indicate that consuming an average of two weekly portions of a range of oily fish could result in intakes in the region of the TDI of 2 pg TEQ/kg bw/day, when the rest of the diet is taken into account. Consuming an average of four weekly portions of a range of oily fish will result in intakes within the guideline value of 8 pg TEQ/kg bw/day, when the rest of the diet is taken into account.

44. It can be seen from Tables 1 and 2 that the inclusion of the brominated substances in the TEQ has a minor impact on the estimates of total exposure, particular taking into account the uncertainty in the estimated intake of the brominated dioxins from the non-fish part of the diet.

45. Tables 3 and 4 demonstrate that whereas most non-oily fish species contribute little to total dietary intake of chlorinated and brominated dioxins some species contain concentrations similar to those found in oily fish, and could make a relatively substantial contribution to total intake if eaten regularly. This particularly applies to wild sea bass, farmed sea bass, farmed halibut, turbot (Greenland), wild turbot (UK) sea bream, dogfish, and crab (brown/white).

Table 1. Estimated upper bound average daily dioxins and dioxin-like PCBs dietary exposure for a 60 kg adult consuming 1-4 portions of oily fish per week.

Species	Concentration in fish (ng WHO-TEQ/ kg fresh weight)	Portion size (g)	Fat content (%)	Total daily dietary intake [a] (pg WHO-TEQ/kg bodyweight/day) Number of portions of fish consumed per week: One portion	Two portions	Three portions	Four portions
Oily fish							
Sprat	4.29	140	9.1	2.1	3.5	4.9	6.4
Herring	3.47	140	19.3	1.8	3.0	4.2	5.4
Farmed salmon	2.51	140	14.1	1.5	2.4	3.2	4.1
Wild salmon	1.51	140	13.5	1.2	1.7	2.3	2.8
Mackerel	2.22	140	16.2	1.3	1.9	2.5	3.2
Sea Trout	1.42	140	9.9	1.1	1.6	2.1	2.6
Farmed Trout	1.02	140	8.8	1.0	1.3	1.6	2.0
Swordfish	0.72	140	6.1	0.9	1.1	1.4	1.6
Salmon (Alaska wild)	0.25	140	3.9	0.7	0.8	0.9	1.0
Tuna (Fresh)	0.07	140	0.7	0.7	0.7	0.7	0.7
Sardine/Pilchard	5.96	70	12.7	1.7	2.6	3.6	4.6
Whitebait	3.13	70	4.5	1.2	1.7	2.2	2.7
Canned sardines	2.34	70	11.3	1.0	1.4	1.8	2.2
Herring (Rollmops)	1.67	70	10.9	0.9	1.2	1.5	1.8
Eel	1.31	70	22.1	0.9	1.1	1.3	1.5
Canned mackerel	1.28	70	14.8	0.9	1.1	1.3	1.5
Canned pilchards	1.25	70	10.5	0.9	1.1	1.3	1.5
Canned salmon	0.65	70	9.7	0.8	0.9	1.0	1.1

[a] Assuming a 60 kg adult with a 0.7 pg WHO-TEQ/kg bw/day dietary intake from non-fish part of diet.

Exceeds TDI by up to 2-fold (green shading: Sprat one and two portions; Herring and Farmed salmon two portions; Wild salmon, Mackerel, Sea Trout three and four portions; Sardine/Pilchard two and three portions; Whitebait three and four portions; Canned sardines four portions)

Exceeds TDI by 2- to 4-fold (grey shading: Sprat and Herring three and four portions; Farmed salmon four portions; Sardine/Pilchard four portions)

Table 2. Estimated upper bound average daily dioxins, dioxin-like PCBs, brominated dioxins and dioxin-like PBBs dietary exposure for a 60 kg adult consuming 1-4 portions of oily fish per week.

Species	Concentration in fish (ng TEQ/kg fresh weight)	Portion size (g)	Fat content (%)	Total daily dietary intake * (pg -TEQ/kg bodyweight/day) — Number of portions of fish consumed per week: One portion	Two portions	Three portions	Four portions
Oily fish							
Sprat	4.31	140	9.1	2.5	3.9	5.4	6.8
Herring	3.69	140	19.3	2.3	3.5	4.8	6.0
Farmed salmon	2.63	140	14.1	1.9	2.8	3.7	4.6
Wild salmon	1.66	140	13.5	1.6	2.2	2.7	3.3
Mackerel	1.96	140	16.2	1.7	2.4	3.0	3.7
Sea Trout	1.45	140	9.9	1.5	2.0	2.5	3.0
Farmed Trout	1.02	140	8.8	1.4	1.7	2.1	2.4
Swordfish	0.74	140	6.1	1.3	1.6	1.8	2.1
Salmon (Alaska wild)	0.28	140	3.9	1.2	1.3	1.3	1.4
Tuna (Fresh)	0.09	140	0.7	1.1	1.1	1.2	1.2
Sardine/Pilchard	5.99	70	12.7	2.1	3.1	4.1	5.1
Whitebait	3.16	70	4.5	1.6	2.1	2.6	3.2
Canned sardines	2.37	70	11.3	1.5	1.9	2.2	2.6
Herring (Rollmops)	1.70	70	10.9	1.3	1.6	1.9	2.2
Eel	1.34	70	22.1	1.3	1.5	1.7	2.0
Canned mackerel	1.33	70	14.8	1.3	1.5	1.7	2.0
Canned pilchards	1.28	70	10.5	1.3	1.5	1.7	1.9
Canned salmon	0.68	70	9.7	1.2	1.3	1.4	1.5

* Assuming a 60 kg adult with a 1.1 pg TEQ/kg bw/day dietary intake from non-fish part of diet made up of 0.7 pg WHO-TEQ/kg bw/day for chlorinated dioxins and DL-PCBs and 0.4 pg TEQ/kg bw/day (range of lower to upper bound 0.08 - 0.4 pg TEQ/kg bw/day) for brominated dioxins and DL-PBBs

Exceeds TDI by up to 2-fold (green: Sprat, Herring one–two portions; Farmed salmon two–three portions; Wild salmon, Mackerel two–four portions; Sea Trout, Farmed Trout three–four portions; Swordfish four portions; Sardine/Pilchard one–two portions; Whitebait two–four portions; Canned sardines three–four portions; Herring (Rollmops) four portions)

Exceeds TDI by 2- to 4-fold (grey: Sprat, Herring three–four portions; Farmed salmon four portions; Sardine/Pilchard three–four portions)

Table 3. Estimated upper bound average daily dioxins and dioxin-like PCBs dietary exposure for a 60 kg adult consuming 1-4 portions of non-oily fish per week.

Species	Concentration in fish (ng WHO-TEQ/ kg fresh weight)	Portion size (g)	Fat content (%)	Total daily dietary intake [a] (pg WHO-TEQ/kg bodyweight/day) – Number of portions of fish consumed per week: One portion	Two portions	Three portions	Four portions
Non-oily fish							
Wild Sea Bass	3.71	140	6.8	1.9	3.1	4.4	5.6
Farmed Sea Bass	1.46	140	8.5	1.1	1.6	2.1	2.6
Farmed Halibut	2.43	140	4.2	1.5	2.3	3.1	3.9
Wild Halibut	1.09	140	4.5	1.0	1.4	1.7	2.1
Turbot (Greenland)	2.33	140	10.4	1.4	2.2	3.0	3.8
Dogfish	2.15	140	7.3	1.4	2.1	2.8	3.5
Wild Turbot (UK)	1.54	140	1.5	1.2	1.7	2.2	2.7
Farmed turbot	1.01	140	9.6	1.0	1.3	1.7	2.0
Sea Bream	1.48	140	1.5	1.2	1.6	2.1	2.6
Plaice	0.70	140	2.3	0.9	1.1	1.4	1.6
Hake	0.59	140	2.4	0.9	1.1	1.2	1.4
Lemon Sole	0.43	140	1.1	0.8	0.9	1.1	1.2
Coley	0.16	140	1.9	0.7	0.8	0.8	0.9
Shark	0.13	140	1.2	0.7	0.7	0.8	0.8
Red snapper	0.12	140	1.9	0.7	0.7	0.8	0.8
Cod	0.10	140	0.4	0.7	0.7	0.8	0.8
Whiting	0.09	140	0.7	0.7	0.7	0.8	0.8
Haddock	0.07	140	0.9	0.7	0.7	0.7	0.7
Crab (brown/white)	3.59	70	6.0	1.3	1.9	2.5	3.1
Oysters	0.45	70	2.0	0.7	0.8	0.9	1.0
Mussels	0.28	70	2.8	0.7	0.8	0.8	0.8
Scampi	0.24	70	1.1	0.7	0.7	0.8	0.8
Canned crab (white)	0.15	70	1.1	0.7	0.7	0.7	0.8
Prawns cold	0.10	70	1.7	0.7	0.7	0.7	0.7
Scallops	0.07	70	1.5	0.7	0.7	0.7	0.7
Prawns warm	0.07	70	1.5	0.7	0.7	0.7	0.7
Canned tuna	0.02	70	2.3	0.7	0.7	0.7	0.7
Fish paste	2.40	30	10.1	0.8	1.0	1.2	1.3
Canned anchovy	0.58	30	13.7	0.7	0.7	0.8	0.8
Surimi	0.02	30	1.3	0.7	0.7	0.7	0.7

[a] Assuming a 60 kg adult with a 0.7 pg WHO-TEQ/kg bw/day dietary intake from non-fish part of diet.

Exceeds TDI by upto 2-fold (green shading: Wild Sea Bass two portions; Farmed Sea Bass three and four portions; Farmed Halibut two, three and four portions; Wild Halibut four portions; Turbot (Greenland) two, three and four portions; Dogfish two, three and four portions; Wild Turbot (UK) three and four portions; Sea Bream three and four portions)

Exceeds TDI by 2- to 4-fold (grey shading: Wild Sea Bass three and four portions)

Table 4. Estimated upper bound average daily dioxins, dioxin-like PCBs, brominated dioxins and dioxin-like PBBs dietary exposure for a 60 kg adult consuming 1-4 portions of non-oily fish per week.

Species	Concentration in fish (ng TEQ/kg fresh weight)	Portion size (g)	Fat content (%)	Total daily dietary intake [a] (pg TEQ/kg bodyweight/day) — Number of portions of fish consumed per week: One portion	Two portions	Three portions	Four portions
Non-oily fish							
Wild Sea Bass	3.73	140	6.8	2.3	3.6	4.8	6.0
Farmed Sea Bass	1.49	140	8.5	1.6	2.1	2.6	3.1
Farmed Halibut	2.45	140	4.2	1.9	2.7	3.5	4.3
Wild Halibut	1.13	140	4.5	1.4	1.8	2.2	2.5
Turbot (Greenland)	2.35	140	10.4	1.8	2.6	3.4	4.2
Dogfish	2.17	140	7.3	1.8	2.5	3.2	3.9
Wild Turbot (UK)	1.56	140	1.5	1.6	2.1	2.6	3.1
Farmed turbot	1.03	140	9.6	1.4	1.8	2.1	2.4
Sea Bream	1.51	140	1.5	1.6	2.1	2.6	3.0
Plaice	0.72	140	2.3	1.3	1.5	1.8	2.0
Hake	0.61	140	2.4	1.3	1.5	1.7	1.9
Lemon Sole	0.45	140	1.1	1.2	1.4	1.5	1.6
Coley	0.18	140	1.9	1.1	1.2	1.2	1.3
Shark	0.15	140	1.2	1.1	1.2	1.2	1.2
Red snapper	0.14	140	1.9	1.1	1.1	1.2	1.2
Cod	0.12	140	0.4	1.1	1.1	1.2	1.2
Whiting	0.11	140	0.7	1.1	1.1	1.2	1.2
Haddock	0.10	140	0.9	1.1	1.1	1.1	1.2
Crab (brown/white)	3.63	70	6.0	1.7	2.3	2.9	3.5
Oysters	0.71	70	2.0	1.1	1.2	1.3	1.4
Mussels	0.42	70	2.8	1.1	1.2	1.3	1.3
Scampi	0.26	70	1.1	1.1	1.2	1.2	1.2
Canned crab (white)	0.17	70	1.1	1.1	1.1	1.1	1.2
Prawns cold	0.12	70	1.7	1.1	1.1	1.1	1.1
Scallops	0.10	70	1.5	1.1	1.1	1.1	1.1
Prawns warm	0.09	70	1.5	1.1	1.1	1.1	1.1
Canned tuna	0.04	70	2.3	1.1	1.1	1.1	1.1
Fish paste	2.44	30	10.1	1.2	1.4	1.6	1.8
Canned anchovy	0.62	30	13.7	1.1	1.2	1.2	1.2
Surimi	0.04	30	1.3	1.1	1.1	1.1	1.1

[a] Assuming a 60 kg adult with a 1.1 pg TEQ/kg bw/day dietary intake from non-fish part of diet made up of 0.7 pg WHO-TEQ/kg bw/day for chlorinated dioxins and DL-PCBs and 0.4 pg TEQ/kg bw/day (range lower to upper bound 0.08 - 0.4 pg TEQ/kg bw/day) for brominated dioxins and DL-PBBs.

Exceeds TDI by upto 2-fold — Exceeds TDI by 2- to 4-fold

Tribrominated dioxin and furan

46. The concentrations of 2,3,7-triBDD and 2,3,8-triBDF are also reported in these surveys. The trichlorinated congeners, have short half-lives, and therefore do not have TEFs. There are no data on the half-lives for the brominated compounds and they have not been included in the combined TEQ.

47. Analysis of the 2003 TDS samples estimated the upper bound adult dietary intake for an average consumer of 2,3,7-triBDD from the non-fish part of the diet to be 0.09 pg/kg bw/day (lower bound 0 ng/kg bw/day). Consumption of only four species of shell fish, crab (white and brown), canned crab (white), mussels and oysters would increase the dietary intake. The maximum concentration detected in oysters was 6.7 μg/kg freshweight. Assuming that a 60 kg person consumes a weekly portion of 70 g of oysters containing this concentration of 2,3,7-triBDD, the total dietary intake from the oysters and the rest of the diet would be 1.1 pg/kg bw/day.

48. For the tribrominated dibenzofuran, 2,3,8-triBDF, the non-fish part of the diet would contribute an estimated upper bound adult dietary intake for an average consumer of 0.25 pg/kg bw/day (lower bound 0.13 pg/kg bw/day). Concentrations detected in surveyed fish ranged from 0.001 – 0.078 μg/kg freshweight, with oysters having the highest concentrations. Consumption of four portions of oysters containing 0.078 μg/kg freshweight per week could increase the total dietary intake of 2,3,8-TriBDF by 0.05 pg/kg bw/day for a 60 kg person.

Ortho-*polybrominated biphenyls*

49. The concentrations of the seven *ortho*-PBBs detected in all species were similar, with PBB-52 being detected at the highest concentration of 0.05 μg/kg freshweight in sprats. Sprats also showed the highest concentration of PBBs when all seven congeners were summed (0.1 μg/kg freshweight).

50. Assuming that a 60 kg person consumes a weekly portion (140g) of sprats with a total PBB concentration of 0.1 μg/kg freshweight the total dietary intake including the non-fish part of the diet would be approximately 0.4 ng/kg bw/day, which is considerably lower than the TDI of 0.15 μg/kg bw/day proposed by WHO[17]. Estimated lower bound intakes from the non-fish part of the diet for the *ortho*-PBBs were <0.001 ng/kg bw/day for PBBs 49, 52, 80, 101, and 153, 0.002 ng/kg bw/day for PBB 15 and 0.18 ng/kg bw/day for PBB 209.

Polybrominated diphenyl ethers

51. In total 17 PBDE congeners were analysed in fish and the 2003 TDS samples, consisting of 2 triBDEs, 5 tetraBDEs, 5 pentaBDEs, 3 hexaBDEs, 1 heptaBDE and decaBDE.

52. The congeners present at the highest levels in the sampled fish were, in order of decreasing concentrations, PBDE-47 (2,2',4,4'-tetraBDE), PBDE-209 (decaBDE), PBDE-100 (2,2',4,4',6-pentaBDE), and PBDE-49 (2,2',4,5-tetraBDE).

53. Fish with the highest concentrations of the sum of the measured PBDEs were dogfish (8.71 μg/kg freshweight) and eel (5.4 μg/kg freshweight). Farmed salmon, herring, sprat and whitebait had concentrations ranging from 4.0 to 4.5 μg/kg freshweight.

54. Estimated upper bound adult dietary exposure to the sum of the measured PBDEs from the non-fish part of the diet is 5.6 ng/kg bw/day (lower bound 5.5 ng/kg bw/day). Assuming a 60 kg person consumes one weekly portion of dogfish containing the highest total PBDE concentration detected, the total intake from the diet would be 8.5 ng/kg bw/day.

55. COT set a target MoE of 1000 for liver toxicity of pentaBDE on the basis of the NOAEL for liver effects in rats (450 μg/kg bw/day). Above this MoE, risks to health would not be expected. The MoE for the intake levels described above is approximately 53,000. JECFA proposed using a reference dose at which adverse effects were not expected, 100 μg/kg bw/day, on which to base the MoE (2005). In this case, consumption of one weekly portion of dogfish will result in a MoE of approximately 11,000.

Hexabromocyclododecane

56. Eels had the highest maximum concentration of HBCD detected, the α-HBCD concentration was 5.1 μg/kg freshweight, with the sum of all three isomers being 5.3 μg/kg freshweight. This level is significantly lower than the maximum concentration of 9432 μg/kg freshweight detected in eels from the Skerne-Tees river system in 2003[22].

57. For the non-fish part of the diet, the upper bound concentration of HBCD (sum of all diastereoisomers) in the 2004 TDS samples was 5.8 ng/kg bw/day (lower bound 1.9 ng/kg bw/day). Assuming that a 60 kg person consumes a weekly portion (70 g) of eel containing 5.3 μg/kg freshweight of HBCD, the intake would be 6.6 ng/kg bw/day.

58. COT has previously used a margin of exposure approach in the risk assessment of HBCD, using a LOAEL of 100 mg/kg bw/day as the basis for the calculation. The uncertainty factors of 100 to allow for inter and intra-species differences, 10 to allow for gaps in the data and 3-10 for extrapolation from the LOAEL to a NOAEL produce a target MoE of 3,000-10,000. Applying the MoE approach to the most recent intake data for HBCD produces MoEs of approximately 15,000,000.

Tetrabromobisphenol A

59. Mackerel was identified as having the highest concentrations of TBBPA (0.21 μg/kg freshweight). The average concentration for all species of fish was 0.04 μg/kg freshweight (range 0.03 – 0.21). On the basis of the 2004 TDS samples, intake of TBBPA from the non-fish part of the diet is 1.5 ng/kg bw/day. Assuming that a 60 kg person consumes a weekly portion (140 g) of mackerel containing 0.21 μg/kg freshweight, the daily dietary intake of TBBPA would be 1.6 ng/kg bw/day.

60. In 2004 COT recommended a TDI of 1 mg/kg bw/day for TBBPA[25]. The estimated dietary intake from the total diet including one weekly portion of mackerel is considerably below the TDI.

COT Evaluation

61. The COT reviewed the new information in the light of previous COT conclusions and paid particular attention to possible combined effects of the different contaminants.

62. The COT noted that where comparison is possible concentrations of chlorinated dioxins and dioxin-like PCBs in fish from the most recent survey are generally lower than detected in fish sampled in 1994 to 1996. In the case of some species of oily fish (herring, mackerel and farmed salmon) the decreases were particularly marked (for herring, up to 50% lower).

63. There is increasing evidence that the brominated dioxins, furans and coplanar and mono-*ortho* polybrominated biphenyls are dioxin-like in respect to their effects in *in vitro* and *in vivo* mammalian test systems. However, there still remain some significant data gaps for a number of the congeners, in particular in terms of repeat-dose studies and the toxicokinetics of these compounds in man. The available data indicates that the brominated congeners are equally or less toxic compared to the chlorinated dioxins, and in rodents the few tested congeners have similar half-lives to the chlorinated congeners.

64. The Committee agreed that in light of this evidence, and the absence of an alternative approach, it would be prudent to apply the TEFs assigned to the chlorinated dioxins to the brominated congeners. The TEQs for the brominated contaminants should be combined with the WHO-TEQs for the chlorinated dioxins to provide an indication of the total intake of chemicals with dioxin-like properties.

65. Including the brominated congeners in the TEQs for intake from fish and the rest of the diet did not raise additional toxicological concerns. As there are no new toxicity data giving rise to new concerns, it was considered unnecessary to alter the COT's previous advice on oily fish consumption.

66. The data for the *ortho*-PBBs, PBDEs, HBCD and TBBPA were considered separately from the dioxin-like compounds as they were considered to have different modes of action. The Committee agreed that the concentrations of these contaminants in the sampled fish did not raise toxicological concerns. In the cases of the *ortho*-PBBs and TBBPA the estimated dietary intake from the total diet including a portion of the species of fish with the highest concentration of the respective contaminant were considerably below the TDIs (WHO-proposed TDI for the PBBs). For the PBDEs and HBCDs the MoE for the intake levels described earlier in this statement were above the target MoE set previously by COT.

Conclusions

67. We consider that the concentrations of PBDEs, HBCD and TBBPA detected in these surveys do not raise toxicological concerns.

68. We conclude that concentrations of dioxin-like compounds detected in these surveys are not a concern for the health of the majority of UK consumers, who do not eat fish frequently. For those who choose to eat more than two portions of fish per week, the data reconfirm the previous SACN/COT guidance on upper levels of oily fish consumption.

69. The concentrations of dioxin-like compounds in some species of non-oily fish (sea bass, sea bream, halibut, turbot, dogfish and crab) are similar to those commonly found in some oily fish. Frequent consumption of these species of fish in addition to the recommended amounts of oily fish could result in exceedance of the intake guidelines for dioxin-like compounds.

70. We welcome the decrease in concentrations of chlorinated dioxin-like compounds in most fish species for which comparative data are available. Concentrations in wild fish can be reduced only in the long term by control of emissions to the environment. Controls on contaminant levels in feed for farmed fish are important for reducing dietary exposure of people who eat fish frequently.

71. We consider that the new survey data do not indicate a need for a change in the Food Standards Agency's current advice on consumption of oily fish.

72. There are considerable uncertainties in the data which indicate that this assessment might be over-precautionary. The risk assessment could be improved by refinement both of exposure assessment and of the toxicological basis for the TEFs, using probabilistic approaches. Modelling the available information may help to determine where the greatest uncertainty lies, in order to prioritise future research.

COT statement 2006/06
April 2006

References

1. SACN/COT. (2004). Advice on fish consumption: benefits & risks. Scientific Advisory Committee on Nutrition and Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. The Stationery Office, London.

2. Ferre-Huguet, N., Nadal, M., Schuhmacher, M., Domingo, J.L. (2006). Environmental impact and human health risks of polychlorinated dibenzo-p-dioxins and dibenzofurans in the vicinity of a new hazardous waste incinerator: a case study. *Environ Sci Technol* 40: 61-66.

3. COT. (2001). COT statement on the tolerable daily intake for dioxins and dioxin-like polychlorinated biphenyls. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. COT statement 2001/07. Available at http://www.food.gov.uk/multimedia/pdfs/cot-diox-full.pdf

4. MAFF. (1997). Dioxins and polychlorinated biphenyls and foods and human milk. Ministry of Agriculture, Fisheries and Food. Food Surveillance Information Sheet 105. London.

5. MAFF. (1998). Dioxins and polychlorinated biphenyls in farmed trout in England and Wales. Ministry of Agriculture, Fisheries and Food. Food Surveillance Information Sheet 145. London.

6. MAFF. (1999). Dioxins and polychlorinated biphenyls in UK and imported marine fish. Ministry of Agriculture, Fisheries and Food. Food Surveillance Information Sheet 184. London.

7. WHO. (1998). Polybrominated dibenzo-*p*-dioxins and dibenzofurans. Environmental Health Criteria 205, IPCS, Geneva.

8. Kedderis, L.B., Diliberto, J.J., Birnbaum, L.S. (1991). Disposition and excretion of intravenous 2,3,7,8-tetrabromodibenzo-p-dioxin (TBDD) in rats. *Toxicol Appl Pharmacol* 108: 397-406.

9. Golor, G., Yamashita, K., McLachlan, M, Hutzinger, O, Neubert, D. (1993). Comparison of the kinetics of chlorinated and brominated dioxins and furans in the rat. *Organohalogen Compounds* 25: 203-206.

10. Nagao, T., Yamashita, K., Golor, G., Bittmann, H., Korner, W., Hagenmaier, H., Neubert, D. (1996). Tissue distribution after a single subcutaneous administration of 2,3,7,8-tetrabromodibenzo-p-dioxin in comparison with toxicokinetics of 2,3,7,8-tetrachlorodibenzo-p-dioxin in female Wistar rats. *Life Sci* 58: 325-336.

11. Birnbaum, L.S., Morrissey, R.E., Harris, M.W. (1991). Teratogenic effects of 2,3,7,8-tetrabromodibenzo-p-dioxin and three polybrominated dibenzofurans in C57BL/6N mice. *Toxicol Appl Pharmacol* 107: 141-152.

12. Mason, G., Zacharewski, T., Denomme, M.A., Safe, L., Safe, S. (1987). Polybrominated dibenzo-p-dioxins and related compounds: quantitative *in vivo* and *in vitro* structure-activity relationships. *Toxicology* 44: 245-255.

13. Behnisch, P.A., Hosoe, K., Sakai, S. (2003). Brominated dioxin-like compounds: *in vitro* assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds. *Environ Int* 29: 861-877.

14. Behnisch, P.A., Fujii, K., Shiozaki, K., Kawakami, I., Sakai, S. (2001). Estrogenic and dioxin-like potency in each step of a controlled landfill leachate treatment plant in Japan. *Chemosphere* 43: 977-984.

15. Brown, D. J., Van Overmeire, I., Goeyens, L., Denison, M. S., De Vito, M. J., Clark, G. C. (2004). Analysis of Ah receptor pathway activation by brominated flame retardants. *Chemosphere* 55, 1509-1518.

16. Curran, C.P., Miller, K.A., Dalton, T.P., Vorhees, C.V., Miller, M.L., Shertzer, H.G., Nebert, D.W. (2006). Genetic differences in lethality of newborn mice treated in utero with coplanar versus non-coplanar hexabromobiphenyl. *Toxicol Sci* 89: 454-464.

17. WHO. (1994). Polybrominated biphenyls. Environmental Health Criteria 152, IPCS, Geneva.

18. van den Berg, M., Birnbaum, L., Bosveld, A.T., Brunstrom, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X., Liem, A.K., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T. (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect* 106: 775-792.

19. NTP. (1993). NTP Toxicology and Carcinogenesis Studies of Polybrominated Biphenyls (CAS No. 67774-32-7) (Firemaster FF-1(R)) in F344/N Rats and B6C3F1 Mice (Feed Studies). *Natl Toxicol Program Tech Rep Ser* 398: 1-235.

20. Tennant, R.W., Stasiewicz, S., Spalding, J.W. (1986). Comparison of multiple parameters of rodent carcinogenicity and *in vitro* genetic toxicity. *Environ Mutagen* 8: 205-227.

21. Kavanagh, T.J., Rubinstein, C., Liu, P.L., Chang, C.C., Trosko, J.E., Sleight, S.D. (1985). Failure to induce mutations in Chinese hamster V79 cells and WB rat liver cells by the polybrominated biphenyls, Firemaster BP-6, 2,2',4,4',5,5'-hexabromobiphenyl, 3,3',4,4',5,5'-hexabromobiphenyl, and 3,3',4,4'-tetrabromobiphenyl. *Toxicol Appl Pharmacol* 79: 91-98.

22. COT. (2003). COT statement on brominated flame retardants in fish from the Skerne-Tees river system. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. COT statement 2003/04. Available at http://www.food.gov.uk/multimedia/pdfs/bfrstatement.pdf

23. JECFA. (2005). Sixty fourth meeting: Summary and Conclusions. Joint FAO/WHO Expert Committee on Food Additives. Available at http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf

24. EU. (2002). Hexabromocyclododecane. Draft EU Risk Assessment.

25. COT. (2004). Tetrabromobisphenol A – a review of toxicological data. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. COT statement 2004/02. Available at http://www.food.gov.uk/multimedia/pdfs/cotstatements04tbbpa.pdf

26. NDNS. (2001). National Diet and Nutrition Survey: Adults aged 19 to 64.

Statement on the tolerable daily intake for perfluorooctanoic acid

Introduction

1. The Food Standards Agency has commissioned research to determine the concentrations of perfluorooctanoic acid (PFOA) in the 2004 Total Diet Study (TDS) samples. The Committee was invited to assess the toxicology of PFOA in order to advise on any health implications arising from the results of the survey.

Background

2. The unexpected discovery of fluorinated organic compounds of anthropogenic origin, identified as predominantly perfluorooctane sulfonate (PFOS), in biological and environmental samples[1,2] has resulted in the toxicology of structurally similar perfluorocarbons being investigated.

3. Perfluorooctanoic acid (PFOA) and its salts are fully fluorinated organic compounds produced synthetically or through degradation of some PFOS-related substances[3] and fluorotelomer alcohols[4]. PFOA is primarily used as an emulsifier in industrial applications, for example in the production of fluoropolymers such as polytetrafluoroethylene (PTFE). PFOA may also be found at low levels in some fluorotelomers, as an unintended by-product of the manufacturing process. Fluorotelomer derivatives are ingredients of fire-fighting foams and coatings, and are intermediates in the manufacture of stain-, oil-, and water-resistant additives for some textiles, coatings and food contact papers.

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

Evidence considered in this evaluation

5. The COT has not previously evaluated PFOA or its salts. From an initial assessment of the relevant information it was considered essential to have advice from the Committees on Mutagenicity (COM) and Carcinogenicity (COC) regarding the genotoxicity of PFOA and whether it was appropriate to assume the existence of a threshold for carcinogenicity. The recommendations provided by the COM and COC are summarised in this statement.

6. The evaluation of PFOA considered toxicological data in the published literature and unpublished final reports. Access to the unpublished reports was through the US EPA Office of Pollution Prevention and Toxics (OPPT) Administrative Record AR-226.

Chemical information

7. The high ionization potential and low polarizability of fluorine lead to weak inter- and intra-molecular interactions. This is reflected by the extremely low surface tension of the perfluoroalkyl acids. Their partitioning behaviour is unique; when mixed with water and hydrocarbons, three immiscible phases are formed, indicating that perfluoroalkyl acids are hydrophobic and oleophobic in nature. Consequently, these compounds are ideal surfactants. The strength of the carbon-fluorine bonds makes PFOA and its salts highly stable and, therefore, persistent in the environment.

8. The structure of PFOA ($C_8HF_{15}O_2$, CAS registry number 335-67-1) is shown in Figure 1. The typical structure has a linear chain of eight carbon atoms, but dependent on the manufacturing process branched chain PFOA may also be produced. The electrochemical fluorination process generally gives a product with up to 30% branched PFOA, whereas production by oxidation of perfluorooctyl iodide leads to 100% linear PFOA. Ammonium perfluorooctanoate is of most widespread use and is commonly referred to in the literature as APFO, C8 or FC-143.

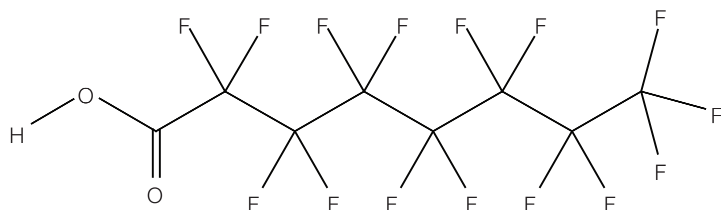

Figure 1 – Structure of perfluorooctanoic acid (PFOA)

9. In water the free acid will completely dissociate to perfluorooctanoate. Water solubility is published as 3.4 g/L, and a 1 g/L solution has a pH of 2.6. An octanol/water partition coefficient cannot be determined for PFOA due to the fact that, rather than being soluble, PFOA forms microdispersion micelles.

Toxicological profile

10. The majority of the toxicological studies have been conducted using ammonium perfluorooctanoate and in most cases the test material was a mixture of the ammonium salts of several perfluorinated acids as manufacturing residues. The typical composition profile is 93-97% ammonium perfluorooctanoate, 1-3% ammonium perfluoropentanoate, 1-3% ammonium perfluoroheptanoate and 1-3% ammonium perfluorohexanoate.

Toxicokinetics – rodents

11. PFOA is well absorbed by rats following oral dosing. Male rats absorbed 93% of an 11 mg/kg bw gavage dose of ^{14}C-PFOA within 24 hours[5]. In this study the ^{14}C total elimination half-life was estimated at 4.8 days (115 hours), whereas, other studies have estimated that the elimination half-life for PFOA in male rats is in the range of 138 to 350 hours[6,7,8]. There is a clear sex-related difference in clearance of PFOA in rats. In female rats, PFOA is more efficiently cleared from the body, primarily via rapid excretion in the urine with a plasma half-life of approximately 10 hours[9,10]. Following oral gavage of female rats with 2 mg PFOA, the quantity of non-ionic fluorine recovered in the urine was 89% of the administered dose within 96 hours.

12. Distribution studies, using administration of PFOA via gavage, and intravenous and intraperitoneal injection, have shown that PFOA does not partition to the lipid fraction or adipose tissue, but is primarily found in the liver, plasma, and kidney[6].

13. Following PFOA administration by gavage, Kemper[8] determined tissue concentrations at the time of maximum plasma concentration (T_{max}) and when plasma concentration had fallen to 50% maximum (i.e. at 11 and 171 hours post-dosing for males and at 1.3 and 4 hours post-dosing for females). In males, tissue concentrations remained constant or decreased with time, in all but the liver where PFOA levels increased. The fraction of the dose present in all female rat tissues remained constant or decreased with time. Kidney to blood PFOA concentration ratios at T_{max} were approximately 2 at all dose levels in females and remained constant with time.

14. PFOA crosses the placenta. The concentration of PFOA in fetal plasma on gestation day (GD) 21, following continuous maternal exposure from GD 4 was approximately half the steady state concentration in maternal plasma. PFOA was also detected in the milk of rats, at levels approximately 10% those of maternal plasma concentrations[11].

15. PFOA undergoes enterohepatic circulation. By disrupting enterohepatic circulation (with cholestyramine treatment) in male rats, the level of PFOA eliminated in faeces increased by approximately 9.6-fold[12]. A concomitant decrease in the proportion of the PFOA dose excreted in the urine was seen (from 67% to 41% over 14 days).

16. There is evidence that PFOA is not metabolised in the rat.

17. In the 24 hours following treatment with a single i.v. dose (mean doses: 16.7 and 13.1 mg/kg bw for female and male CD rats, respectively) of ^{14}C-PFOA, female rats had excreted essentially all the administered dose via the urine, whilst the males had only excreted 20% of the dose[9]. In total, over the course of 36 days the male rats excreted 83% of the total dose via the urine and 5.4% via the faeces.

18. Studies investigating the sex-related difference in elimination of PFOA have shown that the female rat possesses an active secretory mechanism which rapidly eliminates PFOA from the body. Administration of probenecid (an inhibitor of the renal active secretion system for organic acids) reduced the PFOA/inulin clearance ratio in female rats from 15 to 0.46[13]. PFOA clearance was reduced from 5.8 to 0.11 mL/min/100 g bw, a level similar to male rat PFOA clearance which was virtually unaffected by probenecid administration.

19. In male rats, testosterone has been shown to exert a suppressive effect on renal excretion of PFOA[14]. Castration of male rats increased the elimination of PFOA in the urine. Kudo *et al.*,[7] demonstrated that organic anion transporter 2 (OAT2) mRNA levels in male rats were only 13% those in female rats. Castration or oestradiol treatment increased the levels of OAT2 mRNA whereas treatment of castrated rats with testosterone reduced them. Evidence was also obtained for the involvement of OAT3 in PFOA excretion.

20. Comparison of area under concentration-time curve in plasma for oral and intravenous doses of PFOA (1 mg/kg bw) in Sprague-Dawley rats indicated that oral bioavailability is approximately 100%[8]. Plasma elimination curves for PFOA following gavage at doses of 0.1, 1, 5, and 25 mg/kg bw were log-linear with respect to time in male rats, while elimination kinetics were biphasic in the 5 and 25 mg/kg bw female dose groups. Estimated plasma elimination half-lives were approximately 277 hours in males and 3.4 hours in females, using non-compartmental pharmacokinetic models. In contrast, Kudo *et al.*,[7] using a two-compartment open model, found the terminal elimination half-lives in Wistar rats to be 137 and 1.9 hours in males and females, respectively. Females appeared to exhibit biphasic elimination. The main component was rapid (half-life of 1.9 hours), with a slower minor component

21. Hormonal changes associated with pregnancy have been shown not to alter the rate of elimination of PFOA[15].

22. An unpublished comparative study with male and female rats, mice, hamsters and rabbits[16] dosed by oral gavage (10 mg/kg bw ^{14}C-PFOA) and sacrificed 5-7 days later, showed significant differences in PFOA elimination between the species. Male hamsters and both sexes of rabbits excrete PFOA as rapidly as female rats, however, the female hamster cleared the chemical more slowly, having excreted only 58% of the administered dose in 5 days. Male and female mice retained between 50-70% of the administered dose 5 days after dosing.

23. Lau *et al.*[17] compared body burdens of PFOA between rat and mouse after subchronic exposure. A clear sex-related difference in PFOA accumulation was confirmed in the rat; a serum level of 111 µg PFOA/mL was reached in male rats 24 h after the last of 20 daily 10 mg/kg bw/day doses, while only 0.7 µg PFOA/mL was detectable in female rat serum. A steady state level of PFOA in mice was reached in one week of exposure. PFOA concentrations in serum following 7 daily 20 mg/kg bw/day doses were approximately 180 µg PFOA/mL for male and female mice.

Toxicokinetics – dog

24. The renal clearance rate of PFOA in beagles (3/sex) following an i.v. administration (30 mg/kg bw) was approximately 0.03 mL/min/kg bw[18]. Probenecid significantly reduced clearance rates in both sexes, indicating an active excretion mechanism. Plasma half-life was 473 hours in male dogs and 202 hours in female dogs.

Toxicokinetics – non-human primates

25. The toxicokinetics of PFOA following a single i.v. dose (10 mg/kg bw) have been assessed in cynomolgus monkeys (3 monkeys/sex)[19]. Body weights were unaffected between days 1 and 28. PFOA serum concentrations 0.5 hours post-dosing were similar in males and females. By day 123, male PFOA serum levels were at or slightly above 0.02 μg/mL (the limit of quantitation), and female serum concentrations were between 0.89 and 4.7 μg/mL. There were no obvious sex differences in the urinary excretion of PFOA, which was slow (less than 20% of the administered dose was excreted in the urine over the first 48 hours). However, although the number of monkeys in this study was limited, estimated half-life and total body clearance values indicated elimination in males may occur at a slightly faster rate than females. The average terminal elimination phase half-lives were approximately 21 and 33 days for males and females, respectively.

26. In a six month oral capsule dosing study of PFOA male cynomolgus monkeys (4-6/group) were administered 0, 3, 10 or 30/20 (reduced on day 22 from 30 to 20) mg/kg bw/day[19]. During the first week of dosing monkeys in the 30 mg/kg bw/day group showed general signs of toxicity, including low food consumption, significant loss of body weight, and 4 of the 6 monkeys also had few or no faeces. Dosing was suspended on Day 12 and reinitiated at 20 mg/kg bw/day on Day 22. Steady state was reached within four weeks in serum, urine and faeces. Serum PFOA followed first-order elimination kinetics following the last dose, with a half-life of approximately 20 days. Urine was the primary elimination route. The i.v. study would predict that steady state in the daily oral dosing study would not be reached until at least eight weeks of daily oral dosing. The reasons for the apparent achievement of steady state before this time are not known.

Toxicokinetics – humans

27. Human toxicokinetic data on PFOA are limited in number and conflicting.

28. A number of studies have assessed the levels of PFOA in blood of occupationally and non-occupationally exposed populations. Serum PFOA levels in 3M workers have been measured since 1993. In the Cottage Grove, Minnesota production plant, PFOA serum levels were highest amongst the 3M plants (geometric mean = 1.7 μg/mL, range, 0.07-33 μg/mL)[20].

29. Ubel *et al.*,[21] reported an approximate half-life of PFOA of 18 months based on one PFOA worker. A report from 3M on nine fluorochemical production plant retirees suggested a serum half-life of 4.4 years, with a range of 1.5 to 14 years[22]. This study suffered from significant limitations, such as small sample size, reference material purity unchecked, and unreplicated serum measurements.

30. In a study of Japanese maternal and cord blood samples[23], PFOA was detected in only 3 of the 15 maternal blood samples (range 0.0005-0.003 μg/mL) and not in any fetal blood samples (limit of detection <0.0001 μg/mL).

31. In contrast to the large active renal excretion of PFOA in female rats[7], renal clearance of PFOA is almost negligible in both sexes in humans[24].

32. PFOA has also been found in the serum of children, adults and the elderly in the general population[25,26]. In the US, serum concentrations follow a log-normal distribution with geometric mean concentrations of 0.004-0.005 μg/mL, and over 90% of serum samples had quantifiable levels of PFOA. The upper bound of the 95th percentile estimate of the population, below which the 95th percentile serum concentration of the samples falls with 95% confidence, was 0.014 μg/mL.

33. An assessment of the prevalence of organic perfluorochemicals in the blood of Swedish mothers and sons concluded that PFOA levels in the general population of Sweden and the US are simila[27]. In whole blood samples (*n* = 66) PFOA concentrations ranged from 0.0005 to 0.0124 μg/mL with an arithmetic mean of 0.0027 μg/mL. There was no significant difference between males and females.

34. In 473 human blood/serum/plasma samples collected in various countries worldwide (USA, Colombia, Brazil, Belgium, Italy, Poland, India, Malaysia, and Korea) PFOA was seen at a low frequency[23]. Samples (*n* = 50) from Italy did not have quantifiable levels of PFOA, whereas PFOA was quantifiable in all samples from Poland (*n* = 25). Two Korean females in particular showed PFOA levels greater than 0.1 μg/mL.

35. Concentrations of PFOA in sera sampled from a small number (*n* = 23-60) of female residents of three Japanese cities have increased by 14-fold over the last 25 years[28]. In 2003, the geometric mean concentrations of PFOA in sera, from both sexes, ranged from 0.0028 to 0.012 μg/mL, with significantly higher levels reported in males.

36. Olsen *et al.*,[26] compared PFOA levels in samples taken from the same subjects, that were part of two large community-based cohorts established in Maryland, US, in 1974 and 1989 and reported that concentrations were two-fold higher in 1989. However, the trend may not have continued, since more recent samples (taken in 2001) appeared similar to the 1989 levels.

Acute and sub-acute toxicity

Rodents

37. There is reasonable consistency between several acute oral LD_{50} studies, which indicate PFOA is moderately toxic.

38. Oral studies with PFOA indicate an oral LD_{50} in rats ranging between 430 and 680 mg/kg bw[29]. There are no reported differences in the sensitivity of castrated or ovariectomised versus intact rats (male or female) to PFOA[30]. Newborn rats (<2 days old) (LD_{50} ~250 mg/kg bw) were more sensitive to PFOA than weanlings and adult animals[31]. Pre-treatment of rats with phenobarbital[29] (an enzyme inducer, in particular of CYP2B1 and CYP2B2) or proadifen hydrochloride (a cytochrome P450 inhibitor) did not alter the LD_{50} of PFOA.

39. PFOA administered to ChR-CD mice for 28 days[32] resulted in dose-related reductions in mean body weight and in muscular weakness after 9, 6 and 4 days at 18, 60 and 200 mg/kg bw/day, respectively. Absolute and relative liver weights were also increased in both sexes in a dose-related manner. Treatment-related changes in the liver included hepatic enlargement and/or discoloration of one or more liver lobes. Histopathological examination revealed panlobular hypertrophy accompanied by focal to multifocal cytoplasmic lipid vacuoles of variable size.

40. In a similar 28-day study[33] with ChR-CD rats PFOA induced absolute liver weight increases in both sexes. The severity and degree of tissue involvement were more pronounced in males than females. Panlobular, multifocal to diffuse, hypertrophy was observed, with focal to multifocal cytoplasmic enlargement of hepatocytes in the centrilobular and midzonal areas. The hypertrophy was associated with acidophilic degeneration and necrosis of scattered hepatocytes with no lobular distribution.

41. Goldenthal *et al.*[34] administered ChR-CD rats (5/sex/dose group) dietary levels of 0, 10, 30, 100, 300 and 1000 ppm PFOA for 90 days, equivalent to dietary intakes of 0.6, 1.7, 5.6, 18, and 64 mg/kg bw/day in males and 0.7, 2.3, 7.7, 22.4 and 76 mg/kg bw/day in females. In male rats body weight gain was reduced at 18 and 64 mg/kg bw/day. Relative kidney weights were significantly increased in males at 5.6 mg/kg bw/day and above, which was dose-related. Absolute and relative liver weights were increased in males of the 18 and 64 mg/kg bw/day groups and also 76 mg/kg bw/day treated females. Males in the 1.7 mg/kg bw/day dose group also had increased absolute liver weights. Hepatocellular hypertrophy (focal to multifocal in the centrilobular to midzonal regions) was observed in males of the 5.6 mg/kg bw/day and higher dose groups, with hepatocellular necrosis in the 1.7 mg/kg bw/day and above dose groups. Based on liver effects in this study, the no observed adverse effect level (NOAEL) was 0.56 mg/kg bw/day in males, and in females the NOAEL was 22 mg/kg bw/day.

42. A 13-week study performed in male ChR-CD rats (45-55/group) at dietary intakes equivalent to 0.06, 0.64, 1.94, and 6.4 mg PFOA/kg bw/day reported no treatment-related clinical signs[35,36]. Effects on the liver (increased hepatic palmitoyl CoA oxidase levels, increased relative liver weights and hepatocellular hypertrophy) were observed in the 0.64, 1.94 and 6.4 mg/kg bw/day dosed animals. In the 0.64 mg/kg bw/day group liver effects were statistically significant at 4 weeks of dosing, but not at 7 or 13 weeks. Hypertrophy at 0.64 mg/kg bw/day was described in the pathology report as minimal and "characterised by an accentuated centrilobular pattern in which the hepatocytes appear to have a more homogenous cytoplasm and the cell borders are more rounded giving the cells a more 'plump' appearance. Except for this 'enlarged' appearance the cells are otherwise 'normal'."

43. The unpublished report[35] concluded that the no observed adverse effect level was 6.4 mg/kg bw/day, the highest tested dose, and the no effect level was 0.06 mg/kg bw/day based on increases in absolute and relative liver weights. In the published report[36] the authors do not establish a NOAEL, but refer to a no effect level of 0.06 mg/kg bw/day with doses of 0.64 mg/kg bw/day and higher producing adaptive and reversible liver changes.

44. The COT modelled the absolute and relative liver weight data using US EPA Benchmark Dose Software to estimate a benchmark dose for a 10% response[†] (BMD_{10}) and its lower confidence limit ($BMDL_{10}$) for these effects. BMD_{10} and $BMDL_{10}$ for absolute liver weights were 0.60 and 0.40 mg/kg bw/day (week 4), 0.66 and 0.29 mg/kg bw/day (week 7) and 0.89 and 0.44 mg/kg bw/day (week 13), respectively. For liver weight relative to body weight, the BMD_{10} and $BMDL_{10}$ were 0.50 and 0.36 mg/kg bw/day (week 4), 0.58 and 0.33 mg/kg bw/day (week 7) and 0.84 and 0.54 mg/kg bw/day (week 13), respectively. In the 1.94 mg/kg bw/day dosed animals the hepatocyte hypertrophy was grade 2 (mild) or higher in 2 of 15 animals. BMD modelling of grade 2 or higher hepatocellular hypertrophy estimated the $BMDL_{10}$ at 0.95 mg/kg bw/day. The duration of exposure did not appear to increase severity of the hypertrophy and liver effects were reversed following an 8 week recovery period.

Non-human primates

45. In a 90-day study in rhesus monkeys[39] (2/sex/group) doses of 0, 3, 10, 30 and 100 mg/kg bw/day PFOA were administered by gavage. By week 5 all monkeys in the highest dose group had died and by week 13 three monkeys from the 30 mg/kg/day group had died. Absolute and relative heart weights of females from the 10 mg/kg bw/day group were significantly decreased as were absolute brain weights of females. No associated morphological changes were observed. No treatment-related lesions were seen in the organs of animals from the 3 and 10 mg/kg bw/day groups. The surviving 30 mg/kg bw/day dose group male had moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen. The LOAEL was 3 mg/kg bw/day on the basis of soft stools or moderate to marked diarrhoea or frothy emesis.

46. The study in male cynomolgus monkeys, described in paragraph 26 above, also reported effects of 0, 3, 10 and 30/20 mg/kg bw/day PFOA for 26 weeks[40]. Dose-dependent increases in absolute liver weight associated with mitochondrial proliferation occurred in all PFOA-treated groups, although histopathological evidence of liver toxicity was not seen at 3 or 10 mg/kg bw/day. A liver-to-body weight ratio percentage of 2.4 for a 3 mg/kg bw/day monkey found in moribund condition was comparable to that of the high-dose monkeys. The moribund 3 mg/kg bw/day monkey was sacrificed on day 137,

† The benchmark dose (BMD) approach[37,38] aims to provide an approach to dose-response assessment that is more quantitative than the current NOAEL process. This approach constructs mathematical models to fit all data points in the dose-response study and uses the best fitting model to interpolate an estimate of the dose that corresponds to a particular level of response (a benchmark response), often 10%. A measure of uncertainty is also calculated, and the lower confidence limit on the benchmark dose is called the BMDL. This accounts for the uncertainty in the estimate of the dose-response that is due to characteristics of the experimental design such as sample size. The BMDL can be used as the point of departure for derivation of a health-based guidance value or a margin of exposure.

When the COT has performed benchmark dose modelling as part of this assessment the US Environmental Protection Agency's Benchmark Dose Software (2000) was used.

however, a complete review of the in-life monkey history including review of the clinical and microscopic pathology failed to identify the cause of this monkey's extreme poor health. These findings were non-specific and, largely, were not consistent with the findings observed for monkeys in the high dose group. There was considerable variation in PFOA concentrations measured in serum and liver with no linear relationship with dose detected. Two control and 10 mg/kg bw/day monkeys were designated as recovery group monkeys and over the course of a 90-day recovery period, PFOA concentrations returned to pre-treatment levels. Markers of tumour formation in the liver, pancreas and Leydig cells, i.e. replicative DNA synthesis in the liver, and serum cholecystokinin (CCK), alkaline phosphatase, bilirubin, bile acids, oestradiol, oestriol and testosterone concentrations were also assessed. There was a two-fold increase in hepatic palmitoyl CoA oxidase activity in the high-dose group and the other markers were unaffected. The study authors acknowledge significant limitations in this study, but suggested that it demonstrates a "*dramatic demarcation in dose-response between a relatively mild response (liver weight increase at the 3 and 10 mg/kg bw/day doses) and serious toxicity (dramatic weight loss and one death at the 30/20 mg/kg bw/day dose)*". As the cause of the 3 mg/kg bw/day monkey's moribund state was not established it was not possible to identify a NOAEL.

Mutagenicity and Carcinogenicity

47. The COM considered the mutagenicity of PFOA in May 2005. PFOA has no apparent structural alerts for mutagenicity and the evidence from animal studies is that absorbed material is not metabolised.

48. Members concluded that the plate incorporation bacterial mutagenicity tests using strains of *Salmonella typhimurium* and *Eschericia coli* using sodium[41] or ammonium[42] perfluorooctanoate were adequate and gave negative results. The *in vitro* hprt assay in Chinese hamster ovary (CHO) cells using ammonium perfluorooctanoate[43] also gave negative results.

49. PFOA (sodium salt) at high concentrations induced a reproducible response in the *in vitro* chromosomal aberration assay in CHO cells in the presence of metabolic activation[44]. No evidence for chromosomal aberrations was documented in the absence of exogenous metabolic activation. It was not clear to what extent the positive results reported were due to cytotoxicity. Ammonium perfluorooctanoate also increased chromosomal aberrations in CHO cells in the presence of exogenous metabolic activation[45]. However, for this study there was clear evidence of cytotoxicity at the same dose level. The COM concluded that the positive results were likely to represent a cytotoxic response.

50. Sodium perfluorooctanoate in the absence and presence of metabolic activation had not induced chromosomal aberrations in cultured human whole blood lymphocytes when tested up to doses that were cytotoxic[46].

51. No evidence for a mutagenic effect was found in mouse bone marrow micronucleus assays testing single oral gavage doses of up to 5000 mg/kg bw sodium perfluorooctanoate[47] or 1990 mg/kg bw ammonium perfluorooctanoate[48]. The COM considered the *in vivo* bone marrow mouse micronucleus studies had been adequately conducted. However, it was noted that there was no direct measure of exposure of the bone marrow in the test materials.

52. Overall, the COM concluded that PFOA was not mutagenic, however, a plausible *in vitro* mechanism for the positive response in the *in vitro* chromosomal aberration assay in CHO cells was required to reassure COM about this conclusion.

53. The carcinogenicity of PFOA has been investigated in two dietary exposure studies in Sprague-Dawley rats[49,50]. Ammonium perfluorooctanoate was administered to Sprague-Dawley rats at levels of 0, 30 ppm (mean achieved dose levels 1.3 and 1.6 mg/kg bw/day in males and females, respectively) or 300ppm (mean achieved dose levels 14.2 and 16.1 mg/kg bw/day in males and females, respectively) in the diet for 104 weeks[49]. Dose-related non-neoplastic liver effects included megalocytosis, cystoid degeneration and portal mononuclear infiltration. Red blood cell counts, haemoglobin and haematocrit values were minimally decreased in the high-dose male rats compared to control values. There were statistically significant decreases in the following parameters: erythrocytes at 6, 12 and 18 months; haemoglobin at 3 and 18 months; and haematocrit at 3, 12 and 18 months. In high dose females, erythrocyte count, haemoglobin and haematocrit were statistically significantly decreased at 12 months. Mean leucocyte counts were increased in the low-dose male group compared to control values, throughout the first year, but not in the high dose group. Statistically significant increases were observed: in lymphocyte counts at 3 months in the high- and low-dose groups, and at 6 and 18 months in the low-dose group; and in neutrophil counts at 12 months in both groups. No statistically significant haematological changes were evident in low dose males and females at 24 months. On the basis of increases in liver weight and hepatic changes in males, and reduced body weight gain and haematological changes in females the NOAEL was 1.3 mg/kg bw/day in males and 1.6 mg/kg bw/day in females. BMD modelling of induced hepatocytic megalocytosis in male rats, by COT, estimated the BMD_{10} and $BMDL_{10}$ at 1.1 and 0.74 mg/kg bw/day, respectively. A significant increase in female mammary fibroadenomas was considered not to be significant by the study authors when compared to historical control data. PFOA also induced an apparent dose-related increase in Leydig cell adenomas, which was not significant compared to historical control incidence. COC members were concerned to note the occurrence of at least two viral infections in the rats used in this study. This limited the value of the results.

54. Biegel *et al.*,[50] investigated a single high dietary dose of PFOA (300 ppm for 24 months; mean achieved dose level 13.6 mg/kg bw/day) in male rats and reported increased incidences of hepatocellular adenomas, Leydig cell adenomas and pancreatic acinar cell adenomas. Serum estradiol concentrations were significantly increased in treated animals. COC noted that this study was not designed to identify a NOEL. In light of the pancreatic acinar cell adenoma findings the pancreas slides from the earlier study[49] were reassessed and the occurrence of proliferative lesions of the pancreatic acini was confirmed[51].

55. A hypothesis had been put forward which proposed that PFOA induces liver, Leydig cell and pancreatic acinar cell tumours via PPAR-alpha activation and that because of lack of relevance of this mode of action in human carcinogenicity, PFOA is unlikely to induce such tumours in humans[52]. COC agreed with the proposal by Klaunig *et al.* that activation of aromatase and subsequent increases in serum estradiol levels were suggestive that a mode of action (MOA) could be proposed for the Leydig cell tumours. However, studies in PPARα-null mice had shown PFOA-induced liver effects[53], which is not consistent with the proposed MOA for liver tumours[52]. COC did not consider it possible to propose MOAs for the liver and pancreatic tumours reported[50].

56. COC concluded that for the purpose of the risk assessment, it would be acceptable to use a threshold approach, and to select an appropriate NOAEL for a precursor event for the most sensitive tumour and that an uncertainty factor of 100 would be appropriate for this endpoint.

57. In two linked, retrospective cohort studies of mortality in an occupationally exposed population[54,55], small increases were reported in death from cancer of the large intestine and from cancer of the prostate in employees with over 1 year definite exposure to PFOA. Standardised mortality ratios were zero, i.e. there were no cases reported, for tumour types observed in the two year rodent carcinogenicity study. COC considered that none of the effects reported were significant for risk assessment.

Developmental and reproductive toxicity

58. A number of prenatal developmental toxicity studies with PFOA have been conducted in rats, mice and rabbits.

59. Time-mated Sprague-Dawley rats (22/dose group) were administered 0, 0.05, 1.5, 5 and 150 mg/kg bw/day PFOA by gavage on GD 6-15[56]. Animals were sacrificed on GD 20. The only statistically significant sign of maternal toxicity was a reduction in mean maternal body weights (150 mg/kg bw/day dose group). Administration of PFOA during gestation did not affect the ovaries or reproductive tract of the dams. Based on signs of maternal toxicity the NOAEL was 5 mg/kg bw/day. The NOAEL indicated for developmental toxicity was 150 mg/kg bw/day, the highest dose tested.

60. Gortner[57] administered four groups of pregnant New Zealand rabbits (18/dose group) doses of 0, 1.5, 5, and 50 mg/kg bw/day PFOA by gavage on GD 6-18. Fetuses were examined for gross abnormalities and placed in a 37°C incubator for a 24 hour survival check. A transient, but statistically significant, reduction in maternal body weight gain was noted on GD 6-9, but body weights returned to control levels on GD 12-29. The authors concluded that because this was the only sign of maternal toxicity, the NOAEL was 50 mg/kg bw/day. A dose-related increase in a skeletal variation (extra ribs or 13th rib) was the only sign of developmental toxicity. Incidence was 16, 20, 30 and 38% in 0, 1.5, 5, and 50 mg/kg bw/day dose groups and this reached statistical significance in the high dose group. The authors concluded that the NOAEL was 5 mg/kg bw/day on the basis of the skeletal variation observations.

61. Staples *et al.*,[58] administered PFOA (0 and 100 mg/kg bw/day) to pregnant rats in two oral dosing studies for GD 6-15. Study one sacrificed dams at GD 21 and study two allowed parturition and sacrificed pups on postnatal day (PND) 35. Three out of the twenty five dams in study one died in the 100 mg/kg bw/day PFOA dose group (one on GD 11 and two on GD12). Food consumption and body weights were reduced in treated animals compared to controls. No adverse effects were noted in any of the reproductive parameters assessed. Fetal weights and incidences of malformation were similar in the control and treated animals. Study two noted similar clinical signs in dams as in study one and no significant effects of PFOA treatment on reproductive performance or in the pups.

62. In a two-generation rat study PFOA was administered by oral gavage (0, 1, 3, 10 and 30 mg/kg bw/day) to the F_0-generation rats, beginning at 6 weeks of age and at least 10 weeks before cohabitation[59]. F_1-generation rats were treated at the same dosage levels as their respective sires and dams beginning at weaning (lactation day (LD) 22). F_0-generation males were 106-110 days of age at sacrifice and F_1-generation males were 109-120 days of age at sacrifice.

63. At terminal sacrifice F_0-generation male rats showed liver and kidney weight increases at all doses, i.e. for these effects it was not possible to establish a NOAEL and decreased body weights at 3 mg/kg bw/day and above. F_1-generation male rats, also at terminal sacrifice, showed significantly decreased body weights and increased liver weights at all doses. The absolute weights of the left and/or right kidneys were significantly increased in the 1 and 3 mg/kg bw/day dose groups and significantly decreased in the 30 mg/kg bw/day dose group compared to controls. F_0-generation females showed decreases in relative liver weight at 10 mg/kg bw/day, and decreases in absolute and relative kidney weights at 30 mg/kg bw/day. BMD modelling by the COT of the absolute liver weight data in the F_0-generation male rats estimated that the BMD_{10} and $BMDL_{10}$ were below the tested dose range at 0.68 and 0.31 mg/kg bw/day, respectively. For F_1-generation male rats BMD_{10} and $BMDL_{10}$ for absolute liver weight data were estimated to be 0.78 and 0.31 mg/kg bw/day, respectively. Body weights were assessed in directly-dosed rats during different periods of sexual development. Findings showed a greater sensitivity of sexually mature male rats to PFOA-induced bodyweight effects compared to sexually immature rats. Statistically significant decreases in bodyweight were present only at 30 mg/kg bw/day during the juvenile period (from PND 21 to 35) and peripubertal period (PND 36-60) but were present in all dose groups by the last three weeks of dosing in sexually mature male rats. The authors concluded that this may be related to differences in testosterone levels during different development phases. Thus, lower serum testosterone levels in male rats during the juvenile and peripubertal periods of sexual development may be associated with PFOA elimination kinetics similar to that of the female rat, i.e. more rapid renal clearance and shorter serum half-life, as demonstrated by numerous studies[7,14,60].

64. Reproductive endpoints were not affected in either generation. The 30 mg/kg bw/day F_1-generation pups had decreased body weight at birth and a reduced viability, however, F_2-generation pups at the same dosage levels, although somewhat lighter, did not show a loss in viability. Preputial separation and vaginal opening were somewhat delayed at 30 mg/kg bw/day in the F_1- and F_2-generation rats but this had no apparent consequences with regard to reproductive performance of F_1-generation rats. The NOAELs were; 30 mg/kg bw/day for reproductive function of the F_0- and F_1-generation, 10 mg/kg bw/day for F_1-generation pup mortality, birth weight, and sexual maturation, and a NOAEL could not be determined for male body weight and organ weight changes, as effects were observed in the lowest tested dose group (1 mg/kg bw/day). The NOAEL for F_2-generation rats was 30 mg/kg bw/day, the highest tested dose.

65. A recently published study investigated the developmental toxicity of PFOA (0, 1, 3, 5, 10, 20, and 40 mg/kg bw/day by oral gavage daily from GD 1-17) in timed-mated CD-1 mice[17]. Maternal absolute liver weight at term (*n* = 9-45 per dose group) was statistically significantly increased at all dose levels, and BMD_5 and $BMDL_5$ were estimated, by the study authors using the US EPA Benchmark Dose Software, at 0.20 and 0.17 mg/kg bw/day, respectively. In contrast to findings in the rat[59], in mice Lau and colleagues observed statistically significant increases in the incidence of full-litter resorptions and neonatal mortality at 5 mg/kg bw/day and above. No significant increase in malformations was noted in any treatment group. The incidence of live birth was significantly lowered by PFOA: approximately 70% for the 10 and 20 mg/kg bw/day groups compared to 96% for controls. Neonatal survival at postnatal day 23 was significantly compromised at 5 mg/kg bw/day and above. The BMD_5 and $BMDL_5$ for this effect were estimated at 2.84 and 1.09 mg/kg bw/day, respectively. Dose-dependent growth deficits were detected in all PFOA-treated litters except the 1 mg/kg group. Significant delays in eye-opening (up to 2-3 days) were noted at 5 mg/kg and higher dosages. Accelerated sexual maturation was observed in male offspring (preputial separation), but not in females. The authors of this study hypothesise that the species difference in terms of developmental toxicity of PFOA in rats and mice is, in part, due to the differential pharmacokinetic disposition of the chemical. In addition they propose that the lack of a sex-related difference in the pharmacokinetics of PFOA in humans, non-human primates and mice suggests that findings in mice maybe more appropriate for the purposes of species extrapolation in the human health risk assessment. However, the COT noted that it would also be necessary to consider relative sensitivity alongside sex- and species-related differences in pharmacokinetics before concluding studies in one species are more appropriate than in another species. At the request of the COT, the study authors agreed to repeat the BMD modelling in order to provide estimates of BMD_{10} and $BMDL_{10}$ for maternal liver weight at term, which appears to be the most sensitive endpoint in this study. The BMD_{10} and $BMDL_{10}$ were estimated to be 0.52 and 0.46 mg/kg bw/day, respectively.

Mechanistic studies

66. Cook *et al.*[61], carried out a 14-day gavage study to investigate possible mechanisms of induction of the Leydig cell adenomas, reported in the 2-year feeding study[49]. Adult male CD rats were administered 0, 1, 10, 25, or 50 mg/kg bw/day PFOA by gavage for 14 days, with a second control group pair-fed to the 50 mg/kg bw/day group. A dose-dependent decrease in body and relative accessory sex organ (ASO) weights was seen, with the relative ASO weights of the 50 mg/kg/day group significantly less than those of the pair-fed controls. Serum oestradiol levels were elevated in the 10, 25, and 50 mg/kg bw/day dose groups, and levels in the 50 mg/kg bw/day group were 2.7-fold greater than in pair-fed controls. A statistically significant downward trend with dose was seen in serum testosterone levels when compared to *ad libitum* controls. Animals administered PFOA for 14 days were also challenged with human chorionic gonadotropin (hCG), gonadotropin-releasing hormone (GnRH), or naloxone (which antagonises the inhibitory effects of endogenous opioids on GnRH release) one day prior to termination. Results suggested decreases in testosterone levels following PFOA exposure were due to an effect at the level of the testis. The elevated oestradiol levels in treated rats were hypothesised as being responsible for the decreased relative ASO weight and serum testosterone levels seen in this study as well as the increased incidence of Leydig cell adenomas in the 2-year feeding study with PFOA.

67. In a mixture of *in vivo*, *ex vivo* and *in vitro* studies Biegel *et al.*,[62] investigated the mechanism for PFOA induction of Leydig cell tumours. In the *in vivo* and *ex vivo* studies, male CD rats were treated with 0 and 25 mg/kg bw/day PFOA for 14 days by gavage. Findings in the *in vivo* study were statistically significant increases in the serum and testicular interstitial fluid oestradiol concentrations in the treated group. Testicular interstitial TGFα levels were also raised in dosed animals.

68. Leydig cells were isolated from the testes of PFOA-treated and untreated rats for the *ex vivo* and *in vitro* studies, respectively. Treatment of Leydig cells *in vitro* with PFOA (100-1000 μM for 5 hours) followed by hCG stimulation resulted in a dose-dependent decrease in testosterone production. In contrast the *ex vivo* studies, which stimulated Leydig cells from PFOA-treated rats with hCG, demonstrated an increase in testosterone production.

69. Three immunotoxicity studies of PFOA have been conducted in mice[53,63,64]. PFOA in the diet (200 ppm in food, approximately equivalent to 30 mg/kg bw/day) of male C57Bl/6 mice for 2, 5, 7 or 10 days resulted in significant atrophy of the spleen and thymus[63]. The time-course of the thymic and splenic atrophy resembled that of liver weight increases and of peroxisome proliferation. PFOA treatment decreased the number of thymocytes and splenocytes by >90% and about 50%, respectively. Accumulation of thymocytes in the G_0/G_1 phase (assessed by flow cytometric analysis) indicated that thymocyte proliferation had been significantly inhibited by PFOA treatment.

70. Dose-dependency of PFOA effects was tested in male C57Bl/6 mice administered PFOA in the diet (10, 30, 100, 200 and 500 ppm in the diet, approximately equivalent to 1.5, 4.5, 15, 30 and 75 mg/kg bw/day)[65]. Significant increases in peroxisome proliferation (measured as induction of acyl-CoA oxidase) were observed at all doses. Liver weight increased with all doses, reaching statistical significance at doses of 30 mg/kg bw/day and above. Thymus and spleen weights were significantly decreased at 15, 30 and 75 mg/kg bw/day. PFOA-induced atrophy of the thymus was more severe than atrophy of the spleen. Following a 5 or 10 day recovery period after treatment with 30 mg/kg bw/day the weights of spleen and thymus, respectively, recovered to control values whereas liver weight had not returned to control values. Following withdrawal of PFOA no changes were noted in splenocyte or thymocyte numbers during the first 2 days, but cell numbers returned to normal between days 5 and 10. The study authors considered that the effects in the thymus were due to inhibition of cell proliferation.

71. Specific humoral immune responses in male C57Bl/6 mice, administered 200 ppm PFOA in the diet (equivalent to approximately 30 mg/kg bw/day) for 10 days, were assessed using a plaque forming cell assay and serum antibody titre assay[64]. PFOA treatment prevented increased plaque formation and serum IgG and IgM titres in response to immunisation with horse red blood cells. PFOA also exerted immunosuppressive effects on lipopolysaccharide- and concanavalin A-stimulated proliferation of splenic lymphocytes.

72. PPARα-null mice, which do not exhibit peroxisome proliferation or hepatomegaly and hepatocarcinogenesis after exposure to peroxisome proliferators, did not show significant changes in body or spleen weight or the number of splenocytes after administration of 30 mg/kg bw/day for 7 days[53,63,64]. The decrease in thymus weight and cellularity observed in wild-type mice was attenuated, but not totally eliminated, in PPARα-null mice. Significantly the increases in liver weight observed in wild-type mice was virtually unaltered in null mice exposed to PFOA, indicating that this may not be a PPARα mediated effect. However, hepatic peroxisome proliferation was not observed in PFOA-treated PPARα-null mice.

COT evaluation

73. The COT considered it appropriate to take a threshold approach to establishing a tolerable intake for PFOA, in accordance with the advice of COM and COC. This is based upon predominantly negative genotoxicity in standard *in vitro* and *in vivo* assays and equivocal evidence for carcinogenicity. The positive response from the *in vitro* chromosomal aberration assay was considered to most likely represent a cytotoxic response.

74. COC considered that it was not possible to propose a PPARα agonist mode of action for the liver and pancreas tumours induced by PFOA. Therefore, relevance of these tumours to humans could not be discounted. For the purpose of the risk assessment, the COC concluded that selection of a NOAEL for a precursor event for the most sensitive tumour (liver or pancreas) as the critical effect level would be appropriate for the derivation of a safety limit.

75. There is considerable uncertainty regarding the pharmacokinetics of PFOA in rats and humans, especially in relation to human half-life data. Studies have provided some insight into possible mechanisms for the sex-related difference in PFOA elimination in rats. However, the rapid elimination of PFOA by female rats suggests that developmental/reproductive toxicity studies in this species may not be particularly informative for the risk assessment of PFOA for human health.

76. Due to the long half-life of PFOA in humans, estimated on the basis of the available data, the risk assessment for PFOA could be based on a comparison of the internal dose of PFOA from animals, for a specific endpoint, with the internal dose in humans. This approach is somewhat analogous to using a margin of exposure, calculated as the ratio of the NOAEL or LOAEL for a specific endpoint to the estimated human exposure level. However, the toxicokinetics of PFOA in rodents and humans are not yet fully understood. The sex-related difference in half-life of PFOA in rats was particularly noted as a source of uncertainty, as the active renal clearance in female rats is specific to that species. Therefore, the use of internal doses for the risk assessment was not considered appropriate on the basis of available data.

77. Considering the non-hepatic toxicity of PFOA, the lowest LOAEL indicated in the database was 1 mg/kg bw/day for kidney weight increases in the F_0- and F_1-generation males in the two-generation rat reproductive study[59]. Haematological effects were observed in male rats at interim sacrifices, but not at the terminal sacrifice, of a two year carcinogenicity study[49] at the lowest tested dose of 1.3 mg/kg bw/day. In female rats the NOAEL for haematological changes was 1.6 mg/kg bw/day (the lowest tested dose).

78. The database indicates that hepatic effects in rodents may occur at lower doses than non-hepatic effects. The lowest no observed effect level (NOEL) was 0.06 mg/kg bw/day for increased liver weight seen at 0.64 mg/kg bw/day in a 13-week dietary study in rats[35,36]. COT estimated that $BMDL_{10}$s for increased absolute liver weight at week 4, 7 and 13 sacrifices were 0.4, 0.3 and 0.44 mg/kg bw/day, respectively. A $BMDL_{10}$ of 0.74 mg/kg bw/day was estimated for hepatocytic megalocytosis in male rats of the two-year carcinogenicity study[49]. A LOAEL of 1 mg/kg bw/day was identified for increased liver weight, and focal to multifocal hepatic necrosis in the F_0- and F_1-generational male rats in the two-generation reproductive study[59], and $BMDL_{10}$s were 0.31 mg/kg bw/day in both generations. Increased maternal liver weight was also reported in the reproductive toxicity study in mice[17]. The $BMDL_5$ estimated, by the study authors, for increased maternal absolute liver weight was 0.17 mg/kg bw/day (almost one order of magnitude below the lowest tested dose). A benchmark response rate of 10% is more commonly used in order to be within the observed dose range. At the request of the COT, the authors of this study remodelled the data to estimate BMD_{10} and $BMDL_{10}$ for maternal liver weight at term. These were 0.52 and 0.46 mg/kg bw/day, respectively.

79. For deriving a tolerable daily intake (TDI) a dose level of 0.3 mg/kg bw/day was selected as a suitable point of departure expected to be without adverse effect on the basis of a number of endpoints of PFOA toxicity.

80. An uncertainty factor of 100 was applied to allow for inter- and intra-species variation. Therefore, the TDI indicated for PFOA is 3 μg/kg bw/day.

Exposure assessment

81. The Food Standards Agency has completed an analysis of composite food groups samples from the 2004 Total Diet Study (TDS) for a range of fluorinated chemicals, including PFOA and PFOS[66]. The TDS models the typical UK diet and is fully described in Food Survey Information Sheet 38/03[67].

82. PFOA was only detected at a concentration above the limit of detection in the potatoes food group.

83. The estimated average and high level adult intakes of PFOA from the whole diet in 2004 were 0.001-0.07 μg/kg bw/day and 0.003-0.1 μg/kg bw/day (range of lower to upper bound figures)[†], respectively. Estimated high level dietary intake for toddlers was 0.01-0.3 μg/kg bw/day (range of lower to upper bound figures). These estimated intakes of PFOA from the diet are below the TDI recommended by the COT.

† Upper bound concentrations assume that PFOA is present at the reporting limit for those food groups in which PFOA is present at concentrations below the reporting limit (limit of detection), and therefore could be an overestimate of the true concentrations. By contrast, lower bound concentrations assume that PFOA is absent for those food groups in which PFOA is present at concentrations below the limit of detection, and will therefore be an underestimate of the true concentrations. The range between the lower and upper bound values demonstrates the uncertainty in these exposure estimates and the true values will lie somewhere between the upper and lower bounds.

Conclusions

84. We recommend a TDI of 3 μg/kg bw/day be established, based on the range of effects on the liver, kidney, haematological and immune systems. We consider that the TDI is adequate to protect against other potential effects, such as cancer.

85. We note the results of the Food Standards Agency analysis of composite food group samples from the 2004 Total Diet Study (TDS) that estimated high level adult dietary intakes of PFOA are lower than the recommended TDI. The estimated intakes are not of concern regarding human health.

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References

1. Taves, D.R., Guy, W.S., Brey, W.S. (1976). Organic fluorocompounds in human plasma: prevalance and characterization. *In Biochemistry involving carbon-fluorine bonds A symposium sponsored by the Division of Fluorine and Biological Chemistry at the 170th meeting of the American Chemical Society Chicago, IL August 26, 1975 ed Washington DC, American Chemical Society* 117-134.

2. Taves, D.R.(1968). Evidence that there are two forms of fluoride in human serum. *Nature* 217: 1050-1051.

3. Lange, C.C. (2000). The aerobic biodegradation of N-EtFOSE alcohol by the microbial activity present in municipal wastewater treatment sludge. For 3M Company.

4. Wang, N., Szostek, B., Folsom, P.W., Sulecki, L.M., Capka, V., Buck, R.C., Berti, W.R., Gannon, J.T. (2005). Aerobic biotransformation of 14C-labeled 8-2 telomer B alcohol by activated sludge from a domestic sewage treatment plant. *Environ Sci Technol* 39: 531-538.

5. Gibson, S.J. and Johnson, J.D. (1979). Absorption of FC-143-14C in rats after a single oral dose. Riker Laboratories, Inc. Subsidiary of 3M, St. Paul, MN, USA.

6. Vanden Heuvel, J.P., Kuslikis, B.I., Van Rafelghem, M.J., Peterson, R.E. (1991). Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. *J Biochem Toxicol* 6: 83-92.

7. Kudo, N., Katakura, M., Sato, Y., Kawashima, Y. (2002). Sex hormone-regulated renal transport of perfluorooctanoic acid. *Chem Biol Interact* 139: 301-316.

8. Kemper, R.A. (2003). Perfluorooctanoic acid: Toxicokinetics in the rat. Submitted to US EPA Administrative Record 116.

9. Gibson, S.J. and Johnson, J.D. (1980). Extent and route of excretion and tissue distribution of total carbon-14 in male and female rats after a single I.V. dose of FC-143-14C. Riker Laboratories, Inc., Subsidiary of 3M, St. Paul, MN, USA.

10. Ophaug, R.H. and Singer, L. (1980). Metabolic Handling of perfluorooctanoic acid in rats. *Proc Soc Exp Biol Med* 163: 19-23.

11. Hinderliter, P.M., Mylchreest, E., Gannon, S.A., Butenhoff, J.L., Kennedy, G.L., Jr. (2005). Perfluorooctanoate: Placental and lactational transport pharmacokinetics in rats. *Toxicology* 211: 139-148.

12. Johnson, J.D., Gibson, S.J., Ober, R.E. (1984). Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [14C]perfluorooctanoate or potassium [14C]perfluorooctanesulfonate. *Fundam Appl Toxicol* 4: 972-976.

13. Hanhijarvi, H., Ophaug, R.H., Singer, L. (1982). The sex-related difference in perfluorooctanoate excretion in the rat. *Proc Soc Exp Biol Med* 171: 50-55.

14. Vanden Heuvel, J.P., Davis, J.W., Sommers, R., Peterson, R.E. (1992). Renal excretion of perfluorooctanoic acid in male rats: inhibitory effect of testosterone. *J Biochem Toxicol* 7: 31-36.

15. Gibson, S.J. and Johnson, J.D. (1983). Extent and route of total carbon-14 in pregnant rats after a single oral dose of ammonium 14 C-perfluorooctanoate. Riker Laboratories, Inc., subsidiary of 3M, St. Paul, MN, USA.

16. DuPont Haskell Laboratory. (1982). Excretion and disposition of 14C-ammonium perfluorooctanoate in male and female rats, mice, hamsters, and rabbits. U.S. EPA Public Docket AR-226.

17. Lau, C., Thibodeaux, J.R., Hanson, R.G., Narotsky, M.G., Rogers, J.M., Lindstrom, A.B., Strynar, M.J. (2006). Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. *Toxicol Sci* 90: 510-518.

18. Hanhijarvi, H., Ylinen, M., Haaranen, T., Nevalainen, T. (1988). A proposed species difference in the renal excretion of perfluorooctanoic acid in the beagle dog. *New Developments in Biosciences: Their Implications for Laboratory Animal Science* 409-412.

19. Butenhoff, J.L., Kennedy, G.L., Jr., Hinderliter, P.M., Lieder, P.H., Jung, R., Hansen, K.J., Gorman, G.S., Noker, P.E., Thomford, P.J. (2004). Pharmacokinetics of perfluorooctanoate in cynomolgus monkeys. *Toxicological Sciences* 82: 394-406.

20. Olsen, G.W. and Mandel, J.H. (2003). Descriptive analysis of serum fluorochemical concentrations from Cottage Grove employee participants of the 2002 medical surveillance program. 3M Company. Submitted to US EPA AR226-1352.

21. Ubel, F.A., Sorenson, S.D., Roach, D.E. (1980). Health status of plant workers exposed to fluorochemicals – a preliminary report. *Am Ind Hyg Assoc J* 41: 584-589.

22. Burris, J.M., Lundberg, J.K., Olsen, G.W., Simpson, C., Mandel, J. (2002). Determination of serum half-lives of several fluorochemicals. Interim Report #2. 3M Medical Department.

23. Kannan, K., Corsolini, S., Falandysz, J., Fillmann, G., Kumar, K.S., Loganathan, B.G., Mohd, M.A., Olivero, J., Van Wouwe, N., Yang, J.H., Aldoust, K.M. (2004). Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ Sci Technol* 38: 4489-4495.

24. Harada, K., Inoue, K., Morikawa, A., Yoshinaga, T., Saito, N., Koizumi, A. (2005). Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. *Environ Res* 99: 253-261.

25. Olsen, G.W., Church, T.R., Miller, J.P., Burris, J.M., Hansen, K.J., Lundberg, J.K., Armitage, J.B., Herron, R.M., Medhdizadehkashi, Z., Nobiletti, J.B., O'Neill, E.M., Mandel, J.H., Zobel, L.R. (2003). Perfluorooctanesulfonate and other fluorochemicals in the serum of American Red Cross adult blood donors. *Environ Health Perspect* 111: 1892-1901.

26. Olsen, G.W., Huang, H.Y., Helzlsouer, K.J., Hansen, K.J., Butenhoff, J.L., Mandel, J.H. (2005). Historical comparison of perfluorooctanesulfonate, perfluorooctanoate, and other fluorochemicals in human blood. *Environ Health Perspect* 113: 539-545.

27. Karrman, A., van Bavel, B., Jarnberg, U., Hardell, L., Lindstrom, G. (2004). Levels of perfluoroalkylated compounds in whole blood from Sweden. *Organohalogen Compounds* 66: 4058-4062.

28. Harada, K., Saito, N., Inoue, K., Yoshinaga, T., Watanabe, T., Sasaki, S., Kamiyama, S., Koizumi, A. (2004). The influence of time, sex and geographic factors on levels of perfluorooctane sulfonate and perfluorooctanoate in human serum over the last 25 years. *J Occup Health* 46: 141-147.

29. DuPont Company. (1981). Oral LD50 test in rats. Haskell Laboratory, Newark, DE, USA.

30. DuPont Company. (1981). Liver weight comparison in castrated and ovariectomised rats vs. normal rats. Haskell Laboratory, Newark, DE, USA.

31. DuPont Company. (1983). Acute oral toxicity of FC-143 as a function of age. Haskell Laboratory, Newark, DE, USA.

32. Christopher, B. and Martin, J. W. (1977). 28-Day oral toxicity study with FC-143 in albino mice. 8532-10655, T-1742CoC. Industrial Bio-Test Laboratories, Inc.

33. Metrick, M. and Marias, A.J. (1977). 28-Day oral toxicity study with FC-143 in Albino rats. 8532-10654, T-1742CoC. Industrial Bio-Test Laboratories, Inc.

34. Goldenthal, E.I., Jessup, D.C., Geil, R.G., Mehring, J.S. (1978). Ninety-day subacute rat toxicity study. 137-085. International Research and Development Corporation.

35. Palazzolo, M.J. (1993). Thirteen-week dietary toxicity study with T-5180, ammonium perfluorooctanoate (CAS No. 3825-26-1) in male rats. HWI 6329-100. Hazleton Wisconsin, Inc.

36. Perkins, R.G., Butenhoff, J.L., Kennedy, G.L., Jr., Palazzolo, M.J. (2004). 13-week dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats. *Drug Chem Toxicol* 27: 361-378.

37. Crump, K.S.(1984). A new method for determining allowable daily intakes. *Fundam Appl Toxicol* 4: 854-871.

38. Barnes, D.G., Daston, G.P., Evans, J.S., Jarabek, A.M., Kavlock, R.J., Kimmel, C.A., Park, C., Spitzer, H.L. (1995). Benchmark Dose Workshop: criteria for use of a benchmark dose to estimate a reference dose. *Regul Toxicol Pharmacol* 21: 296-306.

39. Goldenthal, E.I. (1978). Final report, ninety day subacute Rhesus monkey toxicity study. 137-090. International Research and Development Corporation.

40. Butenhoff, J., Costa, G., Elcombe, C., Farrar, D., Hansen, K., Iwai, H., Jung, R., Kennedy, G., Jr., Lieder, P., Olsen, G., Thomford, P. (2002). Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. *Toxicological Sciences* 69: 244-257.

41. Lawlor, T.E. (1995). Mutagenicity test with T-6342 in the *Salmonella-Escherichia coli*/mammalian-microsome reverse mutation assay. 17073-0-409. Corning Hazleton, Inc., Vienna, VA, USA.

42. Lawlor, T.E. (1996). Mutagenicity test with T-6564 in the *Salmonella-Escherichia coli*/mammalian-microsome reverse mutation assay with a confirmatory assay. 17750-0-409R. Corning Hazleton, Inc. Vienna, VA, USA.

43. Sadhu, D. (2002). CHO/HGPRT forward mutation assay – ISO (T6.889.7). 01-7019-G1. Toxicon Corporation, Bedford, MA, USA.

44. Murli, H. (1996). Mutagenicity test on T-6342 measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells with a confirmatory assay with multiple harvests. 17073-0-437CO. Corning Hazleton, Inc., Vienna, VA, USA.

45. Murli, H. (1996). Mutagenicity test on T-6564 measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells with a confirmatory assay with multiple harvests. 17750-0-437CO. Corning Hazleton Inc., Vienna, VA, USA.

46. Murli, H. (1996). Mutagenicity test on T-6342 measuring chromosomal aberrations in human whole blood lymphocytes with a confirmatory assay with multiple harvests. 17073-0-449CO. Corning Hazleton, Inc., Vienna, VA, USA.

47. Murli, H. (1995). Mutagenicity test on T-6342 in an *in vivo* mouse micronucleus assay. 17073-0-455. Corning Hazleton, Inc., Vienna, VA, USA.

48. Murli, H. (1996). Mutagenicity test on T-6564 in an *in vivo* mouse micronucleus assay. 17750-0-455. Corning-Hazleton Inc., Vienna, VA, USA.

49. Sibinski, L.J. (1987). Final report of a two-year oral (diet) toxicity and carcinogenicity study of fluorochemical FC-143 (perfluorooctanane ammonium carboxylate) in rats. 0281CR0012, 8EHQ-1087-0394. 3M Company.

50. Biegel, L.B., Hurtt, M.E., Frame, S.R., O'Connor, J.C., Cook, J.C. (2001). Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. *Toxicological Sciences* 60: 44-55.

51. Frame, S. R. and McConnell, E. E. (2003). Review of proliferative lesions of the exocrine pancreas in two chronic feeding studies in rats with ammonium perfluorooctanoate. DuPont-13788. EPA Administrative Record: AR226-1920.

52. Klaunig, J.E., Babich, M.A., Baetcke, K.P., Cook, J.C., Corton, J.C., David, R.M., DeLuca, J.G., Lai, D.Y., McKee, R.H., Peters, J.M., Roberts, R.A., Fenner-Crisp, P.A. (2003). PPARalpha agonist-induced rodent tumors: modes of action and human relevance. *Crit Rev Toxicol* 33: 655-780.

53. Yang, Q., Xie, Y., Alexson, S.E., Nelson, B.D., DePierre, J.W. (2002). Involvement of the peroxisome proliferator-activated receptor alpha in the immunomodulation caused by peroxisome proliferators in mice. *Biochem Pharmacol* 63: 1893-1900.

54. Gilliland, F.D. and Mandel, J.S. (1993). Mortality among employees of a perfluorooctanoic acid production plant. *J Occup Med* 35: 950-954.

55. Alexander, B.H., Olsen, G.W., Burris, J.M., Mandel, J.H., Mandel, J.S. (2003). Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. *Occup Environ Med* 60: 722-729.

56. Gortner, E. G. (1981). Oral teratology study of T-2998CoC in rats. 0681TR0110. Safety Evaluation Laboratory and Riker Laboratories, Inc.

57. Gortner, E. G. (1982). Oral teratology study of T-3141CoC in rabbits. 0681TB0398. Safety Evaluation Laboratory and Riker Laboratory, Inc.

58. Staples, R.E., Burgess, B.A., Kerns, W.D. (1984). The embryo-fetal toxicity and teratogenic potential of ammonium perfluorooctanoate (APFO) in the rat. *Fundam Appl Toxicol* 4: 429-440.

59. Butenhoff, J.L., Kennedy, G.L., Jr., Frame, S.R., O'Connor, J.C., York, R.G. (2004). The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. *Toxicology* 196: 95-116.

60. Kudo, N. and Kawashima, Y. (2003). Toxicity and toxicokinetics of perfluorooctanoic acid in humans and animals. *J Toxicol Sci* 28: 49-57.

61. Cook, J.C., Murray, S.M., Frame, S.R., Hurtt, M.E. (1992). Induction of Leydig cell adenomas by ammonium perfluorooctanoate: a possible endocrine-related mechanism. *Toxicol Appl Pharmacol* 113: 209-217.

62. Biegel, L.B., Liu, R.C., Hurtt, M.E., Cook, J.C. (1995). Effects of ammonium perfluorooctanoate on Leydig cell function: *in vitro*, *in vivo*, and *ex vivo* studies. *Toxicol Appl Pharmacol* 134: 18-25.

63. Yang, Q., Xie, Y., DePierre, J.W. (2000). Effects of peroxisome proliferators on the thymus and spleen of mice. *Clin Exp Immunol* 122: 219-226.

64. Yang, Q., Abedi-Valugerdi, M., Xie, Y., Zhao, X.Y., Moller, G., Nelson, B.D., DePierre, J.W. (2002). Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. *Int Immunopharmacol* 2: 389-397.

65. Yang, Q., Xie, Y., Eriksson, A.M., Nelson, B.D., DePierre, J.W. (2001). Further evidence for the involvement of inhibition of cell proliferation and development in thymic and splenic atrophy induced by the peroxisome proliferator perfluoroctanoic acid in mice. *Biochem Pharmacol* 62: 1133-1140.

66. FSA. (2006). Fluorinated chemicals – UK dietary intakes. Food Survey Information Sheet No. 11/06. Food Standards Agency, UK.

67. FSA. (2003). Dioxins and polychlorinated biphenyls in the UK diet – 2001 Total Diet Study samples. *Food Survey Information Sheet No.38/03*. Food Standards Agency, U.K.

Statement on the tolerable daily intake for perfluorooctane sulfonate

Introduction

1. Perfluorooctane sulfonate (PFOS) has the potential to enter the food chain and could have a negative health impact on humans. The Food Standards Agency commissioned analysis of the 2004 Total Diet Study samples for PFOS and the Committee was invited to consider the toxicology of PFOS and the results of the analysis.

Background

2. Perfluorooctane sulfonate is a member of the large chemical class of fluorochemicals referred to as perfluorinated alkyl compounds. All perfluorinated substances are of anthropogenic origin. These fluorochemicals have excellent surfactant properties and are widely used in the manufacture of plastics, electronics, textile, and consumer material in the apparel, leather, and upholstery industries[1]. The term PFOS covers its anionic, acid and salt forms, and the PFOS-moiety (the $C_8F_{17}SO_2$ group) is incorporated into a variety of compounds (referred to as PFOS-related substances) that have the potential to degrade subsequently to PFOS either metabolically or through environmental processes. PFOS is widely distributed on a global scale and has been identified in various food chains[2].

3. The major US manufacturer 3M announced in 2000 the voluntary cessation of production of PFOS and chemically-related substances due to reports of persistence and widespread exposure of wildlife and humans. Subsequent limited availability of PFOS-related substances and action within relevant industry sectors to decrease dependence on these substances have led to a significant reduction in the use of PFOS across the EU since 2002.

4. A hazard assessment for PFOS has been produced under the Existing Chemicals Programme of the Organisation for Economic Co-operation and Development (OECD)[3]. Given the widespread occurrence of PFOS the OECD evaluation recommended that national or regional exposure information gathering and risk assessment may need to be considered. The Environment Agency for England and Wales consequently reviewed the environmental risks of PFOS use and concluded that PFOS meets the criteria for classification as a Persistent, Bioaccumulative and Toxic (PBT) substance[4]. In June 2005 the Swedish Environment Ministry announced that it will propose a ban for PFOS to the United Nations under the Stockholm Convention. Sweden also filed a national ban on PFOS to the European Commission.

Evidence considered in this evaluation

5. The COT has not previously evaluated PFOS. From an initial assessment of the relevant information it was considered essential to have advice from the Committees on Mutagenicity (COM) and Carcinogenicity (COC) regarding the genotoxicity of PFOS and whether it was appropriate to assume the existence of a threshold for carcinogenicity. The recommendations provided by the COM and COC are summarised in this statement.

6. The evaluation considered published literature and unpublished final reports of toxicology studies largely conducted by, or on behalf of, 3M.

7. Specialist teratology advice was sought from Professor Aldert Piersma (National Institute for Public Health and the Environment, The Netherlands) and his conclusions regarding the reported teratology findings are gratefully acknowledged.

Chemical information

8. The high ionization potential and low polarizability of fluorine lead to weak inter- and intra-molecular interactions that are reflected by the extremely low surface tension of the perfluoroalkyl acids. Their partitioning behaviour is also unique; when they are mixed with water and hydrocarbons, three immiscible phases are formed, indicating the hydrophobic and oleophobic nature of these chemicals. Consequently, these compounds are ideal surfactants. Due to the strength of the carbon-fluorine bonds, these compounds are highly stable leading to their persistence and bioaccumulative properties[2].

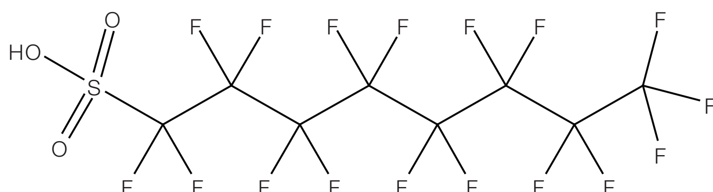

Figure 1 – Structure of perfluorooctane sulfonic acid (PFOS acid)

9. The perfluorooctane sulfonate anion (PFOS), does not have a CAS number, but that of the perfluorooctane sulfonic acid (Figure 1, $C_8F_{17}SO_3H$, molecular weight: 500) is 1763-23-1.

10. The Environment Agency has published a draft list of 96 PFOS-related substances which have the potential to degrade to PFOS[4]. Current information strongly supports a conclusion that PFOS and its salts cannot be broken down further chemically[3]. However, only limited data are available on the toxicology of the PFOS-related substances, such as 2-(*N*-ethylperfluorooctanesulfonamido)ethyl alcohol (*N*-EtFOSE, Figure 2).

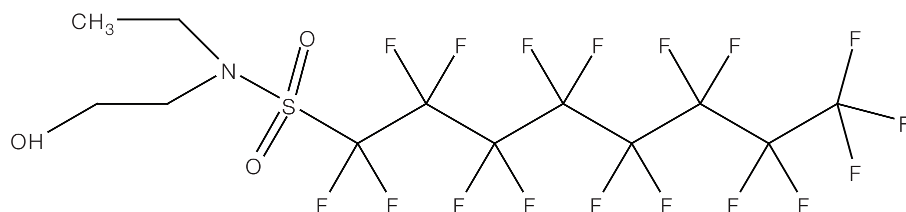

Figure 2 – Structure of N-ethylperfluorooctanesulfonamido-ethanol (N-EtFOSE).

11. PFOS is manufactured by a process known as Simons Electro-Chemical Fluorination (ECF). 3M reports a final product from ECF of approximately 70% linear PFOS and 30% branched impurities, including odd and even chain lengths. Although not specifically reported by 3M, manufacture of PFOS-related substances by ECF is assumed to result in the same proportion of linear and branched products.

12. Two determinations of the water solubility of PFOS have been reported. The average results were 519 mg/L at 20 ± 0.5°C, and 680 mg/L at 24-25°C. The surface active properties of the substance make a direct determination of the octanol-water partition coefficient impossible. In a preliminary study reported by 3M an inseparable emulsion was formed. 3M determined the solubility of PFOS in octanol as 56 mg/L.

Toxicological profile

13. The majority of the toxicology studies of PFOS have been conducted with its potassium salt (approximately 70% linear PFOS and 30% branched impurities), a white crystalline powder at normal temperature and pressure. No data are available on the relative toxicity of the non-linear contaminants of the test chemical.

Toxicokinetics – rat

14. Toxicokinetic data for potassium perfluorooctane sulfonate are available from five studies in the rat. These show that rather than bioconcentrating in the lipid fraction, PFOS tends to bind to plasma proteins.

15. Over 95% of an oral dose (4.2 mg/kg bw) of ^{14}C-PFOS was absorbed within 24 hours by male rats (8 weeks old)[5]. The redistribution half-life from plasma was 179 hours (7.5 days).

16. In two repeat dose studies to investigate the toxicokinetics of PFOS over the course of gestation, non-radiolabelled PFOS was administered by oral gavage to F_0 female rats. In the first study, PFOS was administered daily for 42 days prior to mating and continued through gestation day (GD) 20 at dose levels of 0, 0.1, 0.4, 1.6, and 3.2 mg/kg bw/day[6]. In GD 21 fetuses, serum PFOS levels were comparable to those of dams, but the fetal liver PFOS levels were considerably lower than in dams. In the second study female rats were dosed daily for 43 days prior to mating and through until confirmation of mating at three dose-levels, 0, 0.1, and 1.6 mg/kg bw/day PFOS[7]. As with the earlier study there was a dose-related increase in the levels of PFOS in the liver and serum, with much higher levels present in the liver than in the serum of both dams and pups.

17. Tissue distribution and extent and route of excretion of ^{14}C-PFOS were investigated in 8 week old male rats treated with a single dose of 4.2 mg/kg bw (i.v. tail vein)[8]. By post-dosing day 89, mean urinary excretion was 30% of administered ^{14}C, compared with 13% of administered ^{14}C excreted via faeces. Only liver and plasma contained a substantial percentage of the dose at 89 days post dosing, 25% and 2.8%, respectively. Elimination of only 42.8% of the dose through urine and faeces after 89 days indicated that the terminal half-life of elimination from the body was probably >89 days in the male rat.

18. PFOS undergoes considerable enterohepatic recirculation in the ra[9]. A 9.5-fold greater elimination of PFOS via faeces was observed in rats with disrupted enterohepatic circulation (induced by cholestyramine treatment) than for animals with normal enterohepatic circulation (mean percentage of dose eliminated via faeces in control rats was 8%).

Toxicokinetics – non-human primates

19. The pharmacokinetics and urinary excretion of PFOS, following a single i.v. bolus dose of 2 mg/kg bw, have been reported for male and female cynomolgus monkeys[10]. The serum concentration versus time data were subjected to non-compartmental pharmacokinetic analysis and the authors estimated that the serum terminal half-life of PFOS ranged from 122 to 146 days (mean: 132 days) in male monkeys and from 88 to 138 days (mean 110 days) in female monkeys. The study authors further concluded that these results provided no clear indication that the pharmacokinetics of PFOS were different in male and female monkeys.

20. In a six month study of PFOS toxicity, cynomolgus monkeys (4-6/sex/group) received 0, 0.03, 0.15 and 0.75 mg/kg bw/day by intragastric intubation of a capsule dose for at least 26 weeks[11,12]. Two monkeys/sex/group in the control, 0.15 and 0.75 mg/kg bw/day dose groups were monitored for one year following the end of treatment. Six months after cessation of treatment the control and 0.75 mg/kg bw/day dose group monkeys underwent partial hepatectomy. Serum PFOS concentrations showed a linear increase with time in both the low- and mid-dose groups and a non-linear increase in the high-dose group. Serum PFOS concentrations in the high-dose group monkeys appeared to plateau at approximately 20 weeks. The serum PFOS elimination curves, during the recovery phase, appeared to be multiphasic in the high dose group and linear in the mid-dose group. During the first 23 weeks of the recovery phase serum concentrations in the high dose group decreased at a faster rate (half-life for elimination approximately 120 and 150 days in male and female monkeys, respectively) than in the mid-dose group (approximate half-life for elimination 180 days). However, towards the end of the one-year recovery period the slopes of the two recovery group elimination curves were similar (elimination half-life of approximately 180 days). This study did not show evidence for differences in pharmacokinetics between male and female monkeys.

Toxicokinetics – human

21. In 1976, Taves *et al.*,[13] reported that human serum samples contained non-ionic (organic) fluorine from perfluorocarbons. Preliminary evidence in 1979 from a 3M fluoropolymer production facility, showed that total serum organic fluorine levels for five employees were 4.1-11.8 parts per million (ppm), and that 55-80% of that was PFOS[14]. More recently PFOS was detected in 50% of non-occupationally exposed human blood donor samples in India and 100% of human blood samples in Poland, Italy, Belgium, USA and Japan[15].

22. There is some inconsistency with regard to the half-life of PFOS in humans. One study following 3 retired 3M workers for five and a half years suggested a mean elimination half-life of 1428 days (approximately 4 years)[16].

23. The first report from an ongoing study following 27 retirees from a 3M production plant derived an elimination half-life of 139-640 days[17]. A mean serum half-life for PFOS of 8.7 years (S.D. = 6.1; range 2.3 – 21.3) was reported more recently[18]. The investigators have listed a number of limitations, and a number of attempts have been made to minimise the experimental error using selected subjects. No effort was made to determine, or control for, retiree re-exposure or endogenous metabolism of other perfluorinated chemicals to PFOS, both potentially leading to artificially long half-life estimations.

24. An analysis of PFOS concentrations in Kyoto City residents identified a sex-related pharmacokinetic difference[19]. Pre-menopausal females had higher serum PFOS concentrations than post-menopausal females and males. At an approximate age of 60 years, serum concentrations in post-menopausal females decreased to the level in males. Elimination in urine was approximately one-fifth of total PFOS elimination, assuming a one-compartment model.

25. PFOS can cross the human placenta[20]. PFOS concentrations in Japanese maternal blood samples were 4.9-18 ng/mL, whereas those in fetal samples were 1.6-5.3 ng/mL. The mean ratio of cord to maternal blood PFOS concentrations was 0.32 (range 0.23-0.41), indicating that PFOS may bind to a different extent in the fetal circulation.

26. A number of studies have assessed the levels of PFOS in blood of non-occupationally exposed humans. However, there have been no reports of levels of PFOS in UK subjects. The largest PFOS biomonitoring study of adults in the United States[21] (645 Red Cross blood donors aged 20 – 69), reported a geometric mean serum concentration of 36 ng/mL (ppb). Given the consistency of the data in this large study with that of smaller studies in US and European populations, the authors hypothesised that the average serum PFOS concentrations in non-occupationally exposed populations may range from 30 to 40 ppb with 95% of a population's serum PFOS concentrations below 100 ng/mL.

27. A comparison of PFOS levels in 59 paired samples collected in 1974 (serum) and 1989 (plasma) from volunteer participants of a large community health study indicated serum concentrations of PFOS were statistically significantly higher in 1989 than 1974 (median concentrations of 34.7 ng/mL and 29.5 ng/mL, respectively, representing a 25% increase)[22]. The same study reported only a 9% increase in serum PFOS concentrations from 1974 to 1989 in non-paired samples adjusted for age and sex (120 samples/year) and this was not statistically significant. The levels of PFOS in 1989 were comparable to the levels in the Red Cross blood donor study[21].

28. In blood samples collected from the United States, Colombia, Brazil, Belgium, Italy, Poland, India, Malaysia and Korea PFOS was the predominant perfluorochemical detected[15]. The highest concentrations were in samples from the U.S. and Poland (>30 ng/mL). Levels were lowest in India (<3 ng/mL) and the others were in the range of 3–29 ng/mL. No age- or sex-related differences were found.

29. The primary binding proteins in human plasma have been identified by incubating PFOS with seven separate human-derived plasma protein fractions at two different protein fraction concentrations (10% and 100% physiological concentrations)[23]. The percentage of PFOS bound to each human plasma protein at 100% physiological concentrations was 99.8% for albumin, 95.6% for beta-lipoprotein, 59.4% for alpha-globulin, 24.1% for gamma globulin, and <0.1% for each of fibrinogen, alpha-2-macroglobulin, and transferrin.

Acute and sub-acute toxicity

30. The oral LD_{50} in rat is 230 and 270 mg/kg bw (160-340 and 200-370 mg/kg bw, 95% confidence limits) for males and females, respectively[24].

31. Five sub-acute studies of PFOS have been conducted: two dietary studies in rats (a 90-day study[25] and a combined 4- and 14-week study[26]), two 90-day gavage studies in rhesus monkeys[27,28] and a 26 week study in cynomolgus monkeys[11,12].

Rat

32. In the 90-day study[25], Sprague-Dawley rats (5/sex/group) were administered potassium PFOS in the diet (mean achieved doses; 0, 2, 6, 18, 60, and 200 mg/kg bw/day). All the animals in the 18, 60, and 200 mg/kg bw/day dose groups died. Increased relative and absolute liver weights were reported at 2 and 6 mg/kg bw/day.

33. The second study[26], describes data from interim sacrifices at 4 and 14 weeks of a 2-year cancer bioassay. PFOS (potassium salt) was administered in the diet (mean achieved doses; 0, 0.05, 0.20, 0.42, and 1.6 mg/kg bw/day at 4 weeks, and 0, 0.04, 0.14, 0.37, and 1.40 mg/kg bw/day at 14 weeks) to Sprague-Dawley rats (5/sex/group) for 4 or 14 weeks.

34. Statistically significant effects were reported for the 1.6 mg/kg bw/day dose group at 4 weeks and the 1.4 mg/kg bw/day dose group at 14 weeks. At 4 weeks relative liver weights were significantly increased but absolute liver weights were unchanged. Male rats had lower serum glucose levels and females had elevated aspartate aminotransferase (AST) levels. Palmitoyl CoA oxidase activity in liver was 2-fold higher than in controls.

35. At 14 weeks in the 1.4 mg/kg bw/day dose group, absolute and relative liver weights were significantly higher in males and relative liver weight was significantly higher in females. Concentrations of PFOS in the livers were comparable between the sexes, but PFOS levels in serum were 31-42% higher in females than males. Compared with controls, males showed moderately lower serum cholesterol concentrations, mildly raised alanine aminotransferase (ALT) values and both sexes had mildly raised urea nitrogen values. Palmitoyl CoA oxidase activity in liver was not significantly different from controls. Centrilobular hepatocytic hypertrophy and midzonal to centrilobular vacuolisation were seen in males of the 0.37 mg/kg bw/day and 1.4 mg/kg bw/day dose groups and females of the 1.4 mg/kg bw/day group.

36. Serum and liver PFOS concentrations were used to provide a means of estimating internal doses that can be associated with effects and NOAELs. The mean serum PFOS concentration associated with the NOAEL (0.37 mg/kg bw/day, on the basis of liver weight changes at 14 weeks) was 44 μg/mL in males and 67 μg/mL in females. These doses corresponded to PFOS levels in the liver of 360 μg/g and 670 μg/g in males and females, respectively. A re-analysis of the data derived the lower 95% confidence interval of the benchmark dose[†] at the 10% response level ($BMDL_{10}$) for relative liver weights, the most sensitive endpoint in this study, of 0.20 mg/kg bw/day for males and females.

Non-human primate

37. Two 90-day subchronic studies in rhesus monkeys provide few reliable quantitative data. In the first study[27], animals (2/sex/group) were treated by gavage with PFOS at 0, 10, 30, 100, and 300 mg/kg bw/day. All treated animals died by day 20. Similar signs of toxicity were shown by all dose groups including decreased activity, emesis with some diarrhoea, general body trembling, twitching and convulsions. Necropsy showed yellowish-brown discoloration of the liver (no microscopic lesions on histological examination) in the 100 and 300 mg/kg bw/day groups. Congestion, haemorrhage and lipid depletion of the adrenal cortex were noted in all treatment groups.

38. Goldenthal *et al.*[28] reported on a 90-day subchronic rhesus monkey study of 2 animals/sex/group dosed at 0, 0.5, 1.5, and 4.5 mg/kg bw/day via gavage.

39. All monkeys in the highest dose group (4.5 mg/kg bw/day) died or were sacrificed *in extremis* between weeks 5 and 7 of the study, having exhibited signs of gastrointestinal tract toxicity. After 30 days of treatment, there was a significant decrease in serum cholesterol and a 50% drop in serum alkaline phosphatase activity. There were no differences in mean organ weights compared to controls. In all treated animals there was marked diffuse lipid depletion in the adrenals. Both females and one male had moderate diffuse atrophy of the pancreatic exocrine cells with reduced size and loss of zymogen granules. Both males and one female had moderate diffuse atrophy of serous alveolar cells of the submandibulary salivary gland marked by decreased cell size and loss of cytoplasmic granules.

40. The 1.5 and 0.5 mg/kg bw/day dose groups survived until the end of the study and necropsy showed no treatment related lesions. However, both groups showed signs of gastrointestinal tract effects (soft stools and diarrhoea).

† The benchmark dose (BMD) approach[29,30] aims to provide an approach to dose-response assessment that is more quantitative than the NOAEL process. This approach constructs mathematical models to fit all data points in the dose-response study and uses the best fitting model to interpolate an estimate of the dose that corresponds to a particular level of response (a benchmark response), often 10%. A measure of uncertainty is also calculated, and the lower confidence limit on the benchmark dose is called the BMDL. This accounts for the uncertainty in the estimate of the dose-response that is due to characteristics of the experimental design such as sample size. The BMDL can be used as the point of departure for derivation of a health-based guidance value or a margin of exposure.

When the COT has performed benchmark dose modelling as part of this assessment the US Environmental Protection Agency's Benchmark Dose Software (2000) was used.

41. Cynomolgus monkeys (6/sex/group) were treated with 0, 0.03 (4/sex/group), 0.15, and 0.75 mg/kg bw/day PFOS by intragastric intubation of a capsule dose for at least 26 weeks[11,12]. Two monkeys/sex/group in the control, 0.15 and 0.75 mg/kg bw/day dose groups were monitored for one year following the end of treatment.

42. Two male animals in the high dose group died or were killed *in extremis* before the end of the dosing period, with indications of pulmonary necrosis or hyperkalemia.

43. Females in the high dose group had significantly increased absolute liver weights and males and females in this group had increased relative liver weights. Serum PFOS concentrations showed a linear increase with time in the low- and mid-dose groups but the serum PFOS concentration in the high-dose group was non-linear over time and appeared to plateau. Average liver to serum PFOS concentration ratios were not dose-related and ranged from 0.9:1 to 2.7:1.

44. High-dose group males had lower haemoglobin levels, which was considered to be a treatment-related effect. Serum total cholesterol values were significantly reduced in both sexes of the low- and high-dose groups. HDL cholesterol values were significantly lower for males in the low-dose group, females in the mid-dose group and for both sexes in the high-dose group. Due to the apparent lack of a dose response, the observed decrease in HDL cholesterol values in males given 0.03 mg/kg bw/day was considered, by the authors, unlikely to be a compound-related adverse effect. The significance of the decrease in HDL cholesterol values in 0.15 mg/kg bw/day dosed females was considered difficult to interpret, given the small number of study animals, lack of pre-study and interim HDL values and lack of proportionate changes in total cholesterol.

45. There was a statistically significant increase (50%) in hepatic palmitoyl CoA oxidase activity in the female 0.75 mg/kg bw/day dose group. In the 0.75 mg/kg bw/day dose group some animals presented with centrilobular vacuolisation, hypertrophy and mild biliary stasis.

46. Serum samples collected on days 50, 40 and 27 prior to treatment and days 37, 62, 91, 182 and 184 (necropsy) of treatment were analysed by standard radioimmunoassay (RIA) methods for cortisol, testosterone, estradiol, estrone, estriol, total triiodothyronine (T_3), total thyroxine (T_4), free T_3 and free T_4. Thyroid stimulating hormone (TSH) was measured by a double antibody RIA developed for determination of TSH in non-human primates that used human TSH standards, polyclonal rabbit antihuman TSH antibodies and radiolabelled human TSH. In 0.15 and 0.75 mg/kg bw/day dosed males at 26 weeks, TSH values were increased and total T_3 values were decreased. In the unpublished study report [11] the study authors concluded that the NOAEL was, therefore, 0.03 mg/kg bw/day. Analysis of thyroid hormone values was subsequently repeated by an independent laboratory on some of the archived serum samples taken at necropsy (day 184) using equilibrium dialysis followed by RIA for free T_4 and by standardised chemiluminometric immunoassays for the measurement of T_3, T_4 and TSH and reported by Seacat *et al.*[12] The reductions in T_3 and increases in TSH values in the 0.15 mg/kg bw/day dose group were not statistically significant in the second set of analyses. In both analyses, no dose-related changes were detected in total and free T_4 values.

47. All effects appeared completely reversible on withdrawal of treatment. Taking account of the re-analysis of male thyroid hormone values and acknowledging the uncertainty concerning the significance of lowered HDL observed in females given 0.15 mg/kg bw/day, the authors of the published report considered the study NOAEL was 0.15 mg/kg bw/day[12].

48. The COT considered the application of Benchmark Dose modelling to the analytical results for TSH and total T_3 values from the two laboratories and concluded that the data were insufficiently robust for BMD modelling to be applied with confidence. Therefore, although probably conservative, the Committee considered that a NOAEL in this study of 0.03 mg/kg bw/day was indicated on the basis of the totality of the data from the analysis of thyroid hormone values.

Mutagenicity and Carcinogenicity

49. The COM considered the mutagenicity of PFOS in May 2005. PFOS has no structural alerts apparent for mutagenicity and the evidence from animal studies is that absorbed material is not metabolised.

50. Members concluded that the *in vitro* plate incorporation test using five strains of *Salmonella typhimurium* and the D4 strain of *Saccharomyces cerevisiae* gave negative results[32]. The reverse mutation assay using *Eschericia coli* gave negative results[33]. For the *in vitro* chromosomal aberration assay in human lymphocytes[34], the Committee noted the difficulty in formulating adequate suspensions of PFOS but agreed that this study had yielded negative results. The *in vitro* UDS assay in rat liver primary hepatocytes also gave negative results[35].

51. PFOS has also been tested in the mouse bone-marrow micronucleus test[36]. Members noted that only 1000 micronuclei had been evaluated at each dose level and that there was difficulty in adequately formulating PFOS for oral dosing. However, overall the study was considered to be acceptable and provided negative results.

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

53. The carcinogenicity and epidemiology studies relating to PFOS[37] (and a carcinogenicity study of the PFOS-related substance *N*-EtFOSE[38]) were considered by the COC in July 2005.

54. One dietary carcinogenicity study in Sprague-Dawley rats was available in which PFOS was administered in the diet for 104 weeks[37]. Interim sacrifices were made at 4, 14 (reported in[26]) and 52 weeks. Survival was considered to be adequate in this study. Non-neoplastic effects reported in the liver included increased absolute and relative liver weight, hepatocellular cystic degeneration and hepatocellular hypertrophy (often associated with vacuolation). No signs of hepatotoxicity were evident 52 weeks after cessation of a 52 week high-dose treatment. The NOAEL for non-neoplastic liver pathology was 2 ppm, i.e. a mean achieved dose of 0.16 and 0.14 mg/kg bw/day for males and females, respectively. This was based on the consideration that the low incidence of liver hypertrophy (3/17 and 1/9 in males and females, respectively at 2 ppm compared with 0/11 and 0/25 for males and females in the control group) associated with a lack of any effect on liver weight at this dose did not represent an adverse effect.

55. The incidence of hepatocellular adenomas was significantly increased at 20 ppm (mean achieved dose of 1.43 and 1.50 mg/kg bw/day for males and females, respectively). There was a single hepatocellular carcinoma in the female high dose (20 ppm) group. The incidence of thyroid follicular cell adenoma was significantly increased in the male high-dose recovery group, but not in the male and female high dose groups fed PFOS for 104 weeks.

56. A dietary carcinogenicity study in Sprague Dawley rats was also available in which *N*-ethylperfluorooctanesulfonamido ethanol (*N*-EtFOSE) was administered in the diet for 104 weeks[38]. No significant treatment-related effects were observed on 2-year survival rates, although survival in all groups including the controls was relatively poor. There was evidence of hepatocellular hypertrophy in high dose animals (mean achieved dose of 5.9 and 4.2 mg/kg bw/day for males and females, respectively). The incidence of hepatocellular adenomas was slightly higher in high dose male and female groups than in controls. This difference was statistically significant in the high-dose males. A single hepatocellular carcinoma was observed in a high dose female.

57. Two limited human epidemiological studies (a retrospective mortality study and an 'episodes of care' analysis) have been conducted in occupationally exposed populations. Cohorts were relatively small and also relatively young. In the retrospective cohort mortality study, when restricted to workers with at least one year of employment and high exposure to PFOS, standardised mortality ratios (SMR) were below one for all causes of death and all malignant neoplasms. There were three deaths from malignant neoplasms of the bladder (0.63 expected) in males with over 5 years in high-exposure jobs. This excess was statistically significant (SMR 16.12; 95% CI 3.32-47.14). Members questioned the adequacy of exposure assessment by using job categories. It was noted that there had been potential exposure of the workers to benzidine, a known bladder carcinogen. Members advised that, overall, it was not possible to draw definite conclusions from this study. Further evaluation across all PFOS manufacturing sites would have provided more appropriate information. Members considered that the 'episode of care' analysis was unusual in design and uninformative.

58. In conclusion, the COC agreed that there was equivocal evidence for carcinogenicity limited to hepatocellular adenoma in the animal studies. The NOAEL for tumourigenicity was 0.15-0.57 and 0.19-0.56 mg/kg bw/day in males and females, respectively. COC were not convinced that adequate evidence had been provided for a mode of action incorporating peroxisome proliferation. Considering both the COM conclusions and the carcinogenicity data Members agreed that a threshold approach could be used for risk assessment.

Reproductive toxicity

59. Teratological studies have been conducted in rat, mouse, and rabbit with agreement of observation sacross the species examined. Observed developmental effects include reduction of fetal weight, cleft palate, anasarca, delayed ossification of bones (sternebrae and phalanges), and cardiac abnormalities (ventricular septal defects and enlargement of the right atrium). The majority of these findings were seen in the highest dose groups where significant reductions of weight gain and food consumption were also observed in the pregnant dams.

Rat

60. Time-mated female Sprague-Dawley rats were administered 0, 1, 5, and 10 mg/kg bw/day potassium PFOS by gavage from gestation day (GD) 6 to GD 15[39]. Animals were sacrificed on GD 20. A NOAEL of 5 mg/kg bw/day and a LOAEL of 10 mg/kg bw/day for maternal toxicity were indicated based on significant reductions in mean body weights during GD 12-20. No other signs of maternal toxicity were reported. A LOAEL of 1 mg/kg bw/day for developmental toxicity was indicated on the basis of reductions in fetal weights. Developmental toxicity evident at doses of 10 mg/kg bw/day consisted of reductions in the mean number of implantation sites, corpora lutea, resorption sites and in the mean number of viable male, female and total fetuses, and fetal weights.

61. A repeat study in pregnant Sprague-Dawley rats[40], with the same dosing regime, reported NOAELs for maternal toxicity and developmental toxicity of 1 mg/kg bw/day. The LOAEL for maternal toxicity was 5 mg/kg bw/day, based on clinical signs of toxicity, decreases in body weight and food consumption, decreases in uterine weights, and an increased incidence in gastrointestinal lesions. The LOAEL for developmental toxicity was 5 mg/kg bw/day, based on decreased fetal body weight and increases in external and visceral anomalies and variations. Signs of developmental toxicity included a dose-related trend toward an increased incidence of late resorptions, total resorptions, number of dead fetuses, and fetal loss, although these findings were not statistically significant. Significant decreases in mean fetal weights for both males and females were observed in the 5 and 10 mg/kg bw/day dose groups. Statistically significant increases in incomplete closure of the skull were observed in the low- and high-dose groups. Also observed in the high-dose group were delayed ossification and skeletal variations.

62. Thibodeaux *et al.*,[41] and Lau *et al.*,[42] reported maternal and developmental toxicity studies in rats. Pregnant Sprague-Dawley rats were given 1, 2, 3, 5 or 10 mg/kg bw/day by gavage from GD 2 to GD 21. Maternal weight gains were suppressed by PFOS in a dose-dependent manner (statistically significant in the 2 mg/kg bw/day and higher dose groups), attributed to reduced food and water intake (statistically significantly different from controls at 5 and 10 mg/kg bw/day). Serum PFOS levels increased with dosage and liver levels were approximately four-fold higher than serum levels. Serum T_4 and T_3 in the PFOS-treated dams were significantly reduced (1 week into treatment schedule). However, no feedback response of TSH was seen. Serum triglycerides (though not cholesterol) were significantly reduced, particularly in the high-dose group.

63. Fetuses had detectable levels of PFOS in liver tissue, at almost 50% that in the maternal livers, regardless of dose level. PFOS did not alter the numbers of implantations or live fetuses at term. Birth defects noted included, cleft palate, anasarca, ventricular septal defect and enlargement of the right atrium, primarily in the 10 mg/kg bw/day dose group. Maternal doses estimated, by the study authors, to correspond to the $BMDL_5$s for sternal defects and cleft palate were 0.12 and 3.3 mg/kg bw/day, respectively.

64. In the highest dose group (10 mg/kg bw/day) neonates became pale, inactive and moribund within 1 hour of birth, with death following quickly. Neonates in the 5 mg/kg bw/day dose group survived for between 8 and 12 hours and approximately 50% of offspring died at 3 mg/kg bw/day. Cross-fostering the 5 mg/kg bw/day dose group neonates to control nursing dams failed to improve survival. The maternal dose corresponding to the $BMDL_5$ for survival of rat pups at postnatal day 8 was estimated, by the study authors, at 0.58 mg/kg bw/day.

65. Small but significant and persistent growth lags were detected in surviving pups, and slight delays in eye opening were noted. Serum levels of PFOS in neonates were comparable to those of the dam at term, suggesting that PFOS equilibrated across the placenta. Unlike the situation in the adult there did not appear to be preferential accumulation of PFOS in the neonatal liver.

66. Grasty *et al.*[43] investigated critical windows of PFOS toxicity during gestation. Exposure of pregnant rats to 25 mg/kg bw/day PFOS for a 4 day period during pregnancy demonstrated an increased incidence of neonatal death when administration was later in gestation, reaching 100% mortality in the group treated on GD 17–20.

Mouse

67. Thibodeaux *et al*[41] and Lau *et al.*[42] also reported maternal and developmental toxicity studies in mice. Pregnant CD-1 mice were treated with 1, 5, 10, 15, and 20 mg/kg bw/day from GD 1 to GD 17. Deficits in maternal weight gains were not as pronounced in the mouse as in the rat, and were only statistically significant in the 20 mg/kg bw/day dose group. Serum PFOS levels increased with dosage, and liver levels were approximately four-fold higher than serum levels. Serum T_4 levels were significantly reduced after 1 week of treatment. Serum triglycerides (though not cholesterol) were significantly reduced, particularly in the high-dose groups. Mouse dams in 10 mg/kg bw/day and higher dose groups had markedly enlarged livers.

68. PFOS did not alter the numbers of implantations or live fetuses at term. Birth defects noted were similar to those seen in the rat, namely cleft palate, anasarca, ventricular septal defect and enlargement of the right atrium, primarily in the 20 mg/kg bw/day dose group. The study authors estimated maternal doses corresponding to $BMDL_5$s for sternal and cleft palate defects in fetuses to be 0.016 and 3.5 mg/kg bw/day, respectively.

69. All animals were born alive and initially appeared to be active. In the highest dose group (20 mg/kg bw/day) neonates became pale, inactive and moribund within 1 hour with death following quickly. Neonate mice in the 15 mg/kg bw/day dose group also became moribund but survived for between 8 and 12 hours. Approximately 50% of offspring died at 10 mg/kg bw/day. The maternal dose corresponding to the $BMDL_5$ for survival of pups at postnatal day 6 was estimated at 3.9 mg/kg bw/day, approximately six times higher than that of the rat.

70. Serum levels of PFOS in neonates were comparable to those of the dam at term, suggesting that PFOS equilibrated across the placenta. There was no evidence of preferential accumulation of PFOS in the liver of the neonates.

Rabbit

71. Case *et al.*,[44] carried out oral developmental toxicology studies on mated female New Zealand white rabbits at dose levels of 0, 0.1, 1.0, 2.5, 5.0, 10, and 20 mg/kg bw/day by gavage. Treatment was from GD 6 to GD 20 and rabbits were sacrificed on GD 29. PFOS was not a selective fetal toxicant and did not cause fetal malformations in the rabbit.

72. A NOAEL and LOAEL of 0.1 and 1.0 mg/kg bw/day, respectively, were indicated for maternal toxicity, based on decreases in body weight gains and food consumption. The NOAEL and LOAEL indicated for developmental toxicity were 1.0 and 2.5 mg/kg bw/day, respectively, based on reductions in mean fetal body weight and increased incidences of fetal alterations such as delayed ossification. Abortions occurred in one 2.5 mg/kg bw/day dose group doe (GD 25) and ten of the 3.75 mg/kg bw/day dose group animals (between GD 22 and GD 28).

Two-generation reproductive study

73. A two-generation reproductive toxicity study was conducted in Sprague-Dawley rats[6]. Five groups of 35 rats/sex/dose were administered PFOS by oral gavage at 0, 0.1, 0.4, 1.6, and 3.2 mg/kg bw/day for six weeks prior to and during mating. Treatment in males continued for approximately 22 days, and female rats were treated throughout gestation, parturition and lactation. F_1 generation rats were administered PFOS beginning on lactation day (LD) 22 and continuing through until one day prior to sacrifice. Only the 0, 0.1 and 0.4 mg/kg bw/day dose groups were continued into the F_2 generation because of excessive toxicity seen in the 1.6 and 3.2 mg/kg bw/day F_1 generation pups.

74. No mortality occurred in the F_0 generation females, and there did not appear to be any effects on oestrous cycling, mating and fertility parameters. There were no treatment-related signs of toxicity, effects on mating or on any of the fertility parameters evaluated in the F_0 generation male rats. The 1.6 and 3.2 mg/kg bw/day dose groups did exhibit reductions in body weight gains during the pre-mating period and terminal body weights were also significantly reduced. Absolute weights of seminal vesicles and the prostate in the 3.2 mg/kg bw/day dose group were significantly lower than controls.

75. The most significant finding in the F_1 generation offspring was reduced pup viability at the two highest dose levels. No pups survived beyond LD 1 in the 3.2 mg/kg bw/day dose group and in the 1.6 mg/kg bw/day dose group 10.6% (27/254) of pups were dead on LD1, and an additional 26% (59/227) died between LD 2 and 4. Clinical observations in the 0.1 and 0.4 mg/kg bw/day dose groups F_1 generation male and female rats were unremarkable.

76. Evidence of treatment-related effects in the F_2 generation pups consisted of reductions in mean pup body weights (on a per litter basis) observed at 0.1 and 0.4 mg/kg bw/day on LD 7. Body weights were comparable to control levels by LD 14 (0.1 mg/kg bw/day group) and by LD 21 (0.4 mg/kg bw/day group).

77. Based on reductions in body weight gain and food consumption, the NOAEL was 0.1 mg/kg bw/day for the F_0 generation and female F_1 generation. The NOAEL for the F_1 generation parental males was 0.4 mg/kg bw/day, the highest dose tested, as the 1.6 and 3.2 mg/kg bw/day groups were not continued. The NOAEL for the F_1 generation offspring was 0.1 mg/kg bw/day, based on statistically significant reductions in mean pup weight gain at higher doses. For the F_2 generation offspring the NOAEL was 0.1 mg/kg bw/day, based on statistically significant reductions in mean pup body weight, litter size, pup viability and survival at higher doses.

78. A cross-fostering study was conducted with female Sprague-Dawley rats administered 0 and 1.6 mg/kg bw/day PFOS beginning 42 days prior to mating with untreated males, and continued throughout gestation and into LD 21[7]. Litters were placed with either a control or PFOS-treated dam for rearing, producing four groups of litters: *in utero* exposure only; un-exposed (controls); *in utero* and post-natal exposure; and post-natal exposure only.

79. Pups with post-natal exposure only had a similar mortality rate (1.1%) as pups in the control group (1.6%). Pups exposed to PFOS only *in utero* and those exposed both *in utero* and postnatally had mortality rates of 9.6% and 19.2%, respectively, indicating that *in utero* exposure is the main contributor to reduced pup survival.

Mechanistic studies

80. A small number of recently published studies have investigated more specific effects of PFOS.

81. An acute study demonstrated that PFOS, but not *N*-EtFOSE, administered via a single intraperitoneal injection at 100 mg/kg bw to male Sprague-Dawley rats, induced markers of peroxisome proliferation (induction of lauroyl CoA oxidation and lowering of serum cholesterol) in the absence of hepatomegaly[45]. PFOS did not cause a significant change in liver weight but there was a significant increase in liver-to-body weight ratio (a 12% increase) due to body weight loss.

82. With its highly hydrophobic and rigid perfluorinated carbon tail and strongly polar sulfonyl head group PFOS somewhat resembles a fatty acid. Luebker *et al.*,[46] demonstrated that PFOS and *N*-EtFOSE can interfere with the binding affinity and capacity of liver-fatty acid binding protein for fatty acids.

83. Hepatic gene expression studies in rats treated with PFOS (5 mg/kg bw/day for 3 days or 3 weeks) identified twenty three genes induced significantly and nineteen genes suppressed significantly [47]. Induced genes were primarily genes for fatty acid metabolising enzymes, cytochrome P450s, or genes involved in hormone regulation. One cytosolic enzyme, long-chain acyl-CoA hydrolase, showed a 90-fold induction on treatment. This enzyme cleaves acyl-CoA to free fatty acid and CoA, and leads to increased cytosolic free fatty acid concentrations. PPAR-α mRNA expression levels were unchanged on treatment, however, a number of genes that are indicative of peroxisome proliferation were affected. The activities of the phenobarbital inducible genes carboxyesterases and CYP2B1 were also increased by PFOS treatment, but no evidence for PFOS acting directly on the arylhydrocarbon receptor has been found.

84. One study in mice suggested that PFOS induced increases in peroxisomal fatty acid beta-oxidation, peroxisomal catalase activity, omega-hydroxylation of lauric acid, cytosolic epoxide hydrolase activity and cytosolic DT-diaphorase activity in liver[48], which are effects induced by peroxisome proliferators. The authors proposed that the study results challenge the hypothesis that the first step in peroxisome proliferation is formation of a thioester between the carboxylic group of a proliferator and coenzyme A.

COT evaluation

85. In accordance with the advice of COM and COC, the COT considered it appropriate to take a threshold approach to establishing a tolerable intake for PFOS. This is based upon the negative genotoxicity in standard assays and the equivocal evidence for carcinogenicity.

86. Given the bioaccumulative properties of PFOS it may be more appropriate to relate the toxic effects to a body burden rather than to a daily dose. However, there is incomplete understanding of the pharmacokinetics of PFOS in rodents and humans, and the Committee considered that equilibrium between plasma and target organ concentration is unlikely to have been reached in the sub-acute studies in animals. The use of a body burden approach would therefore involve excessive uncertainty on the basis of the currently available data.

87. Conclusions on the rat and mouse teratology studies[41,42] were:

- The finding of delayed ossification (manifested as bipartite and bilobed sternebrae) would be more appropriately considered a "variation" rather than a "defect" as it regularly occurred in control animals;

- delayed ossification is often a sign of general developmental delay but this is not entirely clear in this study where fetal weight effects only occur in the highest dose group. There is a dose-response in both species (rats and mice) in terms of the number of sternebrae per fetus with the variation. However, in the absence of details about the extent of the effects it is not possible to draw firm conclusions about their significance;

- the authors description of "notable skeletal defects" is not explicitly explained but probably relates to the sternal and phalangeal findings in the rat and to the sternal findings in the mouse. In the mouse, roughly half of the litters show these "notable skeletal defects" in the control and at both highest doses, indicating that this is not a generalized phenomenon throughout all litters, and moreover, a dose-response is not apparent;

- taken together, the sternal findings should not be interpreted as malformations but as indications of delayed development. In view of the above and given the additional fetal observations in this study the sternal findings do not determine the developmental NOAEL in this study.

88. The $BMDL_5$ indicated for sternal defects in the mouse fetus was approximately two orders of magnitude below the lowest dose of PFOS tested[41,42], when modelled by the study authors. Insufficient information was provided on the modelling procedures to verify the validity of this value, which indicated considerable variability. In view of the uncertainties in the BMD modelling, it was considered more practical to define an overall developmental NOAEL, which was 2 mg/kg bw/day in the rat on the basis of anasarca[41], and 5 mg/kg bw/day in the mouse on the basis of heart defects[41].

89. Overall, the data from the mechanistic studies[45-48], the rat carcinogenicity study[26,37] and the 26-week capsule study in cynomolgus monkeys[12] provide evidence that PFOS is not a potent inducer of peroxisome proliferation. Electron microscopy of livers of PFOS-exposed rats did not reveal peroxisome proliferation. The 50-95% increases in liver palmitoyl CoA oxidase levels, although statistically significant, were not considered to be biologically significant. There is evidence for some liver growth inducing agents also increase the incidence of thyroid tumours, however, with respect to PFOS more information is required.

90. Re-analysis by COT of the data reported in a 14-week rodent study[26] derived a $BMDL_{10}$ of 0.20 mg/kg bw/day for increased relative liver weights in males and females, the most sensitive endpoint in this study. Non-neoplastic effects in the two-year rat carcinogenicity study [37] indicated NOAELs of 0.16 and 0.14 mg/kg bw/day for males and females, respectively, and the two-generation reproductive toxicity study in rats [6] indicated a NOAEL of 0.1 mg/kg bw/day for F_0, F_1 and F_2 generations.

91. The 26-week cynomolgus monkey study[11,12] provided the lowest NOAEL, of 0.03mg/kg bw/day for decreased serum T3 levels . This NOAEL was considered to be the most suitable basis for deriving a tolerable daily intake (TDI) for PFOS. The Committee noted that, on the basis of the pharmacokinetic data indicating an elimination half-life of between 110 and 180 days[10-12], the cynomolgus monkeys would be at approximately half steady state at the end of this study.

92. Taking into account that this was a primate study and the effects were mild, the Committee concluded that it was not necessary to apply an additional uncertainty factor to allow for the incomplete attainment of steady state. The Committee applied an uncertainty factor of 100 to allow for inter- and intra-species variation to the NOAEL of 0.03 mg/kg bw/day from the cynomolgus monkey study. Therefore, the TDI indicated for PFOS is 0.3 μg/kg bw/day. This value is provisional and should be reviewed as new information becomes available.

93. Because of the accumulative properties of PFOS, exposure should be averaged over prolonged periods for comparison with the TDI.

Exposure assessment

94. The Food Standards Agency has completed an analysis of composite food groups samples from the 2004 Total Diet Study (TDS) for a range of fluorinated chemicals, including PFOS[49]. The TDS models the typical UK diet and is fully described in Food Survey Information Sheet 38/03[50].

95. PFOS was detected at concentrations above the limit of detection in the potatoes, canned vegetables, eggs and sugars and preserves food groups. Five of the other perfluorinated chemicals were not detected in any food groups and nine were detected only occasionally. Ten different fluorinated chemicals were found in the potatoes food group.

96. The estimated average and high level adult intakes of PFOS from the whole diet in 2004 were 0.01-0.1 μg/kg bw/day and 0.03-0.2 μg/kg bw/day (range of lower to upper bound figures)†, respectively. The highest estimated high level dietary intake was 0.1-0.5 μg/kg bw/day (range of lower to upper bound figure) for 1.5-2.5 year olds. Only 10 to 20% of the estimated intakes is derived from the four food groups in which PFOS was detected. These estimated intakes of PFOS from the diet are below the TDI of 0.3 μg/kg bw/day recommended by the COT, with the exception of the high level intake for children aged 1.5-6 years (0.1-0.5 μg/kg bw/day; range of lower to upper bound figures). As PFOS can be formed by degradation from a large group of related perfluorinated substances, the significance to the exposure assessment of detecting a number of other fluorinated chemicals in different food groups is currently uncertain.

Conclusions

97. We conclude that PFOS has the potential to cause a range of adverse health effects. Given the bioaccumulative properties of PFOS a body burden approach to setting health-based guidance values may be most appropriate, but the current knowledge of the pharmacokinetics of PFOS does not allow adequate estimation of the body burden. We recognise the need for further characterisation of human pharmacokinetics of PFOS but acknowledge that this may not be easily obtained or even feasible. In addition, we recommend that data be generated to support a body burden approach, including a better understanding of the magnitude of enterohepatic recirculation of PFOS in rodents.

98. We recommend a TDI of 0.3 μg/kg bw/day be provisionally proposed for PFOS. We consider that on the basis of available information this provisional TDI is adequate to protect against the range of identified effects.

99. We note the results of the Food Standards Agency analysis of composite food group samples from the 2004 Total Diet Study (TDS) that indicated that some groups of consumers may exceed the recommended TDI. There are considerable uncertainties in the dietary intake estimates, and therefore these potential exceedances do not indicate immediate toxicological concern.

100. We recommend that there is a need for generation of further information to reduce the uncertainties in the exposure assessment, including consideration of the impact of other perfluorinated chemicals in the diet on total PFOS exposure.

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† Upper bound concentrations assume that PFOS is present at the reporting limit for those food groups in which PFOS is present at concentrations below the reporting limit (limit of detection), and therefore could be an overestimate of the true concentrations. By contrast, lower bound concentrations assume that PFOS is absent for those food groups in which PFOS is present at concentrations below the limit of detection, and will therefore be an underestimate of the true concentrations. The range between the lower and upper bound values demonstrates the uncertainty in these exposure estimates and the true values will lie somewhere between the upper and lower bounds.

References

1. Key, B.D., Howell, R.D., Criddle, C.S. (1997). Fluorinated organics in the biosphere. *Environ Sci Technol* 31: 2445-2454.

2. Giesy, J.P. and Kannan, K. (2001). Global distribution of perfluorooctane sulfonate in wildlife. *Environ Sci Technol* 35: 1339-1342.

3. OECD. (2002). Hazard Assessment of Perfluorooctane Sulfonate (PFOS) and Its Salts. Available at URL: http://www.oecd.org/dataoecd/23/18/2382880.pdf.

4. Brooke, D., Footitt, A, Nwaogu, T.A. (2004). Environmental risk evaluation report: Perfluorooctanesulphonate (PFOS). Available at URL: http://www.environment-agency.gov.uk/commondata/105385/pfos_rer_sept04_864557.pdf.

5. Johnson, J.D., Gibson, S.J., Ober, R.E. (1979). Absorption of FC-95-14C in rats after a single oral dose. 890310200. Riker Laboratories, Inc.

6. Christian, M.S., Hoberman, A.M., York, R.G. (1999). Combined (oral) gavage fertility, developmental and perinatal/postnatal reproduction toxicity study of PFOS in rats. 418-008: 6295.9. Argus Research Laboratories, Inc.

7. Christian, M.S., Hoberman, A.M., York, R.G. (1999). Oral (gavage) cross-fostering study of PFOS in rats. 418-014: T-6295.13. Argus Research Laboratories, Inc.

8. Johnson, J.D., Gibson, S.J., Ober, R.E. (1979). Extent and route of excretion and tissue distribution of total-14 in rats after a single intravenous dose of FC-95-14C. Riker Laboratories, Inc.

9. Johnson, J.D., Gibson, S.J., Ober, R.E. (1984). Cholestyramine-enhanced fecal elimination of carbon-14 in rats after administration of ammonium [14C]perfluorooctanoate or potassium [14C]perfluorooctanesulfonate. *Fundam Appl Toxicol* 4: 972-976.

10. Noker, P.E. and Gorman, G.S. (2003). A pharmacokinetic study of potassium perfluorooctanesulfonate in the cynomolgus monkey. Southern Research Institute, Birmingham, Alabama., Souther Research Institute Study ID: 9921.6.

11. Thomford, P. (2002). 26-Week capsule toxicity study with perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) in cynomolgus monkeys. *Covance 6329-223*. Covance Laboratories Inc. Wisconsin, USA.

12. Seacat, A.M., Thomford, P.J., Hansen, K.J., Olsen, G.W., Case, M.T., Butenhoff, J.L. (2002). Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. *Toxicological Sciences* 68: 249-264.

13. Taves, D.R., Guy, W.S., Brey, W.S. (1976). Organic fluorocompounds in human plasma: prevalance and characterization. In*: Biochemistry involving carbon-fluorine bonds A symposium sponsored by the Division of Fluorine and Biological Chemistry at the 170th meeting of the American Chemical Society Chicago, IL August 26, 1975 ed Washington DC, American Chemical Society* 117-134.

14. 3M. (1999). Perfluorooctane sulfonate: Current summary of human sera, health and toxicology data. Available on US EPA Administrative Record Public Docket AR-226-0548.

15. Kannan, K., Corsolini, S., Falandysz, J., Fillmann, G., Kumar, K.S., Loganathan, B.G., Mohd, M.A., Olivero, J., Van Wouwe, N., Yang, J.H., Aldoust, K.M. (2004). Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ Sci Technol* 38: 4489-4495.

16. 3M. (2000). Determination of serum half-lives of several fluorochemicals. FYI-0700-1378. 3M Company.

17. Burris, J. M., Olsen, G., Simpson, C., Mandel, J. (2000). Determination of serum half-lives of several fluorochemicals. Interim Report #1 FYI-0700-1378. 3M Medical Department.

18. Burris, J.M., Lundberg, J.K., Olsen, G.W., Simpson, C., Mandel, J. (2002). Determination of serum half-lives of several fluorochemicals. Interim Report #2. 3M Medical Department.

19. Harada, K., Inoue, K., Morikawa, A., Yoshinaga, T., Saito, N., Koizumi, A. (2005). Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. *Environ Res* 99: 253-261.

20. Inoue, K., Okada, F., Ito, R., Kato, S., Sasaki, S., Nakajima, S., Uno, A., Saijo, Y., Sata, F., Yoshimura, Y., Kishi, R., Nakazawa, H. (2004). Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. *Environ Health Perspect* 112: 1204-1207.

21. Olsen, G.W., Church, T.R., Miller, J.P., Burris, J.M., Hansen, K.J., Lundberg, J.K., Armitage, J.B., Herron, R.M., Medhdizadehkashi, Z., Nobiletti, J.B., O'Neill, E.M., Mandel, J.H., Zobel, L.R. (2003). Perfluorooctanesulfonate and other fluorochemicals in the serum of American Red Cross adult blood donors. *Environ Health Perspect* 111: 1892-1901.

22. Olsen, G.W., Huang, H.Y., Helzlsouer, K.J., Hansen, K.J., Butenhoff, J.L., Mandel, J.H. (2005). Historical comparison of perfluorooctanesulfonate, perfluorooctanoate, and other fluorochemicals in human blood. *Environ Health Perspect* 113: 539-545.

23. Kerstner-Wood, C., Coward, L., Gorman, G. (2003). Protein binding of perfluorobutane sulfonate, perfluorohexanesulfonate, perfluorooctane sulfonate and perfluorooctanoate to plasma (human, rat, and monkey), and various human-derived plasma protein fractions. 9921-7. Southern Research Corporation.

24. Dean, W.P., Jessup, D.C., Thompson, G., Romig, G., Powell, D. (1978). Fluorad fluorochemical surfactant FC-95 acute oral toxicity (LD50) study in rats. 137-083. International Research and Development Corporation.

25. Goldenthal, E.I., Jessup, D.C., Geil, R.G., Mehring, J.S. (1978). Ninety-day subacute rat toxicity study. 137-085. International Research and Development Corporation.

26. Seacat, A.M., Thomford, P.J., Hansen, K.J., Clemen, L.A., Eldridge, S.R., Elcombe, C.R., Butenhoff, J.L. (2003). Sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats. *Toxicology* 183: 117-131.

27. Goldenthal, E.I., Jessup, D.C., Geil, R.G., Mehring, J.S. (1979). Ninety-day subacute rhesus monkey toxicity study. 137-087. International Research and Development Corporation.

28. Goldenthal, E.I., Jessup, D.C., Geil, R.G., Mehring, J.S. (1978). Ninety-day subacute rhesus monkey toxicity study. 137-092. Internation Research and Development Corporation.

29. Crump, K.S.(1984). A new method for determining allowable daily intakes. *Fundam Appl Toxicol* 4: 854-871.

30. Barnes, D.G., Daston, G.P., Evans, J.S., Jarabek, A.M., Kavlock, R.J., Kimmel, C.A., Park, C., Spitzer, H.L. (1995). Benchmark Dose Workshop: criteria for use of a benchmark dose to estimate a reference dose. *Regul Toxicol Pharmacol* 21: 296-306.

31. 3M. (2003). Environmental and health assessment of perfluorooctane sulfonic acid and its salts.

32. Litton Bionetics, I. (1978). Mutagenicity evaluation of T-2014 CoC in the Ames Salmonella/microsome plate test.

33. Mecchi, M. S. (1999). *Salmonella – Eschericia coli*/mammalian-microsome reverse mutation assay with PFOS. 20784-0-409. Covance Laboratories Inc. (Covance).

34. Murli, H. (1999). Chromosomal aberrations in human whole blood lymphocytes with PFOS. Covance Laboratories Inc. 20784-0-449.

35. Cifone, M.A. (1999). Unscheduled DNA synthesis in rat liver primary cell cultures with PFOS. 20780-0-447. Covance Laboratories Inc.

36. Murli, H. (1996). Mutagenicity test on T-6295 in an *in vivo* mouse micronucleus assay. 17403-0-455. Corning Hazleton Inc.

37. Thomford, P.J. (2002). 104-week dietary chronic toxicity and carcinogenicity study with perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) in rats. 6329-183. Covance Laboratories Inc.

38. Thomford, P.J. (2001). 104-Week dietary carcinogenicity study with narrow range (98.1%) N-ethyl perfluorooctanesulfonamido ethanol in rats. 6329-212. Covance Laboratories Inc.

39. Gortner, E.G. (1980). Oral teratology study of FC-95 in rats. 0680TR0008. Safety Evaluation Laboratory and Riker Laboratories, Inc.

40. Wetzel, L.T. (1983). Rat teratology study, T-3351, Final Report. 154-160. Hazleton Laboratories America, Inc.

41. Thibodeaux, J.R., Hanson, R.G., Rogers, J.M., Grey, B.E., Barbee, B.D., Richards, J.H., Butenhoff, J.L., Stevenson, L.A., Lau, C. (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: maternal and prenatal evaluations. *Toxicological Sciences* 74: 369-381.

42. Lau, C., Thibodeaux, J.R., Hanson, R.G., Rogers, J.M., Grey, B.E., Stanton, M.E., Butenhoff, J.L., Stevenson, L.A. (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. *Toxicological Sciences* 74: 382-392.

43. Grasty, R.C., Grey, B.E., Lau, C.S., Rogers, J.M. (2003). Prenatal window of susceptibility to perfluorooctane sulfonate-induced neonatal mortality in the Sprague-Dawley rat. *Birth Defects Res B Dev Reprod Toxicol* 68: 465-471.

44. Case, M.T., York, R.G., Christian, M.S. (2001). Rat and rabbit oral developmental toxicology studies with two perfluorinated compounds. *Int J Toxicol* 20: 101-109.

45. Berthiaume, J. and Wallace, K.B. (2002). Perfluorooctanoate, perflourooctanesulfonate, and N-ethyl perfluorooctanesulfonamido ethanol; peroxisome proliferation and mitochondrial biogenesis. *Toxicol Lett* 129: 23-32.

46. Luebker, D.J., Hansen, K.J., Bass, N.M., Butenhoff, J.L., Seacat, A.M. (2002). Interactions of fluorochemicals with rat liver fatty acid-binding protein. *Toxicology* 176: 175-185.

47. Hu, W., Jones, P.D., Celius, T., Giesy, J.P. (2005). Identification of genes responsive to PFOS using gene expression profiling. *Environ Toxicol Pharmacol* 19: 57-70.

48. Sohlenius, A.K., Eriksson, A.M., Hogstrom, C., Kimland, M., DePierre, J.W. (1993). Perfluorooctane sulfonic acid is a potent inducer of peroxisomal fatty acid beta-oxidation and other activities known to be affected by peroxisome proliferators in mouse liver. *Pharmacol Toxicol* 72: 90-93.

49. FSA. (2006). Fluorinated chemicals – UK dietary intakes. *Food Survey Information Sheet* No. 11/06. Food Standards Agency, UK.

50. FSA. (2003). Dioxins and polychlorinated biphenyls in the UK diet – 2001 Total Diet Study samples. *Food Survey Information Sheet* No. 38/03. Food Standards Agency, UK.

Statement on risk assessment and monitoring of Paralytic Shellfish Poisoning (PSP) toxins in support of human health

Introduction

1. A number of marine phytoplankton produce biotoxins that can be bioconcentrated by shellfish. Consumption of shellfish sufficiently contaminated with these toxins can result in human illness. Marine biotoxins can be categorised on the basis of clinical signs or chemical structure. Based on clinical signs, the main categories of shellfish poisoning are:

   - Amnesic Shellfish Poisoning (ASP)
   - Paralytic Shellfish Poisoning (PSP)
   - Diarrhetic Shellfish Poisoning (DSP)
   - Neurotoxic Shellfish Poisoning (NSP)

2. The Committee was asked for its view on the risk assessment of PSP toxins, and on the best method(s) of testing for biotoxins responsible for PSP in order to support protection of the health of the consumer.

Background

3. Paralytic shellfish poisoning (PSP) is a neurotoxic syndrome with signs including tingling and numbness of extremities, muscular incoordination, respiratory distress and muscular paralysis leading to death by asphyxiation. The signs of PSP are the result of blockade of voltage-gated sodium channels on excitable membrane[1].

4. The toxins responsible for PSP are saxitoxins (STXs), of which there are around 20 known analogues. STXs have been found worldwide.

5. STXs have varying toxicities, and the relative intraperitoneal (i.p.) toxicities of the major PSP toxins, as determined in mice, have been used to sum the toxicity of the different toxins as STX equivalents (eq).

6. In 2004, a Joint FAO/IOC/WHO *ad hoc* Expert Consultation on Biotoxins in Bivalve Molluscs was asked by the Codex Committee on Fish and Fishery Products (CCFFP) to perform risk assessments for a number of biotoxins that are present in bivalve molluscs, and to provide guidance on methods of analysis and monitoring of these toxins[2]. The COT has also been provided with a copy of the background document that supported the consultation[3].

Previous COT evaluations

7. The COT considered PSP toxins in 1994, when it reviewed a MAFF food surveillance paper on Naturally Occurring Toxicants in Food. The Committee noted that the development of chemical assays, immunological or other *in vitro* methods which are more sensitive and more specific than the bioassays currently used in monitoring for marine biotoxins in the UK, would not only be beneficial from an analytical viewpoint but would also avoid the use of experimental animals. The COT recommended:

   - That the surveillance programme for detecting PSP toxins as described in the surveillance paper be continued

   - That research to develop an assay to complement and/or replace the MBA for PSP be continued

Toxicology

Toxicokinetics

8. A study of PSP patients detected PSP toxin levels of 2.8-47 nM in serum during acute illness and of 65-372 nM in urine following acute symptom resolution, suggesting that urine is a major route of excretion[4]. Clearance of PSP toxins from serum was evident within 24 hours. Compared with cooked mussel samples, serum from individuals that had consumed them had a larger proportion of C1 and a lower proportion of gonyautoxin 2. In a post mortem analysis of tissues and body fluids obtained from two victims of PSP, toxins were detected in the gastric content, body fluids (urine, bile and cerebrospinal fluid), and in tissue samples (liver, kidney, lung, stomach, spleen, heart, brain, adrenal glands, pancreas and thyroid glands)[5]. The PSP toxins found in body fluids appeared to have undergone metabolism in the 3-4 hours following ingestion.

9. Rapid excretion in urine has been observed in rats after i.v. administration of STX at a sub-lethal dose (ca. 2 μg/kg). By 24 hours, approximately 58 percent of the administered dose had been excreted[6]. Experiments in cats indicate that STX excretion primarily involves glomerular filtration[7]. Studies investigating the potential for biotransformation of B1 to its carabamoyl form (STX) indicate that conversion occurs in artificial gastric juice (pH 1.1) but not in rat gastric juice (pH 2.2)[8].

Acute toxicity

10. The LD_{50} values for STX in mouse by different routes of administration are shown in Table 1. The oral LD_{50} values for species other than the mouse are shown in Table 2.

Table 1. Acute toxicity of STX in mice[9]

Route	LD50 in µg/kg bw
oral	260-263
intravenous	2.4-3.4
intraperitoneal	9.0-11.6

Table 2. Oral LD50 values of STX in various species[9]

Species	LD50 in µg/kg bw
rat	192-212
monkey	277-800
cat	254-280
rabbit	181-200
dog	180-200
guinea pig	128-135
pigeon	91-100

11. An i.p. mouse bioassay (MBA) has been used to determine the relative potencies of PSP toxins (see Table 3). Toxins were extracted from contaminated shellfish and separated by chromatographic methods. Each toxin was then tested using an MBA and the toxicity relative to STX (assigned as 1) calculated[3].

12. PSP causes death by asphyxiation due to progressive respiratory muscle paralysis. In animals (cat, rabbit) STX causes a decreased respiratory activity reflected in both a decline in the amplitude and velocity. Death can be prevented by artificial respiration, and depending on the dose, respiration may return spontaneously[9].

13. An intravenous dose of 1-2 µg STX causes a rapid weakening of muscle contractions, affecting contractions by direct stimulation and by indirect motorneurone stimulation in all skeletal muscle tissues. This dose level also induces a decrease of the action potential-amplitude and a longer latency time in the peripheral nervous tissue. Both motor and sensory neurones are influenced but the sensory neurones are inhibited at lower dose levels[9].

14. There are uncertainties about the possible effects of PSP toxins on the central nervous system. Most symptoms can be attributed to peripheral effects. However central effects may occur[9].

Table 3. Specific i.p. toxicities of saxitoxin analogues[3]

Toxin	Relative Toxicity
Saxitoxin (STX)	1
Neosaxitoxin (neoSTX)	0.92
Gonyautoxin 1 (GTX1)	0.99
Gonyautoxin 2 (GTX2)	0.36
Gonyautoxin 3 (GTX3)	0.64
Gonyautoxin 4 (GTX4)	0.73
Decarbamoyl saxitoxin (dcSTX)	0.51
Decarbamoyl GTX 2 (dcGTX2)	0.15
Decarbamoyl GTX 3 (dcGTX3)	0.38
B1 (GTX5)	0.064
C1	0.006
C2	0.096
C3*	0.013
C4*	0.058

* Estimated by the measurement of GTX1, GTX4 formed by acid hydrolysis

15. No data are available on repeat dose toxicity, mutagenicity, carcinogenicity, reproductive toxicity or developmental toxicity.

Human data

Symptomalogy

16. Human PSP cases have been defined as mild, moderately severe and extremely severe[10]. Typical symptoms for each category are:

- *Mild:* Tingling sensation or numbness around lips, gradually spreading to face and neck. Prickly sensation in fingertips and toes. Headache, dizziness, nausea.

- *Moderately severe:* Incoherent speech. Progression of prickly sensation to arms and legs. Stiffness and noncoordination of limbs. General weakness and feeling of lightness. Slight respiratory difficulty. Rapid pulse.

- *Extremely severe:* Muscular paralysis. Pronounced respiratory difficulty. Choking sensation.

17. Patients who survive PSP for 24 hours, with or without mechanical ventilation, have a high probability of a full and rapid recovery. Whether a severe response leads to death will be influenced by medical intervention, and this is likely to affect estimates of lethal doses.

Epidemiology studies

18. A number of reports of PSP cases from a range of countries were reviewed by FAO/IOC/WHO[2,3]. The Committee reviewed these data as a possible basis for setting an acute reference dose. The data reviewed by the Committee are summarised in Table 4 and Figure 1.

19. Except where noted otherwise, toxin levels were determined by MBA, using either leftover food or shellfish samples of a similar origin. Some authors applied a correction factor for the effects of cooking on PSP toxin levels, as studies have indicated that cooking can reduce the toxicity of contaminated shellfish by as much as 70%[10]. The toxins are not completely destroyed but are, in part, leached into the cooking fluids. Steaming tests with mussels and clams indicate that 50% of the toxin present in the raw tissue is present in the bouillon (the liquid left after cooking). While the bouillon is commonly discarded following steaming substantive amounts of PSP toxins may still be ingesting when eating a chowder, as the bouillon forms part of the chowder.

20. Most shellfish contaminated with PSP toxins contain a mixture of several saxitoxins. The toxicity of different toxins may be expressed in mouse units (MU). One MU is defined as the amount of toxin required to kill a 20g mouse 15 minutes after i.p. injection, and has been reported to be approximately equivalent to 0.18 μg STX eq[11]. To aid comparison, values that were reported in mouse units (MU) were converted to STX eq, assuming a conversion factor of 0.18 μg STX eq per MU.

21. From the human case reports, the intake of STXs required to induce PSP symptoms varies greatly. This may be due to differences in susceptibility among individuals, and inaccuracies in exposure assessments due to differences in sampling and analysis of contaminated shellfish at the time of poisoning incidents and uncertainty with respect to amounts consumed.

22. Prakash *et al.*[10] reported data on PSP cases that occurred in New Brunswick, Canada from 1945 to 1957. Records were analysed for 131 individuals who had consumed contaminated shellfish from areas where shellfish toxicities were being monitored and included dates and the size, species and number of shellfish consumed by an individual. This information was combined with data on toxin levels determined by MBA and meat yields of shellfish to estimate the amount of toxins ingested by each person. The authors applied a conversion factor of 0.3 to the toxin concentration of raw shellfish when calculating the intake of individuals who had eaten known quantities of cooked shellfish, when no samples of the cooked shellfish were available.

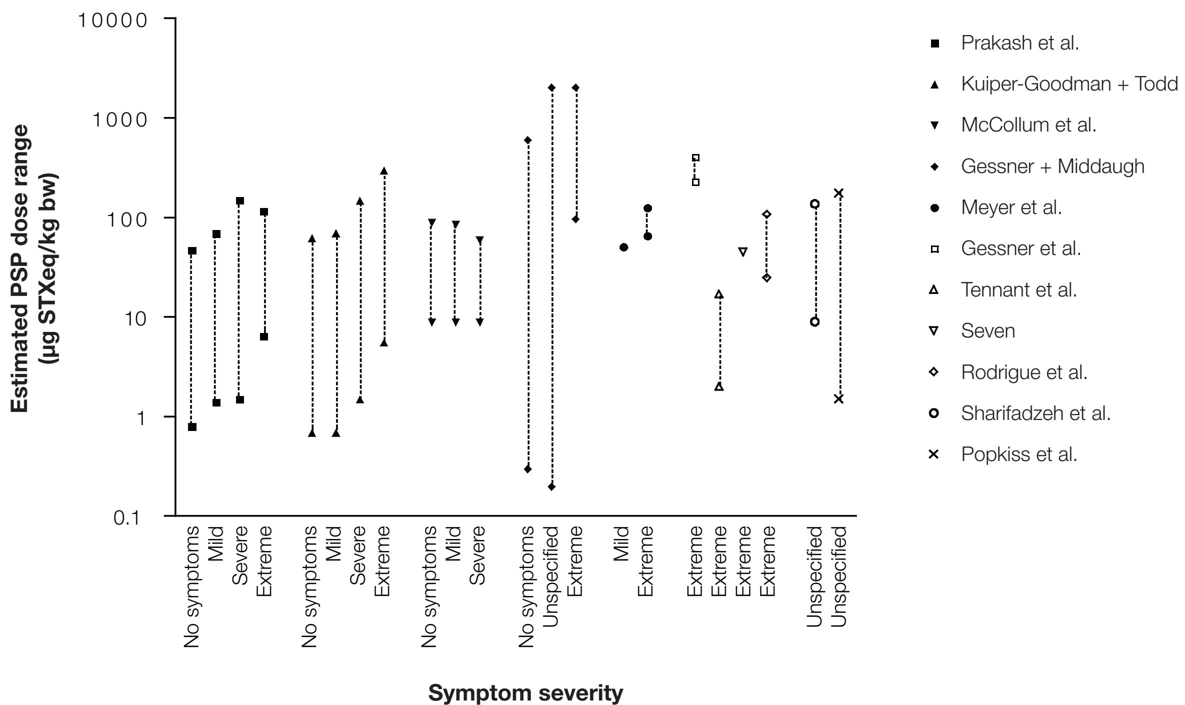
23. Forty-nine cases, including several children, were categorised as having mild, severe, and extreme poisoning. These individuals had ingested toxins within the range of 85-4128, 90-9000 and 390-7000 μg STX eq per person, respectively. Assuming an adult bodyweight of 60 kg, the estimated toxin dose would be 1.4-69, 1.5-150 and 6.5-117 μg STX eq/kg bw, respectively. Only six patients had consumed less than 2 μg STX eq/kg bw. The report also includes information for 82 individuals who did not show any PSP symptoms, who had ingested between 50-2800 μg STX eq per person (0.8-47 μg STX eq/kg). The authors noted that because of the large person-to-person variation in sensitivity, average toxin consumption values are of limited significance.

24. The authors of the report suggested a number of factors that may affect sensitivity to PSP. Noting that two young children aged 2 and 8 years became ill after eating lower than average amounts, they speculated that young children may be more sensitive than adults. It was also suggested that sex may influence susceptibility. However, the COT considered that the variability of the data does not support these conclusions, and any differences in susceptibility related to age or sex appear to be within the range of uncertainty regarding the overall variability in human sensitivity to PSP.

25. The authors also noted that their records suggest PSP symptoms are more acute when contaminated shellfish are eaten alone on an empty stomach than when they are eaten as part of a normal meal, and that consumption of alcohol alongside shellfish accentuates symptoms. However, no details are provided to support these statements.

26. Several other reports have also assessed the effect of alcohol upon PSP. Gessner and Middaugh[17] applied a backward unconditional multiple logistic regression model to information relating to 30 ill and nine non-ill individuals for whom data on alcohol consumption, cooking history and race/ethnicity were available. While the analysis suggested that alcohol consumption may protect Figure 1. Range of PSP toxin intakes associated with human illness against PSP, no details on age or amounts of alcohol consumed are provided in the paper, and the significance of the findings are unclear given the limitations of the study as noted in paragraph 29 below. Popkiss *et al.*[16] did not observe an association between symptom severity and estimated alcohol consumption in a report of a PSP outbreak in South Africa involving 17 individuals (see paragraph 31).

Figure 1: Range of PSP toxin intakes associated with human illness



Symbols indicate the highest and lowest intake of PSP toxins associated with illness of varying severity, as noted in the human case reports. Values reported in mouse units have been converted to STX eq, as described in paragraph 20.

27. An unpublished Health Canada report[14] analysed data on Canadian cases of PSP from 1970-1990, together with information on outbreaks in Canada from 1944-1970, and from Guatemala in 1987 (see paragraph 31). Case histories were used to classify cases as mild, moderately severe or extremely severe. A number of assumptions were made in order to derive PSP toxin intakes when the data were incomplete. When the actual consumption of shellfish by an individual was unknown, typical values from the literature were used. Toxin concentrations in raw shellfish were adjusted for the effects of cooking. Cases for which the overall information was judged inadequate were not included in the final assessment. Intakes were reported as μg STX eq/kg bw, but it is not clear what bodyweight measurements or assumptions were made. However, in one section of the report, adult and child bodyweights of 60 and 25 kg, respectively, are mentioned.

28. Intakes for patients with mild, moderately severe and extremely severe PSP ranged from 0.7-70, 1.5-150 and 5.6-300 μg STX eq/kg bw, respectively. One patient with moderately severe symptoms had a reported intake of 0.3 μg STX eq/kg bw, but this was considered an outlier by the authors of the report. In addition, there were some individuals who did not develop symptoms after apparently consuming doses up to approximately 63 μg STX eq/kg bw. The authors noted that only two cases, both non-fatal, were reported where the PSP toxin dose was less than approximately 1.4 μg STX eq/kg bw. Therefore, they proposed a tolerable single intake of 1.4 μg STX eq/kg bw.

29. Gessner and Middaugh[17] reviewed 54 outbreaks of PSP that had occurred in Alaska between 1973 and 1992, involving 117 patients. PSP toxin levels were determined by MBA from either leftover shellfish collected from persons involved in an outbreak, or from shellfish collected from the same beach where shellfish implicated in an outbreak had been harvested. Data collection was performed over 20 years by several individuals and was not standardised. In addition, no correction appears to have been applied for the effects of cooking, and the authors acknowledged that they may have miscalculated the amount of toxin consumed by assuming that toxin levels from tested shellfish were identical to levels in ingested shellfish, and by assuming uniform weight within shellfish species. The estimated amounts of toxin ingested were reported as μg STX eq per person, and have been converted to μg STX eq/kg bw assuming an adult bodyweight of 60 kg. The estimated dose for 33 ill people (mean age 38 yrs) ranged from 0.2-2058 μg STX eq/kg bw. For 10 non-ill people (mean age 36 yrs), the estimated toxin dose was 0.3-610 μg STX eq/kg bw. Two persons with respiratory arrest ingested 98-2058 μg STX eq/kg bw.

30. A large-scale outbreak of PSP occurred in Guatemala in July-August 1987, affecting 187 individuals between 9 months and 86 years of age[18]. The overall case fatality rate was 14%, but was highest in children under 6 years of age, at 50%. A case study involving 57 patients and 43 healthy family members from 19 households implicated clams harvested from local beaches as the source of the PSP toxins. Of the controls, five had consumed clams, but no information on their dose of PSP was provided. Analysis of clams harvested from the affected area on 1 August by MBA indicated a PSP concentration of 30,000 mouse units (MU)/100 g. Assuming a conversion factor of 0.18 μg STX eq per MU, this corresponds to a concentration of 5400 μg STX eq/100 g clam meat. HPLC analysis of a clam sample indicated a concentration of 7500 μg STX eq/100 g clam meat. A sample of soup collected from one of the affected households was analysed by MBA. The estimated intake of PSP toxins from this soup for one child who died, reported as MU and converted to STX eq, was 25 μg STX eq/kg bw. Four adult patients who died had each consumed 30-85 g clam meat. The authors calculated the amount of PSP toxins consumed by these individuals using the HPLC estimate of 7500 μg STX eq/100 g clam meat, but there appears to be an error in their calculations. The dose range for these individuals has been therefore been recalculated, assuming a 60 kg bodyweight, as 38-106 μg STX eq/kg bw.

31. An outbreak of 17 cases of PSP, none of which were fatal, occurred in South Africa in 1978[16]. The amount of PSP toxins ingested were based on MBA analysis of toxin concentrations in mussels collected from restaurants or affected coastal areas, and estimated mussel consumption. A factor of 0.3 was applied to adjust for the effects of cooking. The estimated dose ranged from 500-58,500 MU per person. Assuming an adult bodyweight of 60 kg and using the conversion factor of 0.18 μg STX eq per MU, these doses equate to 1.5-176 μg STX eq/kg bw. Only one patient had ingested less than 5 μg STX eq/kg bw. The authors did not observe an association between symptom severity and ingested dose of PSP toxins.

32. In 1954, a family of six adults and one child aged 12 years collected and consumed clams containing PSP toxins[20]. All members of the family experienced PSP symptoms, and two of the adults died. The authors of the report had sampled shellfish from the area where the family had obtained the clams on several of the days preceding and following the day that shellfish implicated in the incident were collected. PSP toxin levels within these samples were calculated by MBA and graphed. As no shellfish had been sampled on the day when the clams involved in the incident had been collected, toxin levels were estimated by interpolation on the graph, and a correction factor of 0.3 was applied to adjust for the effects of cooking. The amount of PSP toxins ingested by the patients was calculated by applying the estimated PSP toxin concentrations to the estimated shellfish consumption of the patients.

33. The authors estimated that one of the patients who died had consumed approximately 5800 MU, while the second fatality and one surviving adult had probably consumed 2400 MU. Assuming an adult bodyweight of 60 kg and using the conversion factor of 0.18 μg STX eq per MU, these doses equate to 17 and 7 μg STX eq/kg bw, respectively. The remaining patients were estimated to have probably consumed between 650 and 1000 MU (2 and 3 μg STX eq/kg bw).

34. In 1994, four outbreaks of PSP due to mussel consumption involving two, two, one and six ill individuals were reported in Alaska, USA[4]. Mussel toxin concentrations were calculated by MBA. For outbreaks 1-3, PSP toxin levels were determined from mussels collected within 24 hours of the onset of the outbreak from the implicated beach, while for outbreak 4, all persons had eaten boiled mussels and toxin concentrations were determined from left-over cooked and uncooked mussels.

35. Shellfish toxin concentrations for the four outbreaks ranged from 1778-19,418 μg STX eq/100 g. For 10 individuals for whom dose estimates were available, the lowest dose that caused illness was estimated to be 21 μg STX eq/kg bw, with a median dose of 167 μg STX eq/kg bw. Among four persons with respiratory arrest, who the authors suggested may be considered to have consumed a lethal dose, the estimated dose ranged from 230-411 μg STX eq/kg bw.

36. An outbreak of PSP occurred in the summer of 1968 in 78 individuals who had eaten mussels harvested from the Northumbrian coast, UK[15]. In total, the authors conducted interviews with 71 of the affected individuals. MBA analysis was performed on raw mussels obtained from the retailer that supplied 65 of the individuals, and also on samples that were cooked by the same method the retailer had employed. Toxin concentrations were estimated in MU, and converted to STX eq/kg bw using the conversion factor of 0.18 μg STX eq per MU and an adult bodyweight of 60 kg. Of the 22 persons who consumed an estimated dose of 9-30 μg STX eq/kg bw, 18% did not experience symptoms, while 23% and 59% reported mild and moderate symptoms, respectively. For the 42 individuals estimated to have ingested 30-60 μg STX eq/kg bw, 19% did not report symptoms, while 19% and 62% experienced mild and

moderate symptoms, respectively. Only seven people ingested more than 60 μg STX eq/kg bw. Five experienced moderately severe symptoms, while two experienced no symptoms whatsoever. No fatalities were reported in this incident.

37. The Australia New Zealand Food Authority published a risk assessment on shellfish toxins in 2001[22]. This report claims that 2-3 μg STX eq/kg bw can produce moderate symptoms, 6.7-18 μg STX eq/kg bw can cause death, but 33-167 μg STX eq/kg bw is more likely to constitute a fatal dose, although no references are cited to support this statement. These values have been converted from STX eq per person, assuming an adult bodyweight of 60 kg.

38. The case fatality rate for PSP varies considerably. In relatively recent outbreaks in North America and Western Europe involving over 200 people, there were no deaths. However, in similar outbreaks in Southeast Asia and Latin America, case fatality rates of 2-14% have been recorded. Part of this difference may be related to how readily victims have access to hospital care[1].

39. Other estimated intakes resulting in illness or death, derived from case studies describing a single or small number of incidents or review papers are summarised in table 4.

FAO/IOC/WHO Evaluation

40. The unpublished data from Health Canada[14] (see paragraphs 27-28) was specifically mentioned in the FAO/IOC/WHO Consultation's conclusions[2]. It was noted that analysis of this report indicated that mild cases had generally consumed 2-30 μg STX eq/kg bw, while more severe cases generally involved an exposure of >10-300 μg STX eq/kg bw. On this basis, the Consultation proposed a provisional lowest observed adverse effect level (LOAEL) of 2.0 μg STX eq/kg bw. Considering that mild illness is readily reversible, and the epidemiology data represents a range of individuals with varying susceptibilities, the Consultation applied a safety factor of 3 to this LOAEL, establishing a provisional acute reference dose of 0.7 μg STX eq/kg bw.

COT Evaluation of toxicological data

41. The Committee noted that the available animal and human data are limited. A tolerable daily intake (TDI) could not be derived as the data all related to acute exposure. The acute exposure data were assessed in order to consider establishment of an acute reference dose.

42. The COT noted a large number of uncertainties in the human data. These relate to uncertainties in exposure assessments, for example due to disparities between toxin levels in tested shellfish compared with the levels present in shellfish that were actually consumed. While leftover cooked shellfish were analysed in some incidents, other reports were based on toxin concentrations determined in uncooked shellfish, either from the same batch of shellfish that had been consumed, or that had been collected from areas where consumed shellfish were obtained. In some reports, samples were collected on the same day as the shellfish implicated in the PSP outbreak, while in others shellfish had been collected on a different day.

43. Further uncertainties in exposure assessments relate to uncertainties with respect to amounts of shellfish reportedly consumed, and assumptions regarding the weight of the edible portions of specific shellfish species. While some studies had applied a correction factor to adjust for the effects of cooking, the precise effects of individual cooking practices on toxin levels are uncertain. In the majority of studies, PSP toxin levels in the shellfish were calculated using an MBA, and the identity of the specific toxins that had been consumed was unknown.

44. The Committee observed that some PSP cases have been reported following consumption of PSP toxins below the FAO/IOC/WHO's provisional LOAEL of 2 μg STX eq/kg bw. However, relatively few patients had been ill after consuming such amounts, and these studies were subject to the uncertainties noted above. FAO/IOC/WHO had considered that mild cases had generally consumed 2-30 μg STX eq/kg bw while more severe cases involved an exposure of >10-300 μg STX eq/kg bw[2].

45. Based on an overview of all the available data, and given the limitations regarding the exposure data, the Committee concluded that the FAO/IOC/WHO approach was reasonable.

46. FAO/IOC/WHO had applied a safety factor of 3 to the LOAEL of 2 μg STX eq/kg bw cited for mild effects to establish a provisional acute reference dose of 0.7 μg STX eq/kg bw. The value of 3 had been selected rather than a larger value because the epidemiological data on PSP represented a wide range of individuals with varying susceptibilities, and because mild illness is readily reversible. In addition, the COT noted that the reported dose range was likely to represent individuals at the extreme ends of sensitivity. The Committee noted that the proposed acute reference dose was about one-tenth of the dose range associated with severe illness and was therefore unlikely to be overly conservative.

47. The limited animal data would appear to support this approach. Applying an uncertainty factor of 1000 to allow for differences between species, for human variability and for extrapolation from a lethal dose to the oral LD_{50} of STX in monkeys of 277-800 μg/kg bw (table 2) would indicate an acute reference dose in the region of 0.3-0.8 μg/kg bw.

48. FAO/IOC/WHO assumed a portion size of 250 g would cover 97.5% of consumers in most countries. The Committee noted that this value was a reasonable estimate for high level shellfish consumption in the UK, based on analysis of information on consumption of cockles, mussels, oysters and whelks from the UK National Diet and Nutrition Survey Programme (NDNS)[23]. Given the acute effects of PSP, the Committee considered it essential to refer to high level portion size as the comparator in the risk assessment.

49. For a 60 kg adult, consumption of 250 g of shellfish containing 17 μg STX eq/100 g shellfish meat would result in an intake of PSP at the acute reference dose of 0.7 μg STX eq/kg bw. Because of the uncertainty and lack of precision in the data, the COT concluded that this value should be rounded to a single significant figure of 20 μg STX eq/100 g shellfish meat, which would be the maximum concentration considered to be without appreciable health risk.

50. The current regulatory limit for PSP toxins in shellfish is 80 μg STX eq/100 g shellfish meat, which could result in some individuals consuming greater than the proposed acute reference dose. There have been no reported incidents of PSP resulting from consumption of UK shellfish since the official UK monitoring programme was introduced. This could be interpreted as suggesting the current regulatory limit may provide adequate protection for human health. However, the Committee agreed that it would be imprudent to conclude that mild cases of PSP have not occurred in the UK, as they may go unreported. Furthermore, given the potential for PSP to result in severe illness or even death, the proposed acute reference dose should be supported.

51. Although some reports had suggested factors that may affect sensitivity to PSP, the variability in the available data does not allow identification of any specific susceptibility factors.

52. The Committee agreed with FAO/IOC/WHO that that there is a need for better collection of implicated samples and patient information in future PSP outbreaks, as well as more detailed information on the effects of food processing on toxin levels.

Monitoring of PSP toxins and regulatory levels

53. Current legislation requires shellfish containing 80 μg STX eq/100 g shellfish meat to be withdrawn from sale. A maximum concentration of 20 μg STX eq/100 g shellfish meat would be required in order to ensure that a 60 kg adult consuming 250 g of shellfish would not exceed the proposed acute reference dose of 0.7 μg STX eq/kg bw.

54. Mouse bioassays (MBAs), involving intraperitoneal injection of shellfish extract, are prescribed as the reference methods in EU legislation (Commission Regulations (EC) No 854/2004 and (EC) No 2074/2005) for detection of PSP biotoxins, and are used in the UK PSP monitoring programme. MBAs were developed in the 1930s for the detection of marine biotoxins in protection of public health, when specific analytical methodology was not available. Recent progress in development of certified reference material and alternative methods means it is timely to reconsider the most appropriate way of protecting public health.

55. The COT was asked to comment on the extent to which the available methods for detecting PSP toxins are appropriate for protecting public health. The COT consideration focussed on the MBA and two alternative methods, a high performance liquid chromatography (HPLC) technique and an immunoassay known as the Jellett Rapid Test (JRT).

56. The current MBA for PSP toxins in the UK is carried out using a method based on the updated Association of Analytical Chemists (AOAC) official method, and has a limit of detection of approximately 30 μg STX eq/100 g shellfish meat[24]. The HPLC method, developed by Lawrence et al.[25,26], has a substantially lower detection limit than the MBA currently employed, and is able to identify and quantify a range of PSP toxins. This method has recently undergone interlaboratory validation and has received AOAC approval for the determination of STX, GTX2,3 (together), GTX1,4 (together), dcSTX, B1, C1,C2 (together) and C3,4 (together) in some shellfish species (mussels, clams, oysters and scallops)[27].

57. The JRT, unlike the MBA and HPLC methods, is not a quantitative assay, which the manufacturer claims can be used to screen out samples found to contain approximately ≤40 μg STX eq/100 g shellfish flesh.

58. Tables 5-7 show the available data on the performance characteristics of the three methods. In general, these have been generated from testing of a relatively small number of samples.

Standards and reference materials

59. The use of methods based on HPLC for PSP monitoring programmes has previously been limited by a lack of availability of commercial standards for all the known PSP toxins. Since 2003, however, standards covering all the carbamate and most of the decarbamoyl saxitoxin families, which comprise the PSP compounds that are most toxic in the MBA, have been available. The predominant toxins that have been detected in UK samples are STX, GTX2 and 3, GTX 1 and 4, C1, C2, and NEO[28,29].

Comparative data

60. The Committee noted that a number of trials had been published in which two or three of the methods had been performed concurrently[26,28,37,38,39,40,24,41].

61. Evaluation of alternatives to the MBA by comparison with the MBA is problematic, given the MBA's inherent variability and that the method is unable to identity the specific toxins present within a sample.

62. A further complexity is evident in studies comparing the MBA with HPLC. The authors of the various studies have converted toxin levels determined by HPLC into STX eq, by multiplying the measured toxin concentrations by a relative toxicity factor, as determined by MBA. Although the same source has generally been cited for these relative toxicity values[42], the precise figures used differ in several reports.

63. As part of the interlaboratory study on the Lawrence pre-column method[26], a set of samples used in the study was also analysed by the MBA and by Jellett Rapid Test (JRT) in a single laboratory. To compare with the MBA result, individual PSP toxin levels obtained by HPLC were converted to STX eq using relative toxicity values[42]. In this study, similar results were generally obtained with the MBA and HPLC methods, although one sample that was negative in the MBA was found to contain 54 μg STX eq/100 g shellfish meat by HPLC.

64. In 2005, FSA Scotland funded a short project, which employed the Lawrence HPLC method to verify the presence or absence of PSP toxins in 147 extracts giving positive and negative results using the JRT in Scotland[28]. HPLC results agreed with the absence of toxins in JRT negative extracts, and revealed that the predominant toxins in JRT positive extracts were saxitoxin and GTX 2,3. Higher toxicity values were recorded using HPLC when compared to the MBA data. Similar results have been observed in previous comparisons of HPLC and MBA data, and are considered to be due to the underestimation of PSP toxicity by the MBA[38,43,42]. Underestimation by MBA is thought to be due to high salt levels in the extracts and matrix effects[44,45].

65. Comparative HPLC and MBA data are available from the Portuguese PSP monitoring programme, where the Lawrence HPLC method has been implemented alongside an MBA since 1996. Concentrations of the different PSP toxins were summed as STX eq by conversion of measured PSP toxin levels to their relative toxicity in the MBA. For 79 tested samples, agreement between the MBA and HPLC was 87.3%, with a 12.7% incidence of a 'negative' MBA (defined in the report as ≤80 μg STX eq/100 g shellfish meat) alongside a 'positive' (defined as ≥80 μg STX eq/100 g shellfish meat) HPLC result. There were no incidences of a 'positive' MBA combined with a 'negative' HPLC result[38]. The authors of this report noted that problems had been experienced with the HPLC method due to the presence of two interfering compounds, one eluting close to STX and the other eluting close to dcSTX. However, introduction of solid-phase extraction, as recommended in the Lawrence method, removed one of the interfering peaks completely while the other was reduced by approximately 80%.

66. Data are also available from parallel trials of the JRT alongside the MBA comprising over 2000 samples including a wide range of shellfish species sampled from Alaska, Maine, Washington State, British Columbia, New Zealand and the UK[37]. In these trials, the JRT detected 100% of toxic extracts, defined as those containing ≥80 μg STX eq/100 g shellfish meat. One borderline toxic sample, determined to contain 78 and 86 μg STX eq/100 g shellfish meat in two separate MBAs, was interpreted as positive in one JRT and negative in the second. In addition, between 85-100% of extracts found to contain 32-80 μg STX eq/100 g in the MBA were also positive in the JRT test. The overall rate of JRT positives that were MBA negative was around 14%.

67. To date, 2939 shellfish extracts have been tested in tandem by MBA and JRT by the three UK shellfish monitoring laboratories. Of these samples, 70 were found to contain levels ≥40 μg STX eq per 100 g of shellfish flesh by MBA, all of which tested positive by JRT. Of the remaining 2869 extracts, 350 tested positive by JRT, but were negative in the MBA.

68. New data comparing MBA and JRT results from the Californian PSP monitoring programme have recently been published[39]. The JRT was introduced to screen for shellfish containing PSP toxins in California following an initial study involving parallel testing of 232 mussel and oyster extracts by MBA and JRT. There were no instances of a negative JRT for a sample positive in the MBA, while 29% had a positive JRT result and a negative MBA result.

COT Evaluation of PSP detection methods

69. The Committee concluded that HPLC was currently the only method sensitive enough to detect PSP toxins at the concentration of 20 μg STX eq/100 g shellfish meat, considered by the Committee to be necessary for protection of public health. It was important for the methodology to support detection of all toxins considered likely to be relevant to public health.

70. Potency of the different PSP toxins is currently compared based on the i.p. toxicity by MBA. However, it is not known how this relates to the oral toxicity of these toxins.

71. The COT considered that HPLC should be used for quantification of PSP toxins, subject to appropriate quality control measures and method validation in the testing laboratories, including investigation of possible interfering peaks that could mask the presence of toxins in different matrices.

72. The existing data comparing MBA and HPLC at the current regulatory limit provided reassurance that public health would not be compromised by not using the MBA. However, taking into account the inherent variability in results from bioassays, uncertainty with respect to the relevance to health of discordant results and the inability of the MBA to identify individual PSP toxins, comparative testing was not considered appropriate for validation of alternative methods.

73. As HPLC detects individual PSP toxins, relative i.p. toxicity values have been used to calculate the STX eq concentration within shellfish samples for comparison with a regulatory limit. Consideration should be given to the most appropriate method of summing the concentration of PSP toxins within shellfish samples.

74. The Committee was informed that it might be possible for the JRT to be re-engineered to detect lower concentrations of PSP. The Committee agreed that if this was possible it could be used as a screen, using HPLC for quantification of positive results.

75. At the current regulatory limit of 80 μg STX eq/100 g shellfish meat, the COT considered that, based on the data presented, the JRT was appropriate for use as a pre-screen to identify samples for quantitative testing, subject to appropriate quality control measures.

Conclusions

76. We note that the available animal and human data relate to acute exposure, and are therefore not suitable for the derivation of a tolerable daily intake for PSP toxins. The potential for long-term health effects arising from repeated exposure to PSP toxins is unknown.

77. We consider that human case reports should be used as a basis for risk assessment, while noting the uncertainties related to the amount and nature of PSP toxins actually consumed in cases of human illness.

78. We consider that 2 μg STX eq/kg bw is the best estimate of a LOAEL for mild illness in humans, taking into account the uncertainties in the available data. More severe cases may occur above 10 μg STX eq/kg bw.

79. We conclude that the LOAEL can be used as the basis for deriving an acute reference dose of 0.7 μg STX eq/kg bw, by applying an uncertainty factor of 3 to the LOAEL in order to allow for the absence of a no observed adverse effect level (NOAEL). A larger uncertainty factor is not required because the epidemiological data on PSP represent a wide range of individuals and are likely to include information relating to those who are most sensitive. This value for the acute reference dose is supported by the available data relating to oral toxicity in animals.

80. We note that a portion size of 250 g is a reasonable estimate for high level consumption of shellfish in the UK. We conclude that a PSP toxin concentration of 20 μg STX eq/100 g shellfish meat would be the maximum concentration considered to be without appreciable health risk, assuming a 60 kg adult bodyweight.

81. HPLC is currently the only method sensitive enough for the detection of PSP toxins at a concentration of 20 μg STX eq/100 g shellfish meat.

82. We conclude that HPLC should be used for quantification of PSP toxins subject to appropriate quality control measures and method validation in the testing laboratories, including investigation of possible interfering peaks for different matrices. The methodology should support detection of all toxins that are likely to be relevant to public health.

83. At the current regulatory limit, the JRT could be used as a pre-screen to identify samples for quantitative testing, subject to appropriate quality control measures.

84. We agree that it would be appropriate to review this advice when information on the distribution of PSP toxins in UK shellfish becomes available from the more sensitive HPLC analyses.

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July 2006

Table 4. Summary of PSP epidemiology data

Cases	Reported intake of PSP toxins	Derived dose of PSP calculated as µg STX eq/kg bw*	Assumptions
Reports based on measurement of PSP toxins in cooked shellfish samples left over from meal.			
3 adult cases, 2 men and 1 woman[12]	Mild symptoms (male patient): 17,000 MU ingested Respiratory failure (female): 22,000 MU ingested Fatality (male): 42,000 MU ingested	Mild symptoms: 51 Respiratory failure: 66 Fatality: 126	Exact number of mussels eaten known by number of shells left. Cooked and raw shellfish analysed by MBA. MU converted to STX eq/kg bw using conversion factor of 0.18 µg STX eq per MU, and assuming a 60 kg bodyweight.
6 male cases.[13,3]	Consumption of 3-48 cooked mussels containing 4280 µg STX eq/100 g.	9-137	Assuming an edible mass of 4 g per mussel, and a bodyweight of 60 kg. Method of analysis unspecified.
Reports based on measurement of toxins in shellfish samples, exact source of shellfish unspecified. Adjustment made for effects of cooking on toxin levels.			
49 cases, male and female, including a child of 2 years old. 82 individuals without symptoms[10]	Mild symptoms: 85-4128 µg STX eq/person Severe symptoms: 90-9000 µg STX eq/person Extreme symptoms: 390-7000 µg STX eq/person No symptoms: 50-2800 µg STX eq/person Illness in 2-year old: 96 µg STX eq/person	Mild symptoms: 1.4-69 Severe symptoms: 1.5-150 Extreme symptoms: 6.5-117 No symptoms: 0.8-47 Illness in 2-year old: 8	PSP intake calculated by combining information on species, size and number of shellfish consumed with data on toxicity (from MBA data) and meat yield of shellfish. Correction factor applied for effects of cooking. Estimated dose calculated assuming an adult bodyweight of 60 kg, and 12 kg for a 2-year old.
91 cases in Canada from 1944-1990, and the outbreak in Guatemala detailed below. Details on age and sex not provided.[14]	Mild symptoms: 0.7-70 µg STX eq/kg bw Severe symptoms: 1.5-150 µg STX eq/kg bw Extreme symptoms: 5.6-300 µg STX eq/kg bw No symptoms: 0.7-63 µg STX eq/kg bw		Assumptions included: edible portion sizes for various species; if number of shellfish consumed unknown literature values used; toxin levels corrected for effects of cooking. Adult bodyweights of 60 and 25 kg appear to have been assumed. Only cases judged to have adequate data included in the assessment. **NB**: only 2 cases reported where toxin doses <1.4 µg STX eq/kg bw, tolerable single intake of 1.4 µg STX eq/kg bw proposed
Reports based on measurement of toxins in shellfish samples collected from affected beach, restaurant or retailer. Testing performed on cooked samples or results for raw samples adjusted for effects of cooking			

Table 4. Summary of PSP epidemiology data (continued)

Cases	Reported intake of PSP toxins	Derived dose of PSP calculated as µg STX eq/kg bw*	Assumptions
Reports based on measurement of toxins in shellfish samples collected from affected beach, restaurant or retailer. Testing performed on cooked samples or results for raw samples adjusted for effects of cooking.			
71 patients, details of age and gender not provided.15 NB Only UK PSP outbreak identified.	Mild symptoms: 3000-20,000 MU per person Moderate symptoms: 3000-28,700 MU per person No symptoms: 3000-30,000 MU per person	Mild symptoms: 9-60 Moderate symptoms: 9-86 No symptoms: 9-90	Toxin concentrations determined by MBA analysis of samples supplied by the retailer who supplied majority of patients. Samples analysed when raw and cooked. Intake assessment appears to be based on patient interviews. MU converted to STX eq/kg bw using conversion factor of 0.18 µg STX eq per MU, and assuming a 60 kg bodyweight.
17 cases, 10 male, 7 female.[16]	500-58,500 MU per person	1.5-176	Toxin levels determined by MBA in shellfish collected from restaurants or affected coastal areas. Correction applied for effects of cooking. Intake assessment appears to be based on patient interviews. MU converted to STX eq/kg bw using conversion factor of 0.18 µg STX eq per MU, and assuming a 60 kg bodyweight.
Reports based on measurement of toxins in leftover cooked or raw shellfish samples, or shellfish collected from affected beach or restaurant. Some samples were collected after the incident. No reported adjustment for effects of cooking.			
Dose estimates provided for 33 ill people (mean age 38 yrs) and 10 non-ill people (mean age 36 yrs)[17]	Ill persons: 13-123,427 µg STX eq/person Respiratory arrest: 5863 and 123,427 µg STX eq/person Non-ill persons: 17-36,580 µg STX eq/person	Ill persons: 0.2-2058 Respiratory arrest: 98-2058 Non-ill persons: 0.3-610	Estimated PSP levels determined by MBA from either leftover shellfish or shellfish collected from affected beaches. Estimated dose calculated assuming an adult bodyweight of 60 kg. Authors acknowledged that toxin intakes might be miscalculated due to assumption that toxin levels in tested shellfish identical to those in ingested shellfish.
Four outbreaks involving 11 patients, 6 female, age ranging from 13-61 years. Dose estimates provided for 10 individuals.[4]	Median intake: 167 µg STX eq/kg bw (9176 µg STX eq) Respiratory arrest: 230-411 µg STX eq/kg bw Lowest dose causing illness: 21 µg STX eq/kg bw		Toxin levels in mussels determined by MBA from samples collected from affected beach within 24 hours of outbreak (3 outbreaks), or from left-over cooked and uncooked mussels (1outbreak). Conversion of median intake to dose, based on a 55 kg bodyweight; details not provided for other dose estimates.

Table 4. Summary of PSP epidemiology data (continued)

Cases	Reported intake of PSP toxins	Derived dose of PSP calculated as µg STX eq/kg bw*	Assumptions
Reports based on measurement of toxins in leftover cooked or raw shellfish samples, or shellfish collected from affected beach or restaurant. Some samples were collected after the incident. No reported adjustment for effects of cooking. (continued)			
Outbreak in Guatemala affecting 187 individuals; intakes estimated for 1 child and 4 adults, all of whom died.[18]	Fatality (adult): consumption of 30-85 g shellfish meat containing 7500 µg STX eq/100 g shellfish meat. Fatality (child): 140 MU/kg bw	Fatality (adult): 38-106 Fatality (child): 25	Intake of adults based on HPLC analysis of a clam sample; 60 kg bodyweight assumed. Intake in child based on MBA analysis of soup consumed. Weight of patient given as 25 kg. MU converted to STX eq using conversion factor of
0.18 µg STX eq per MU. 2 adult patients, 1 male and 1 female.[19]	>15,000 MU per person	>45	Unconsumed shellfish analysed by MBA (cooked or uncooked unspecified). Empty shells used to estimate shellfish consumption. MU converted to STX eq/kg bw using conversion factor of 0.18 µg STX eq per MU, and assuming a 60 kg bodyweight.
Toxin levels extrapolated from graph of levels measured in shellfish harvested from the affected area before and after the incident. Correction factor applied for effect of cooking.			
6 adult patients aged 27-69 yrs (2 male and 4 female) 1 female child aged 12 yrs. 2 adult fatalities.[20]	Fatalities and 1 surviving patient: Approximately 2400--5800 MU per person Other patients: Approximately 650-1000 MU per person	7-17 2-3	Estimated PSP levels determined by MBA in samples collected from the implicated beach on days preceding and following the day shellfish involved in incident were collected. Values graphed and toxin concentrations estimated by interpolation. Correction applied for effects of cooking. MU converted to STX eq/kg bw using conversion factor of 0.18 µg STX eq per MU, and assuming a 60 kg bodyweight.
Review data; source of estimate unspecified.			
Review data[21]	Lethal dose: 500-12,400 µg STX eq/person	8-207	No details provided on source of data. Estimated dose calculated assuming an adult bodyweight of 60 kg.
Australia New Zealand Food Authority[22]	Moderate symptoms: 120-180 µg STX eq per person 'May cause death': 400-1060 µg STX eq per person Fatal dose: 2000-10,000 µg STX eq per person	2-3 6.7-18 33-167	No reference given for estimate. Estimated dose calculated assuming an adult bodyweight of 60 kg.

*Derived PSP toxin doses (µg STX eq/kg bw) estimated by COT based on reported intake data, where data on doses was not included in the original paper.

Table 5. Performance characteristics reported for the MBA

Method	Specificity	Within lab precision	Between lab precision	HORRAT value	Recovery %	Standard of validation	LOQ (µg/100 g)	Reference materials available
Mouse Bioassay (MBA)	Used for detection of PSP toxins in shellfish	5-10% (RSD^r)*[30] 95% confidence interval for result of 77 µg STX eq/100g shellfish flesh reported as 65-94 µg++[10] Results ranging from 0-202 µg STX eq/100g observed in 9 mice injected with 140 µg STX eq/100g[31]	8-40% (RSD_R)*[30] 14-27 % (CV)+[32] Statistical evaluation not performed in a FAPAS interlab study[33] due to variable nature of results. (Results in 9 labs for samples spiked with 80 µg STX eq/100 g shellfish flesh ranged from 1-383 µg STX eq/100 g.) **NB** – MBA protocol not standardised between laboratories.	None reported	35-47**[30]	AOAC standardised method published[32]	LOD = 33-40 µg STX eq/100 g shellfish meat[24,34]	Assay standardised using STX dihydrochloride standard solution

Key:

RSD^r = relative standard deviation of repeatability (within laboratory variation)

RSD^R = relative standard deviation of reproducibility (between lab variation)

NB – Values in table have been rounded to whole numbers.

HORRAT value: HORRAT values for interlaboratory studies provide a measure of the acceptability of the reproducibility of a method. They compare the observed reproducibility (RSD_R) with a theoretical value calculated from the Horwitz equation, which was derived from observed reproducibility values from thousands of collaborative trials. Values below 2 are considered acceptable for between-laboratory precision (Horwitz, 1982).

* Based on a proficiency study involving eight French laboratories. PSP toxin levels were determined in four shellfish (oyster) samples; one control sample, one naturally contaminated with PSP toxins, and two samples spiked with low (152.8 µg STX/100 g shellfish meat) and moderate (334.7 µg STX/100 g shellfish meat) amounts of STX. Samples were analysed in duplicate. **NB** – Variation in between-laboratory results postulated to be due to variation in the toxin dilution factors selected by the different laboratories.

** Mean recovery reported by the eight laboratories involved in the above proficiency study for shellfish samples spiked with 152.8 and 334.7 µg STX/100 g shellfish meat.

\+ Coefficient of variation calculated for determination of PSP toxins in a collaborative study involving 11 laboratories. PSP levels were determined in 8 shellfish (clam) samples spiked with 0, 100, 400 or 800 µg purified PSP standard solution/100 g shellfish meat (2 samples per toxin concentration). Details were not provided on this standard solution used in the studies, STX.2HCl is used now.

++ 95% Confidence interval calculated from the statistical analysis of 120 MBAs performed with 18 shellfish extracts giving median times to death of 4.0-6.5 minutes.

Outline of method:

Shellfish samples (100 g) are extracted by boiling in 0.1M HCl (1:1; pH should be <4.0, preferably ca. 3.0) for 5 minutes, and adjusted to pH 2.0-4.0. Supernatant can be separated from solid particles by centrifugation or filtration. The principle is for three mice to be injected i.p with 1 ml of shellfish extract. Median time to death used to calculate toxin level. In practice fewer mice are used in some laboratories.

Table 6. Performance characteristics reported for HPLC[26]

Method	Specificity	Toxin	Mean (µg/kg)	Within lab precision (RSD_r [%])*	Between lab precision (RSD_R [%])*	HORRAT value*	Recovery (%)***	Standard of validation	LOQ (µg/100 g)	Reference materials available
HPLC	Applicable for determination of STX NEO GTX1, 4 GTX2, 3 DcSTX B1 C1, 2 C3, 4 in shellfish (Interlaboratory validation study performed in mussels, clams, oysters and scallops)	STX	Clams: 520 Mussel: 313 Oyster: 140	Clams: 6 Mussel: 22 Oyster: 18	Clams: 14 Mussel: 23 Oyster: 31	Clams: 0.78 Mussel: 1.23 Oyster: 1.46	74-93	Validated through collaborative trial to AOAC standard	2	STX NEO GTX1, 4 GTX2, 3 DcSTX B1 C1,2++
		NEO	Clams: 41 Mussel: 263	Clams: 19 Mussel: 26	Clams: 39 Mussel: 34	Clams: 1.78$ Mussel: 1.75	38-77		4	
		GTX1, 4	Clams: 74 Mussel: 660	Clams: 15 Mussel: 13	Clams: 26 Mussel: 20	Clams: 1.19$ Mussel: 1.20	67-79		5	
		GTX2, 3	Clams: 117 Mussel: 785 Oyster: 347	Clams: 14 Mussel: 17 Oyster: 19	Clams: 20 Mussel: 28 Oyster: 23	Clams: 0.90$ Mussel: 1.69 Oyster: 1.25	76-88		13+	
		DcSTX	Clams: 8	Clams: 9	Clams: 27	Clams: 1.22$	64-84		1	
		B1	Clams: 42 Mussel: 331 Oyster: 39	Clams: 13 Mussel: 8 Oyster: 8	Clams: 19 Mussel: 15 Oyster: 30	Clams: 0.85$ Mussel: 0.79 Oyster: 1.38$	76-86		3	
		C1,2	Clams: 241 Mussel: 101 Oyster: 169	Clams: 16 Mussel: 28 Oyster: 32	Clams: 22 Mussel: 38 Oyster: 54	Clams: 1.12 Mussel:1.75$ Oyster: 1.52	74-78		9	
		C3, 4	Mussel (spikeA): 725 Mussel (spikeB): 1425	N/A	Mussel (spikeA): 25 Mussel (spikeB): 21	Mussel (spikeA): 1.49 Mussel (spikeB): 1.40	79-81		73	

* PSP toxins determined in blind duplicate samples by 8-16 laboratories. **NB** – It should be noted that not all laboratories were able to detect all toxins at the limits in the test materials. These data were selectively excluded from the analysis.

** Based on determination in spiked mussel samples.

*** Recovery based on interlaboratory data for spiked mussel samples.

+ Lowest concentration tested.

++ Standard for C1,2 not currently available commercially.

RSD_r = relative standard deviation of repeatability (within laboratory variation)

RSD_R = relative standard deviation of reproducibility (between lab variation)

NB – Values in table have been rounded to whole numbers.

HORRAT value: HORRAT values for interlaboratory studies provide a measure of the acceptability of the reproducibility of a method. They compare the observed reproducibility (RSD_R) with a theoretical value calculated from the Horwitz equation, which was derived from observed reproducibility values from thousands of collaborative trials. Values below 2 are considered acceptable for between-laboratory precision[35].

NB: HORRAT values marked $ have been adjusted according to recent guidelines for concentrations below 120 µg/kg.

Outline of method:

Test portions are extracted by heating with acetic acid solution. Extracts are cleaned up using solid phase extraction (SPE) C18 cartridges. After periodate and peroxide oxidation, they are analysed by high performance liquid chromatography (HPLC) with fluorescence detection. Most toxins (STX, C1,2, B1, dcSTX and GTX2,3) can be quantified after simple SPE-C18 cleanup. Extracts containing the toxins NEO, GTX1,4, C3,4 and B2 must be further purified by using SPE-COOH cleanup/separation. This method is also suitable for shellfish samples extracted by the MBA HCl extraction protocol[36] (Jim Lawrence, personal communication).

NB: A standard is also available for dcGTX2,3; production of a dcNEO standard is in progress.

Table 7. Performance characteristics reported for the JRT

Method	Specificity	Within lab precision	Between lab precision	HORRAT value* #	Recovery %	Standard of validation	LOQ (µg/100 g)	Reference materials available
Jellett Rapid Test	Cross-reactivity to all known PSP toxins is claimed. Shown to detect PSP toxin profiles in UK shellfish. Cannot be used for individual toxins	3-9% CV*[37] 86% agreement for triplicate analyses**[2] (100% agreement for samples >80 µg STX eq/100 g shellfish meat[24])	No difference in results for 72 samples tested at two separate laboratories[37]	None reported	Not applicable to qualitative assay	Not internationally validated. Accepted by some competent authorities for screening out negative samples	Qualitative assay: LOD = approximately 40 µg STX eq/100 g shellfish meat. Sensitivity varies with toxin profile, but studies indicate able to detect all samples with toxin levels >80 µg STX eq/100 g shellfish meat	Routine use not specified, but those available for HPLC could be used if required

NB: Majority of data generated by manufacturer of kit

* Coefficient of variation in line intensity, determined by testing of shellfish extracts spiked with a STX standard during company QC procedures. Values indicate the coefficient of variation of line intensity over ten replicate tests, and the range indicates results across nine production lots.

** Percentage of sixty-four shellfish extracts giving identical responses when tested in triplicate[24].

NB – Values in table have been rounded to whole numbers.

Outline of method:

The HCl extraction method employed for MBA is also used for JRT. An alternative method for field use, using 10 g of shellfish tissue rather than the 100 g recommended by AOAC, has been developed and is awaiting validation.

The test works on the principle of lateral flow immunochromatography using a strip format. The assay uses a mixture of polyclonal antibodies raised against PSP toxins, and provides a qualitative (yes/no) indication of the presence of PSP toxins within a shellfish extract within 20 minutes.

References

1. Kao, C. Y. Paralytic shellfish poisoning. Falconer, I. R. Algal toxins in Seafood and Drinking Water. [4], 75-86. 1993. Academic press, New York.

2. FAO/IOC/WHO(2004). Ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs.FAO/IOC/WHO.

3. FAO/IOC/WHO. Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs – Background Document. *Oslo, Norway, Sept 26-30 2004.* 2004.

4. Gessner, B.D., Bell, P., Doucette, G.J., Moczydlowski, E., Poli, M.A., Van Dolah, F., Hall, S. (1997). Hypertension and identification of toxin in human urine and serum following a cluster of mussel-associated paralytic shellfish poisoning outbreaks. *Toxicon* 35: 711-22.

5. Garcia, C., del Carmen, B.M., Lagos, M., Lagos, N. (2004). Paralytic shellfish poisoning: post-mortem analysis of tissue and body fluid samples from human victims in the Patagonia fjords 14. *Toxicon* 43: 149-158.

6. Stafford, R.G. and Hines, H.B. (1995). Urinary elimination of saxitoxin after intravenous injection. *Toxicon* 33: 1501-1510.

7. Andrinolo, D., Michea, L.F., Lagos, N. (1999). Toxic effects, pharmacokinetics and clearance of saxitoxin, a component of paralytic shellfish poison (PSP), in cats. *Toxicon* 37: 447-464.

8. Harada, T., Oshima, Y., Yasumoto, T. (1984). Assessment of potential activation of gonyautoxin V in the stomach of mice and rats. *Toxicon* 22: 476-478.

9. Mons, M.N., Van Egmond, H.P., Speijers, G.J.A. (1998). Paralytic shellfish poisoning: a review. 388802005.

10. Prakash A, M. J. T. A. Paralytic shellfish poisoning in eastern Canada. *Fisheries Research board of Canada* 177. 1971.

11. Lehane, L. Paralytic Shellfish Poisoning: A review. *National Office of Animal and Plant Health, Agriculture, Fisheries and Forestry – Australia Canberra* , 1-56. 2000.

12. Meyer, K.F.(1953). Food poisoning (concluded). *N Engl J Med* 249: 843-852.

13. Sharifadzeh, K., Ridley, N., Waskiewicz, R., Luongo, P., Grasy, G. F., et al. Paralytic shellfish poisoning – Massachusetts and Alaska, 1990. *Morbidity and Mortality Weekly Report, Centers for Disease Control* 40, 157-161. 1991.

14. Kuiper-Goodman, T. T. Health hazard assessment of PSP in Canadian shellfish. *Health Canada.* 1991.

15. McCollum, J.P., Pearson, R.C., Ingham, H.R., Wood, P.C., Dewar, H.A. (1968). An epidemic of mussel poisoning in North-East England. *Lancet* 2: 767-770.

16. Popkiss, M.E., Horstman, D.A., Harpur, D. (1979). Paralytic shellfish poisoning. A report of 17 cases in Cape Town. *S Afr Med J* 55: 1017-1023.

17. Gessner, B.D. and Middaugh, J.P. (1995). Paralytic shellfish poisoning in Alaska: a 20-year retrospective analysis. *Am J Epidemiol* 141: 766-770.

18. Rodrigue, B.C., Etzel, R.A., Hall, S., De Porras, E., Vaelasquez, O.H., V, T.R., Kilbourne, E.M., Blake, P.A. (1990). Lethal paralytic shellfish poisoning in Guatemala. *American Journal of Tropical Medicine and Hygeine* 42: 267-271.

19. Seven, M.(1958). Mussel poisoning. *Ann Intern Med* 48: 891-897.

20. Tennant, A.D., Naubert, J., Corbeil, H. (1955). An outbreak of paralytic shellfish poisoning. *Can Med Assoc J* 72: 436-439.

21. Krogh, P.(1983). Algal toxin in seafood and drinking water. *Chemistry international* 5: 45-48.

22. ANZFA. Shellfish toxins in food. A toxicological review and risk assessment. *Technical report series no. 14, Australia New Zealand Food Authority*. 2001.

23. Henderson L, Gregory J, Swan G. National diet and nutrition survey: adults aged 19-64 years. *TSO* Volume 1: types and quantities of foods consumed. 2002.

24. Mackintosh, F.H., Gallacher, S., Shanks, A.M., Smith, E.A. (2002). Assessment of MIST Alert, a commercial qualitative assay for detection of paralytic shellfish poisoning toxins in bivalve molluscs. *J AOAC Int* 85: 632-641.

25. Lawrence, J.F. and Niedzwiadek, B. (2001). Quantitative determination of paralytic shellfish poisoning toxins in shellfish by using prechromatographic oxidation and liquid chromatography with fluorescence detection. *J AOAC Int* 84: 1099-1108.

26. Lawrence, J.F., Niedzwiadek, B., Menard, C. (2004). Quantitative determination of paralytic shellfish poisoning toxins in shellfish using prechromatographic oxidation and liquid chromatography with fluorescence detection: interlaboratory study. *J AOAC Int* 87: 83-100.

27. AOAC. AOAC Official Method 2005.06. Paralytic shellfish poisoning toxins in shellfish: Prechromatographic oxidation and liquid chromatography with fluorescence detection. First Action 2005. *J AOAC Int* 88, 1714. 2005.

28. Smith, E. A., Stobo, L., Lacaze, J-P. Determination of paralytic shellfish poisoning (PSP) toxins in shellfish using prechromatographic oxidation and liquid chromatography with fluorescence detection: Analysis of shellfish extracts from the UK Jellett Rapid Test trial. *Project no.S14001 funded by the Food Standards Agency Scotland*. 2005.

29. Jellett, J. F. and et al. International validation data for Jellett Rapid Test for PSP. 2003.

30. Ledoux, M. and Hall, S. Proficiency testing of eight French laboratories in using the AOAC mouse bioassay for paralytic shellfish poisoning: Interlaboratory collaborative study. *Journal of the AOAC* 83[2], 305-310. 2000.

31. Holtrop, G., Petrie, J., McElhiney, J., Dennison, N. (2006). Can general anaesthesia be used for the Paralytic Shellfish Poison bioassay? *Toxicon* 47: 336-347.

32. McFarren, e. f. Report on collaborative studies of the bioassay for paralytic shellfish poison. *Journal of the AOAC* 42, 263-271. 1959.

33. FAPAS. Marine toxins pilot study report. *FAPAS, Central Science Laboratory, York YO41 1LZ*. 2003.

34. Usleber, E., Donald, M., Straka, M., Martlbauer, E. (1997). Comparison of enzyme immunoassay and mouse bioassay for determining paralytic shellfish poisoning toxins in shellfish. *Food Addit Contam* 14: 193-198.

35. Horwitz W. Evaluation of Analytical Methods used for Regulation of Foods and Drugs. *Anal Chem* 54, 67A-76. 1982.

36. Lawrence, J.F. and Niedzwiadek, B. (2001). Quantitative determination of paralytic shellfish poisoning toxins in shellfish by using prechromatographic oxidation and liquid chromatography with fluorescence detection. *J AOAC Int* 84: 1099-1108.

37. Jellett, J.F., Roberts, R.L., Laycock, M.V., Quilliam, M.A., Barrett, R.E. (2002). Detection of paralytic shellfish poisoning (PSP) toxins in shellfish tissue using MIST Alert, a new rapid test, in parallel with the regulatory AOAC mouse bioassay. *Toxicon* 40: 1407-1425.

38. Vale, P. and Taleb, H. (2005). Assessment of the quantitative determination of paralytic shellfish poisoning toxins by pre-column derivatization and elimination of interfering compounds by solid-phase extraction. *Food Addit Contam* 22: 838-846.

39. Oshiro, M., Pham, L., Csuti, D., Dodd, M., Itiami G.B., Brenden, R. A. Paralytic shellfish poisoning surveillance in California using the Jellett Rapid PSP test. *Harmful Algae* 5, 69-73. 2006.

40. Inami, G.B., Crandall, C., Csuti, D., Oshiro, M., Brenden, R.A. (2004). Feasibility of reduction in use of the mouse bioassay: presence/absence screening for saxitoxin in frozen acidified mussel and oyster extracts from the coast of California with *in vitro* methods 9. *J AOAC Int* 87: 1133-1142.

41. Community Reference Laboratory on Marine Biotoxins(2005). Report on the EU-NRLs 2005 Interlaboratory Interlaboratory Exercise on Paralytic Shellfish Poisoning Toxins Determination.

42. Oshima, Y. Post-column derivatization high performance liquid chromatography method for the analysis of PSP. *J AOAC Int* 78, 795-799. 1995.

43. Asp, T.N., Larsen, S., Aune, T. (2004). Analysis of PSP toxins in Norwegian mussels by a post-column derivatization HPLC method. *Toxicon* 43: 319-327.

44. Schantz, E. J., McFarren, e. f., Schafer M.L., Lewis K.H. Purified shellfish poison for bioassay standardization. *J Assoc Off Anal Chem* 41[1], 160-168. 1958.

45. Park, D.L., Adams, W.N., Graham, S.L., Jackson, R.C. (1986). Variability of mouse bioassay for determination of paralytic shellfish poisoning toxins 1. *J Assoc Off Anal Chem* 69: 547-550.

Statement on risk assessment of marine biotoxins of the okadaic acid, pectenotoxin, azaspiracid and yessotoxin groups in support of human health

Introduction

1. A number of marine phytoplankton produce biotoxins that can be bioconcentrated by shellfish. Consumption of shellfish sufficiently contaminated with these toxins can result in human illness. Marine biotoxins have previously been categorised on the basis of clinical signs, but are increasingly being categorised by chemical structure. The structural toxin groups that are generally considered to be of relevance to shellfish harvested in European waters are:

   - Domoic acid group (DA)
   - Saxitoxin group (STX)
   - Okadaic acid group (OA)
   - Pectenotoxin group (PTX)
   - Azaspiracid group (AZA)
   - Yessotoxin group (YTX)
   - Cyclic imine group

2. Marine biotoxins can also be categorised according to their water solubility which determines the extraction protocol required for analysis. The DA and STX groups are hydrophilic, while the OA, PTX, AZA, YTX and cyclic imine groups are all lipophilic.

3. The DA group is associated with amnesic shellfish poisoning (ASP), the STX group with Paralytic Shellfish Poisoning (PSP) and the OA group with Diarrhetic Shellfish Poisoning (DSP).

4. The Committee was asked for its views on the risk assessment of biotoxins of the STX, OA, AZA, PTX and YTX groups in order to support protection of consumer health. A statement on STXs was published in September 2006[1]. The present statement addresses the OA, PTX, AZA and YTX groups.

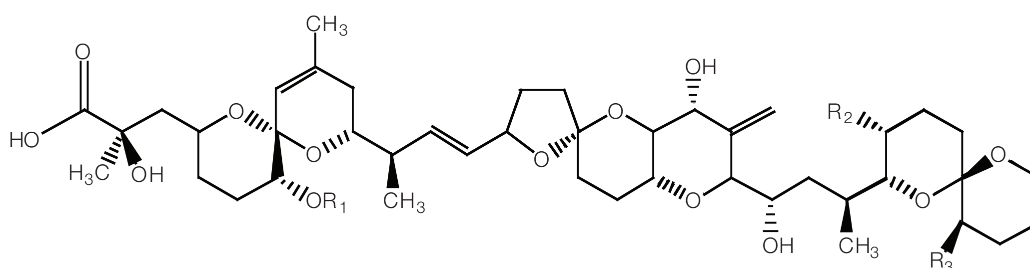
Okadaic Acid group

Background

5. Toxins from the okadaic acid (OA) group are known to cause Diarrhetic Shellfish Poisoning (DSP), a gastrointestinal illness that was first identified in the late 1970s[2]. The effects of OA toxins are considered to be a result of the ability of these compounds to inhibit the activity of the protein phosphatases 1 and 2A[3,4].

6. The OA group comprises OA and its analogues dinophysistoxin (DTX)-1, DTX-2 and DTX-3. 'DTX-3' originally referred to a group of 7-O-acyl derivatives of DTX-1. More recently, however, it has been demonstrated that OA and DTX-2 can also be acylated to give 'DTX-3' compounds[3]. The structures of these compounds are shown in Figure 1. These esters are considered to be relatively unstable and are expected to be hydrolysed in the body to give the free toxins[5,6]. Alkaline hydrolysis of ester forms to the free toxins is required for detection by methods other than *in vivo* assays.

Figure 1. Chemical structures of OA group toxins[7]



Toxin	R_1	R_2	R_3
OA	H	CH_3	H
DTX-1	H	CH_3	CH_3
DTX-2	H	H	CH_3
DTX-3	Acyl	CH_3	CH_3

7. In Japan, DSP outbreaks have mainly been associated with DTX-1, while OA has been more frequently associated with DSP incidents in Europe. DTX-2 has previously been reported to be the predominant diarrhetic shellfish toxin in Ireland[8]. A number of outbreaks associated with DTX-3 toxins have recently been reported in Chile, Norway and Portugal[6,5,9].

8. Current legislation sets out a maximum permitted level for OA toxins together with PTXs of 16 μg OA equivalents (eq)/100 g shellfish meat, although there have been recent proposals to regulate PTXs separately. Mouse bioassays (MBAs), involving intraperitoneal (i.p.) injection of shellfish extract, are prescribed in EU legislation as the reference methods for the detection of these toxins. The regulatory MBA provides a positive/negative result rather than quantitation of toxin concentration, and a positive result is considered to indicate the presence of OA toxins and/or PTXs above the regulatory limit.

Previous COT evaluations

9. The COT previously considered DSP toxins in 1994, when it reviewed a Ministry of Agriculture, Fisheries and Food (MAFF) food surveillance paper on Naturally Occurring Toxicants in Food[10]. The COT recommended:

    - That the surveillance programme for detecting shellfish contaminated with DSP toxins as described in the surveillance paper be continued
    - That exposure to OA should be kept below those concentrations which cause toxicity as any tumour promoter activity would also then be minimised
    - That the mouse bioassay be used for the detection of DSP in shellfish at present but that efforts be made to develop a more quantitative assay

10. Research into alternative methods is ongoing, but a method considered appropriate for use in the UK shellfish monitoring programme for lipophilic biotoxins (incorporating OA toxins, PTXs, YTXs and AZAs) is not yet available. Progress has been limited by a lack of analytical standards for many toxins and problems in achieving the required sensitivity and specificity. However, it is hoped that an appropriate method will become available within a couple of years.

Toxicology

Toxicokinetics

11. Research indicates that OA is widely distributed following oral administration. In Swiss mice, OA was detected in intestinal content (36.3% of given dose) > urine (11.6%) > skin (8.3%) > faeces (6.6%) > blood (4.3%) > muscle (3%) > intestinal tissue (2.6%) > liver and gallbladder > stomach > kidney > brain > lung > spleen > heart (all ≤ 1.0%) 24 hours after administration of a non-diarrhetic dose (50 µg/kg bw)[11]. The distribution was similar in animals given a dose that did cause diarrhoea (90 µg/kg bw), although the OA content was significantly decreased in the stomach and significantly increased in the intestinal tissue and contents compared with animals given 50 µg/kg. The authors suggested that OA largely underwent enterohepatic circulation, which they also observed following intramuscular injection[12].

12. In a later study, OA was detected in the lungs, liver, heart, kidney, stomach and small and large intestines of male ICR mice within 5 minutes of administration of 150 µg OA/kg bw by oral gavage[13]. The site of absorption was reported to be the jejunum. OA continued to be detected in the heart, lung, liver, kidney and blood vessels for 2 weeks following administration. Excretion in urine and from the cecum and large intestine started 5 minutes after administration, and continued via the intestinal contents for 4 weeks.

13. OA has been shown to cross the placental barrier to the fetus following oral administration to pregnant Swiss-Webster mice on day 11 of gestation[14].

14. In humans, faecal samples collected from individuals who developed DSP symptoms following consumption of shellfish contaminated with DTX-3 were found to contain DTX-1[5]. No DTX-3 was detected in the faecal samples, indicating complete transformation into DTX-1 within the body. This transformation was hypothesised to have taken place in the stomach.

Acute toxicity

15. The lethal dose of OA, DTX-1 and DTX-3 following intraperitoneal (i.p.) injection in mice is reported to be 200, 160 and ca. 500 μg/kg bw, respectively[15]. An i.p. lethal dose of 250 μg/kg bw has also been reported for DTX-3[16]. A recent study reported an LD_{50} for DTX-2 of approximately 350 μg/kg bw[17].

16. Estimates of the oral lethality of OA in mice vary considerably. Ito *et al.* reported a lethal oral dose of 400 μg/kg bw[13], while in another study no animals died following oral administration at 1,000 μg/kg bw and 4/5 died after administration of 2,000 μg/kg bw[18]. In a further study inconsistent results were observed, with mortality occurring in all mice given oral doses of 770 μg/kg bw and higher and in animals given 575 μg/kg bw, but not in those administered 610 μg/kg bw or doses of 525 μg/kg bw or lower[19].

17. Following oral administration of DTX-1 to male ddY mice at doses of 100, 200, 300 and 400 μg/kg bw, 1/5, 0/5, 2/4 and 3/4 animals died, respectively[20]. At the higher doses, mice died within 6 hours of dosing, while survival time was 30 hours at 100 μg/kg bw.

18. Diarrhetic effects of OA, DTX-1 and DTX-3 have been reported in several studies with suckling mice [20,21,22]. Intestinal fluid induction has also been reported following administration of single oral doses of 75 μg OA/kg bw and above to 4-week old mice[13], and in adult mice following an oral dose of 90, but not 50 μg OA/kg bw[11]. OA, DTX-1 and DTX-3 have all been found to induce severe diarrhoea in ICR mice and Wistar rats following oral administration at 750 μg/kg bw[23]. Research presented at a recent conference reported a lowest observed adverse effect level (LOAEL) and no observed adverse effect level (NOAEL) for intestinal fluid accumulation by OA following oral administration of 75 and 50 μg/kg bw respectively[24]. In rats, a LOAEL of 400 or 200 μg/kg bw was reported, depending on the vehicle used (saline or triolein-oil, respectively).

19. Damage to the absorptive epithelium of small intestinal villi has been reported in several studies following oral treatment of mice and rats with OA, DTX-1 and DTX-3 [23,25]. All toxins were reported to induce intestinal injury at 750 μg/kg bw, while it was also noted that the minimum dose of DTX-3 that induced collapse of the villous architecture in mice was 150 μg/kg bw. OA and DTX-1 also caused intestinal injury following i.p. administration (≥200 and 375 μg/kg bw, respectively), but DTX-3 only induced significant injury by the oral route. Damage was almost completely repaired within 48 hours. DTX-1 has also been shown to induce mucosal injuries in the small intestine within 1 hour of i.p. administration at doses ranging from 50-500 μg/kg bw[26]. No discernible changes in organs and tissues other than the intestine were observed in this study.

20. Terao *et al.*[23] also examined the effects of i.p. (375 μg/kg bw) and oral (750 μg/kg bw) administration of OA, DTX-1 and DTX-3 to mice and rats on the liver. Adverse effects were observed following administration of DTX-3 by the oral and i.p. route, whereas OA and DTX-1 only induced damage when given intraperitoneally.

21. In a further study, injuries were reported in the lung, stomach, small and large intestines and cecum, but not the liver, of male ICR mice following oral administration of 150 μg OA/kg bw[13]. Degenerative lesions to the small intestine, forestomach and liver have been reported in CD-1 mice following oral administration of OA at doses of 1,000 and 2,000 μg/kg bw, while slight splenic atrophy was also observed at the higher dose[18]. In a recent study, apoptosis was detected in the liver, ileum and kidney of Swiss mice at various time points between 24 and 48 hours after oral administration of 115 and 230 μg OA/kg bw[19].

22. Atrophy and structural alteration of the thymus has been reported in mice 24 hours following consumption of shellfish tissue contaminated with OA or OA and YTX over a 24 hour period (estimated intakes of approximately 18 μg OA and 1.4 μg YTX/kg bw)[27]. Histopathological changes were also observed in the spleen immediately following consumption of contaminated shellfish, but these effects were less marked 24 hours following consumption.

23. A summary of acute toxicity studies is included in table 1.

Mechanism of action

24. OA is a potent inhibitor of the serine-threonine protein phosphatases (PP) 1 and 2A, and the adverse effects of OA group toxins are considered to be mediated by this activity. It has been proposed that OA may induce diarrhoea by stimulating the phosphorylation of proteins that control sodium secretion by intestinal cells, or by enhancing phosphorylation of cytoskeletal or junctional elements resulting in increased permeability to solutes, leading to passive loss of fluids[28].

Table 1. Acute toxicity studies with OA group toxins

Toxin	Species	Parameter	Route of administration	Dose (μg/kg bw)	NOAEL (μg/kg bw)	Comments
OA	Mouse	Lethality	i.p.	200[15]	ND	
OA	Mouse	Lethality	Oral	400-2000[13,18,19]	ND	Varying estimates reported
OA	Mouse	Diarrhoea	Oral	75-750[11,13,23,24]	5024	
OA	Rat	Diarrhoea	Oral	200-400[24]	ND	LOAEL varied depending on vehicle of administration
OA	Mouse	Intestinal injury	i.p.	200[25]	ND	
OA	Rat	Intestinal injury	i.p.	375[23]	ND	
OA	Mouse	Intestinal injury	Oral	150-2000[13,23,25,18]	ND	
OA	Rat	Intestinal injury	Oral	750[23]	ND	
OA	Mouse + Rat	Liver injury	i.p.	375[23]		
OA	Mouse	Liver injury	Oral	115-2000[19,18]	750 (mouse + rat)[23] Apoptosis:	115-1300 Degenerative lesions: 1000-2000
DTX-1	Mouse	Lethality	i.p.	160[15]	ND	
DTX-1	Mouse	Lethality	Oral	100-400[20]	ND	
DTX-1	Mouse	Diarrhoea	Oral	750[23]	ND	
DTX-1	Mouse	Intestinal injury	i.p.	50-500[26,23,25]	ND	Injury observed in mice and rats at 350 μg/kg bw
DTX-1	Mouse + Rat	Intestinal injury	Oral	750[23,25]	ND	
DTX-1	Mouse + Rat	Liver injury	i.p.	375[23]	ND	
DTX-1	Mouse + Rat	Liver injury	Oral		750[23]	
DTX-2	Mouse	Lethality (LD_{50})	i.p.	350[17]	ND	
DTX-3	Mouse	Lethality	i.p.	250-500[15,16]	ND	
DTX-3	Mouse	Diarrhoea	Oral	750[23]	ND	
DTX-3	Mouse + Rat	Intestinal injury	i.p.	375[23,25]	ND	Only minor injury observed with i.p. administration
DTX-3	Mouse	Intestinal injury	Oral	150[25]	ND	
DTX-3	Rat	Intestinal injury	Oral	750[23]	ND	
DTX-3	Mouse + Rat	Liver injury	i.p.	375[23]	ND	
DTX-3	Mouse + Rat	Liver injury	Oral	750[23]	ND	

ND: Not determine

Genotoxicity

25. OA did not induce mutations in the *Salmonella typhimurium* strains TA100 and TA98 in the presence or absence of metabolic activation, but was mutagenic in Chinese hamster lung cells without activation, using diphtheria toxin resistance as a marker[29].

26. OA has been reported to induce DNA adducts with no clear concentration-response relationship using the ^{32}P-postlabelling technique in BHK21 C13 fibroblasts and HESV keratinocytes[30]. OA was negative in the Chinese hamster ovary cell hprt mutation assay conducted to OECD guidelines (with and without metabolic activation) and an *in vitro* unscheduled DNA synthesis (UDS) assay in rat hepatocytes[31].

27. Using the cytokinesis-block micronucleus assay coupled to fluorescence in situ hybridization (FISH), OA has been shown to induce aneuploidy in CHO-K1 cells in the presence and absence of rat liver S9[32,33,34]. The authors suggested that this effect was due to inhibition of chromosome attachment to the mitotic spindle, possibly related to the effects of OA on protein phosphatases. Induction of micronuclei by OA has been reported in Caco-2 human colon cells at doses sufficient to induce apoptosis, but this was not confirmed in colon epithelial cells of mice given OA by oral gavage[19].

28. Overall, the data show some evidence for genotoxicity *in vitro* in non-standard assays, including evidence for DNA adduct formation in mammalian cell lines which are difficult to interpret, and thus it is noted some effects may be related to the toxicity of OA in the *in vitro* assays. There is evidence for aneugenicity *in vitro* in a mammalian cell line which is unlikely to be related to a direct effect of OA on DNA. Standard bacterial reversion, mammalian gene mutation assays and a UDS assay in rat hepatocytes were negative. The *in vivo* relevance of the positive *in vitro* findings is unclear and has not been investigated.

Tumour promoting activity

29. OA and DTX-1 have been shown to act as tumour promoters on mouse skin initiated with 7,12-dimethylbenz[a]anthracene (DMBA)[35,36]. In these studies 100 μg DMBA was applied once to mouse skin, followed by twice weekly application of OA (5 and 10 μg) or DTX-1 (5 μg) for 30 weeks. Administration of OA via drinking water has been found to promote tumour formation in the glandular stomach of rats initiated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in the drinking water for the first 8 weeks of the study (OA administered at 10 μg/rat/day on weeks 9-55 followed by 20 μg/rat/day on weeks 56-72)[37]. In this study, the percentage of rats treated with MNNG and OA, MNNG alone or OA alone bearing neoplastic changes was 75, 46 and 0%, respectively.

30. The tumour promoting activity of OA and DTX-1 is considered to be mediated by inhibition of the protein phosphatases (PP)1 and PP2A, which results in increased protein phosphorylation leading to alterations in gene expression. OA and DTX-1 have been found to induce ornithine decarboxylase activity in mouse skin, with OA also inducing this enzyme in the rat glandular stomach[35,36,37]. In addition, OA has been shown to induce tumour necrosis factor (TNF)-α gene expression in mouse skin[38] and synthesis and secretion of the murine cytokine CXCl1/KC, a promoter of tumour growth, in JB6 cells[39].

31. No data are available on reproductive toxicity or developmental toxicity.

Human data

32. DSP incidents have been reported in many countries around the world, including Japan[40], the Netherlands[41,42], Norway[43,6], Sweden[44], Belgium[45], Portugal[46,47], the UK[48], Canada[49], Chile[50,5] and New Zealand[51].

33. The predominant symptoms of DSP are diarrhoea, nausea, vomiting and abdominal pain. Symptoms are generally reported to occur between 30 minutes and a few hours following shellfish consumption, with patients recovering within 2-3 days.

Epidemiology data

34. Information provided in the majority of reports of DSP outbreaks is very limited. Many do not provide information on the amount of contaminated shellfish consumed by affected individuals, and where exposure assessments are reported, little information is given on how these estimates have been derived. There is also a potential for uncertainty due to disparities in toxin levels in tested shellfish compared with levels present in shellfish that were actually consumed. Limited information is available on the effects of cooking on levels of OA toxins in shellfish, however it is generally considered that cooking does not reduce the levels of these toxins in shellfish due to their chemical stability and lipophilicity[52]. Although many incidents of DSP have been reported globally, this paper focuses predominantly on reports where assessment of toxin intake has been conducted.

35. In several older human case reports, toxin levels in contaminated shellfish have been determined by mouse bioassay (MBA), involving i.p. injection in mice. MBA results are generally reported as either mouse units (MU) or OA equivalents (eq). For OA and DTXs, one MU was defined in these reports as either the minimum amount of toxin required to kill two of three 20 g mice within 24 hours following i.p. injection, or as the amount of toxin required to kill one mouse within 24 hours of i.p. injection. One MU is reported as corresponding to approximately 4 μg OA and 3.2 μg DTX-1, based upon the lethal dose of these toxins following i.p. injection in mice and assuming a 20 g mouse (200 and 160 μg/kg bw, respectively)[53,54]. For DTX-3, 1 MU has been reported as 5 μg, based upon the reported i.p. toxicity in mice of 250 μg/kg bw[55,16].

36. In June and July of 1976 and 1977, a total of 164 individuals in Japan were reported to have developed diarrhoea, nausea, vomiting and abdominal pain following consumption of mussels or scallops[40]. Symptoms occurred between 30 minutes and a few hours following shellfish consumption, with time to onset rarely exceeding 12 hours. Mussels implicated in these incidents were found to contain a toxin, at that time unidentified, that killed mice following i.p. injection.

37. To assess the levels of toxin associated with human illness, three leftover mussel specimens from meals eaten by eight individuals who became ill in 1977 were tested by MBA. Toxin levels were quantified as MU/hepatopancreas, with a definition of 1 MU being the amount of toxin required to kill a mouse in 24 hours following i.p. administration. Details of shellfish consumption, severity of illness and toxin levels within the consumed shellfish are summarised in Table 2.

Table 2. Comparison of human illness with toxin intake[40]

Individual (Age, Sex)	No. Mussels eaten	Toxicity (MU)		Symptom severity
		per g hepatopancreas	Total consumed*	
A (40, F)	3	5.0	12	Mild
B (15, M)	3	5.0	12	Mild
C (45, M)	5	5.0	20	Severe
D (10, M)	5	5.0	20	Severe
E (56, M)	10	5.0	40	Severe
F (52, F)	5	8.5	35	Severe
G (53, M)	10	8.5	70	Severe
H (68, M)	6	4.0	19	Severe

* Toxin intake estimated by study authors based upon an average weight of 0.8 g hepatopancreas per mussel

38. Mild symptoms were experienced by two of the affected individuals; nausea and mild diarrhoea was reported by a female aged 40 years while a male aged 15 years vomited but did not suffer from diarrhoea. The two individuals who experienced mild symptoms had both consumed 3 mussels containing 5 MU of toxin/g hepatopancreas. Assuming an average hepatopancreas weight of 0.8 g/mussel, the authors concluded that 12 MU of toxin was sufficient to induce mild illness in humans. This would correspond to an estimated 48 μg OA or 38 μg DTX-1, assuming that one MU is equivalent to 4 μg OA or 3.2 μg DTX-1. The other six patients, aged between 10 and 68 years, developed severe symptoms following an estimated intake ranging from 19-70 MU per person (corresponding to 76-280 μg OA or 60-224 μg DTX-1). The toxin involved in this incident was subsequently identified as DTX-1[56].

39. In October 1984, cases of gastrointestinal illness developing after consumption of mussels were reported simultaneously in Sweden and Norway[43]. In Norway, cases continued to be reported until April 1985. Mussels described as being 'associated with cases of intoxication' were collected and analysed by MBA. Further details on where these samples were obtained are not reported. In addition, fortnightly sampling took place at three localities on the south-east coast of Norway and south-east coast of Sweden from November 1984 – April 1985.

40. Mussels associated with DSP cases in Norway were found to be 'slightly to highly toxic'[43]. It was also noted that some samples contained 1.5-2 MU of toxin per g of hepatopancreas but it was not specified whether these were samples associated with illness or samples collected from Norway or Sweden in the months following the incident. The amount of mussel meat consumed by affected individuals was reported to range from approximately 30-200 g, and it was suggested that an intake of between 10-15 MU (equivalent to 40-60 μg OA) caused gastrointestinal symptoms[43]. In a separate report, samples involved in the Swedish DSP cases were reported to contain DSP toxins at a concentration greater than 17 MU/100 g shellfish flesh[44], corresponding to 68 μg OA or 53 μg DTX-1/100 g.

41. At an opening ceremony of a new mussel farm in Norway, the 77 guests were served dishes containing blue mussels, and many subsequently developed DSP symptoms[2]. In total, 72 individuals were interviewed by the local Food Control Authority the following day, 39 of whom reported nausea, vomiting, stomach pain, diarrhoea and headache. Analysis of leftover mussels by high performance liquid chromatograpy (HPLC) without hydrolysis of DTX-3 (OA esters) indicated that they contained 55-65 μg OAeq/100 g mussel meat. No precise information on the amount of mussels consumed was available, but a crude estimate of 1-1.5 μg OA eq/kg bw was suggested, based on general information about consumption of blue mussels among Norwegians[57].

42. Several DSP incidents have been reported in the UK. In 1994, two patients developed DSP symptoms 1-2 hours after eating imported mussels[58]. Symptoms persisted for up to 36 hours. Uneaten mussels were tested by MBA and high performance liquid chromatograpy (HPLC), and HPLC confirmed the presence of OA at a concentration of 2030 μg/100 g shellfish flesh. Both individuals were reported to have each consumed 10 mussels weighing approximately 200 g in total including shells[48]. Estimates of meat yields vary, but information provided to the Food Safety Authority of Ireland by Bantry Bay Foods Ltd suggests that in a kg of mussels there are 80-105 mussels that yield between 180 g and 240 g of cooked meat[59], i.e. a meat yield of 18-24%. McCance and Widdowson's 'The Composition of Foods' reports a 30% tissue yield for boiled mussels[60], while an FAO review gives an average edible tissue yield for raw mussels of 24%[61]. Assuming a tissue yield of 25% in the absence of specific data, the individuals may be estimated to have eaten 50 g of mussels providing an OA intake of 1015 μg per person. It is notable that the toxin intake in this incident was considerably higher than that in the other DSP case reports.

43. In June 1997, 49 patients presented with acute onset (within 30 minutes) of DSP symptoms which persisted for more than 8 hours[62]. All individuals had eaten UK-harvested mussels at one of two London restaurants. No pathogenic bacteria or viruses were detected in stool samples taken from some of the patients. HPLC analysis of mussel samples indicated the presence of OA, at concentrations ranging between 25.3 and 36.7 μg/100 g shellfish flesh[48]. No details on the amount of mussels consumed by the affected individuals were reported, although the authors noted that one patient who only developed diarrhoea that lasted for 8 hours had eaten mussel soup. They suggested that this meal may have contained less OA than other dishes.

44. Most recently, a DSP outbreak occurred in June 2006, involving approximately 159 individuals who ate mussels at a chain of restaurants in London. The majority of individuals became ill within 2-12 hours of eating mussels. A report from one restaurant indicates that 407, 242, 265, 239 and 297 mussel dishes were sold on the 17th, 18th, 19th, 20th and 21st of June, with 16 (4%), 25 (10%), 2 (1%), 4 (2%), and 25 (8%) people reporting DSP symptoms, respectively. Three samples obtained from the supplier that had been harvested on 14, 15 and 19 June and served in the restaurants were tested for the presence of norovirus and DSP toxins. Norovirus genogroups I and II were not detected in any sample.

45. The samples harvested on 15 and 19 June tested positive by MBA (indicating the presence of DSP toxins at a concentration >16 μg/100 g shellfish flesh), while the sample collected on 14 June was negative. However, further analysis of the samples by liquid chromatography-mass spectrometry (LC-MS) indicated that all three samples contained OA and OA esters ('DTX-3'), while one sample also contained DTX-1 and DTX-1 esters. The total concentration of OA or OA and DTX-1, following hydrolysis of the samples to convert the esters to their parent compound, was 25.8, 26.5 and 30.2 μg/100 g shellfish flesh in the samples harvested on 14, 15 and 19 June, respectively, i.e. about 70% above the regulatory limit of 16 μg/100 g. These samples were also found to contain PTXs. Analytical results for these samples are summarised in table 3.

Table 3. OA group and PTX concentrations in mussels associated with June 2006 DSP outbreak.

Mussel sample	Date sample collected	MBA+ result	LC-MS results (µg/100 g shellfish meat)			
			OA/DTX	PTX-2	PTX-2 seco acid	7-epi PTX-2 seco acid
BTX/2006/936	19.06.06	+ve	30.2*	43.3	25.6	3.1
BTX/2006/937	15.06.06	+ve	26.5**	51.3	40.3	6.4
BTX/2006/938	14.06.06	-ve	25.8**	30.2	35.4	4.7

+ MBA analysis performed with a 5h observation period rather than the 24h required by EU legislation.
* Concentration of OA and DTX-1 following hydrolysis of shellfish extract (no evidence of DTX-2 or DTX-2 esters).
** Concentration of OA following hydrolysis of shellfish extract (no evidence of DTX-1, DTX-2 or their esters).

46. Information on the exact amount of mussels consumed is not available, although it is known that mussels were served at the restaurants in portion sizes of 500 g and 1 kg including shells. Information from the suppliers of the mussels indicates that the meat yields of the affected batches were 28-30%. Assuming a meat yield of 29% and an OA/DTX concentration of 27.5 µg/100 g shellfish flesh (the average of the 3 values), it is possible that individuals who ate a 500 g portion of mussels may have consumed 145 g of mussels, providing a toxin intake of about 40 µg. Individuals consuming a 1 kg portion of mussels may have eaten 290 g of mussels, providing a toxin intake of about 80 µg. As noted above, these samples also contained PTXs and it is uncertain whether these toxins may have also contributed to illness.

47. In 1998, a DSP outbreak associated with OA esters (DTX-3) was reported in Portugal[46]. In total 18 individuals developed symptoms of DSP after eating *Donax* clams. The severity of symptoms was reported to be related to the amount of clams eaten, with those who ate little experiencing mild symptoms and those who ate 500 g presenting with the most serious symptoms. A sample of these clams was sent for analysis for bacteria and DSP toxins. *Salmonella spp.* were not detected in the clams, and HPLC analysis detected only low levels of OA (10 µg/100 g shellfish meat). However, following alkaline hydrolysis of the extract to release fatty acids from the esters, 130 µg OA/100 g shellfish meat was detected. Noting that the yield of edible tissue from *Donax* clams is approximately 18-20%, the authors calculated that individuals who consumed a 500 g portion would be expected to have eaten 90-100 g of tissue, suggesting a toxin intake of 117-130 µg OA eq per person.

48. A further DSP incident associated with OA esters occurred in Portugal in 2001[9]. Six individuals reported DSP symptoms after eating razor clams and clams that had been obtained locally. In total, 2 kg of razor clams and an unspecified amount of clams had been obtained by the affected individuals. Symptom severity appeared to be related to the amount of shellfish that had been eaten, and took 3 days to resolve in the most severe cases. LC-MS analysis of razor clams harvested the day after the affected individuals had become ill indicated the presence of OA at a concentration of 1 µg/100 g shellfish meat. Following hydrolysis of ester forms, 50 µg OA/100 g shellfish meat was detected.

49. Noting that the edible tissue yield of razor clams is 60%, giving a total edible mass of 1.2 kg from the 2 kg collected by the patients, the authors of the report hypothesised that individuals who reported eating ‘a lot’, ‘little’ or ‘very little’ may have eaten around 350 g, 150 g or 50 g, respectively. On this basis, it was estimated that the respective toxin intakes may have been 175, 75 or 25 µg OA eq per person.

Low levels of domoic acid (DA; 490 µg/100 g), the biotoxin associated with amnesic shellfish poisoning (ASP), were also detected in razor clams harvested commercially at the same period. Although mild symptoms of ASP are similar to those of DSP, it was noted that the level was substantially lower than the regulatory limit of 2000 µg/100 g shellfish flesh and that DA was unlikely to have contributed to the poisoning.

50. In the same month, an individual living in the region developed DSP symptoms after eating green crabs harvested locally. Symptoms started 2-3 hours following ingestion and persisted over 3 days. Leftover crabs from the meal were frozen and analysed by LC-MS 1.5 months later, and found to contain 32.2 µg OA equivalents following hydrolysis. It was estimated that around 30 crabs, consisting of approximately 140 g edible tissue, may have been consumed, which would correspond to an intake of around 45 µg OA eq. Very low levels of DA were detected in the cooked crab sample (40 µg/100 g), but were again not considered to have contributed to the poisoning. Noting the delay between sampling of the crab and analysis, and that OA esters are considered to be quite unstable, the authors suggested that the OA intake may have been underestimated in this case.

51. In 2002, several hundred people became ill after eating self-harvested brown crabs in southern Norway[6]. The symptoms were reported to be typical of DSP, although less severe and with a delayed onset. As no leftovers from any meals were available for analysis, fresh crabs from shallow waters in the same area as the original crabs had been harvested were collected and boiled prior to LC-MS analysis. Crabs move freely from area to area, and it is therefore uncertain how representative the crabs collected by the researchers are to those consumed by affected individuals. No DSP toxins were detected upon initial analysis, but analysis following alkaline hydrolysis indicated the presence of 29 µg OA/100 g and 2 µg DTX-2/100 g. Details of the amount of crabs eaten by affected individuals are not reported, but the report notes that a risk assessment was undertaken on behalf of the Norwegian Food Control Authority, resulting in the establishment of a temporary regulatory limit of 40 µg DTX-3 as OA eq/100 g brown meat in crabs. This limit was set on the basis of the analytical results and reports of illnesses available from the outbreak in 2002, indicating that 75-150 µg DTX-3 as OA eq per person would result in illness, and that an estimated average consumption of 2-3 crabs weighing 500 g at the level of 40 µg OA eq/100 g brown meat would give an intake of 28-42 µg DTX-3 as OA eq. Full details of this risk assessment have been submitted for publication, but are not currently available.

52. A summary of the estimated toxin intakes associated with DSP symptoms is provided in table 4.

53. In view of the tumour promoting effects of OA and DTX-1 in animal studies, researchers in France attempted to assess whether there may be a link between cancer risk and exposure to DSP toxins in humans[63]. Hypothesising that residual levels of OA may be present in shellfish harvested from beds recently re-opened following a contamination episode, the authors assessed mortality rates in coastal areas that had low, medium or high rates of harvesting bed closures for DSP toxin contamination and in areas where no closures had occurred. The authors considered their findings may suggest a possible association between living in areas with a high rate of closures and some digestive cancers, but acknowledged the large number of assumptions that had been made in the study. For example, consumption rates of locally harvested shellfish were not assessed, and it was not possible to confirm whether harvesting bed closure rates actually related to OA toxin exposure. Adjustment for confounding had also not been conducted, apart from for the presence of liver cirrhosis in men as a proxy for alcohol consumption.

Table 4. Summary of DSP epidemiology data

Cases	Reported concentration of OA group toxins in shellfish	Reported intake of OA group toxins	Derived dose calculated as µg OA eq/kg bw*	Assumptions
8 cases, 6 males and 2 females[40]	4-8.5 MU/g hepatopancreas	Mild symptoms: 12 MU/person Severe symptoms: 19-70 MU/person	Mild symptoms: 0.8 Severe symptoms: 1.3-4.7	Toxin intake calculated from reported number of mussels consumed and MBA result of toxin concentration in hepatopancreas of three leftover mussel samples. Average hepatopancreas weight of 0.8 g/mussel assumed. Estimated doses calculated assuming a bodyweight of 60 kg and that 1MU = 4 µg OA
Several hundred individuals[43]	Samples associated with cases of illness reported to be 'slightly to highly toxic' Samples of unspecified source contained 1.5-2 MU/g hepatopancreas	10-15 MU/person	0.7-1	Mussels associated with cases of illness analysed by MBA. Samples from an unspecified source contained 1.5-2 MU/g hepatopancreas. Amount of mussel meat consumed by affected individuals reported as 30-200 g. Value of 10-15 MU estimated by authors without precise basis for this reported. Estimated dose calculated assuming a bodyweight of 60 kg and that 1 MU = 4 µg OA
2 cases, 1 male + 1 female[48]	2030 µg OA per 100 g shellfish		17	Both reported to have consumed 10 mussels weighing 200 g. Estimated dose calculated assuming a body weight of 60kg and assumption of 25% edible tissue yield from mussels. Toxin concentration derived by LC-MS.
49 patients[48]	25.3-36.7 µg/100 g shellfish flesh			No details available on amount of shellfish consumed by affected individuals.
18 cases[46]	130 µg OA eq/100 g shellfish flesh	Severe symptoms: 117-130 µg OA eq/person Mild symptoms: 'ate little shellfish'	Severe symptoms: 1.95-2.2 Mild symptoms: unknown	Individuals who ate 500 g clams reported to have most severe symptoms. Those with mild symptoms reported to have eaten 'little'. Authors estimated edible tissue proportion of Donax clams as being 18-20% of whole shellfish), suggesting consumption of 90-100 g edible shellfish in those who ate a 500 g portion. OA esters reported to be present in leftover shellfish tissue (HPLC data). Estimated dose calculated assuming a body weight of 60kg.

Table 4. Summary of DSP epidemiology data (continued)

Cases	Reported concentration of OA group toxins in shellfish	Reported intake of OA group toxins	Derived dose calculated as μg OA eq/kg bw*	Assumptions
6 cases following consumption of razor clams; one case following consumption of crabs[9]	Razor clams: 50 μg OAeq/100 g shellfish flesh	Razor clams: Symptom severity of +++++: 175 μg OA eq/person Symptom severity of +++: 75 μg OA eq/person Symptom severity of +: 25 μg OA eq/person	Razor clams: Symptom severity of +++++: 2.9 Symptom severity of +++: 1.3 Symptom severity of +: 0.4	Razor clams Toxin concentration measured by LC-MS in razor clams collected day after individuals became sick. Shellfish contained OA and OA esters. Authors estimated individuals eating 'a lot', 'little' or 'very little' clams consumed 350, 150 or 50 g respectively. Estimated doses calculated assuming a body weight of 60kg.
	Crabs: 32.2 μg OAeq/100 g shellfish flesh	Crabs: 45 μg OA eq/person	Crabs: 0.75	Crabs: Toxins determined in leftover crabs that had been frozen for 1.5 months. OA esters present and authors suggest these toxins are unstable and some may have degraded during shellfish storage. Authors estimated individual may have eaten around 30 crabs containing around 140 g edible parts.
Several hundred individuals[6]		75-150 μg OA eq/person	1.25-2.5	Anecdotal mention in paper of risk assessment suggesting individuals became ill following an intake of 75-150 μg OA esters as OA eq/person. Risk assessment in press and currently unavailable. Estimated doses calculated assuming a body weight of 60 kg.
39 cases from 77 individuals served mussels[2,57]	55-65 μg OAeq/100 g shellfish flesh		1.0-1.5	Information on amount of mussels consumed unavailable, but dose estimated on basis of general information about blue mussel consumption by Norwegians.
159 individuals	25.8-30.2 μg OA or OA+DTX-1/100 g shellfish flesh		0.7 (500 g portion size) 1.3 (1 kg portion size)	Toxin concentration of mussels supplied to the restaurant determined by LC-MS. Amount of mussels consumed unknown although it is known that restaurant served 500 g and 1 kg portions. Yield of edible tissue reported as 28-30% of the affected batches, 29% used in estimation. Estimated dose calculated assuming a 60 kg bodyweight, and a toxin concentration of 27.5 μg/100 g shellfish meat, based on the average of the toxin concentration determined in 3 samples implicated in the incident.

*Derived okadaic acid group doses (μg OA eq/kg bw) estimated by COT based on reported intake data, where data on doses were not included in the original paper

Previous Risk Assessments

54. Three risk assessments of marine biotoxins, including those of the OA group, have been conducted by international bodies in recent years. Details of these assessments are summarised below.

55. A European Commission (EC) Working Group on Toxicology of DSP and AZA poisoning[52] based its risk assessment of OA group toxins on the data arising from the Japanese outbreak of 1976 and 1977 involving DTX-1 discussed in paragraphs 36-38 above[40]. The working group noted that 12 MU was the lowest observed adverse effect level (LOAEL) identified in this report, which would correspond to 48 μg OA eq, or 0.8 μg/kg bodyweight (bw) for a 60 kg individual. The working group applied a safety factor of 3 to this value to derive an allowance level of 0.27 μg/kg bw.

56. In 2004, a Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs was asked by the Codex Committee on Fish and Fishery Products (CCFFP) to perform risk assessments for a number of biotoxins that may be present in bivalve molluscs[64]. It was considered that the data from Japan indicated a LOAEL of 1.2-1.6 μg/kg bw, but no details were provided on what bodyweights were used to derive this value. The Expert Consultation also noted that in the outbreak in Norway described in paragraph 41[2] the affected individuals had an estimated toxin intake of 1.0-1.5 μg/kg bw.

57. FAO/IOC/WHO applied a safety factor of 3 to the LOAEL of 1.0 μg OA eq/kg bw to derive an acute reference dose (ARfD; i.e. the amount that can be ingested in a period of 24 hours or less without appreciable health risk) of 0.33 μg OA eq/kg bw. A safety factor of 3 was considered sufficient because of documentation of human cases including more than 40 persons and because DSP symptoms are readily reversible.

58. A further risk assessment was conducted by a Community Reference Laboratory on Marine Biotoxins Working Group on Toxicology in October 2005[65]. The Working Group's report is unreferenced, but notes that the LOAEL of OA in humans is 1 μg/kg bw. A safety factor of 3 was again applied to derive an ARfD of 0.33 μg OA eq/kg bw.

COT Evaluation

59. The Committee assessed the available epidemiology data on OA group toxins with a view to advising on an appropriate ARfD.

60. A large number of uncertainties were noted in the human data, including uncertainties in estimates of the amount of shellfish consumed by affected individuals, and potential disparities in toxin levels in tested shellfish compared with levels present in shellfish that were actually consumed. Reports generally provide only limited information on how exposure assessments have been calculated.

61. The COT considered that the totality of epidemiology data for OA toxins that was available prior to the recent UK DSP outbreak described in paragraphs 43-45 indicated a LOAEL of around 1 μg/kg bw. It was noted that the limited information from the 2006 UK incident may suggest a lower LOAEL of 0.7 μg/kg bw, based on a 60 kg bw. However, the data from this incident were difficult to interpret as the shellfish associated with the outbreak also contained biotoxins of the PTX group. It is uncertain whether the

presence of the PTX toxins may have contributed to illness. In addition, two shellfish portion sizes had been sold at the restaurants involved in the incident, and it was not known which had been eaten by individuals who became ill. Had all individuals eaten the larger portion size, a LOAEL of 1.3 µg/kg bw would be indicated, in line with the earlier epidemiology data.

62. Overall, the Committee considered that 1 µg/kg bw should be viewed as the most appropriate LOAEL for deriving an ARfD for OA toxins. However, the 2006 UK DSP outbreak indicated a reported response rate of up to 10%, suggesting that more than the most susceptible minority were affected at this dose, and therefore that an uncertainty factor of 3 would not be sufficient for extrapolation from a LOAEL to a NOAEL due to potential human variability in susceptibility to the effects of these toxins. It was agreed that an uncertainty factor of 10 should be applied, resulting in an ARfD of 0.1 µg OA eq/kg bw.

Pectenotoxins

Background

63. The presence of pectenotoxins (PTXs) in shellfish was first discovered due to their high acute toxicity in mice following i.p. administration of lipophilic shellfish extracts[3]. More than 12 PTXs have been identified to date, and the structures of some of these toxins are shown in Figure 2.

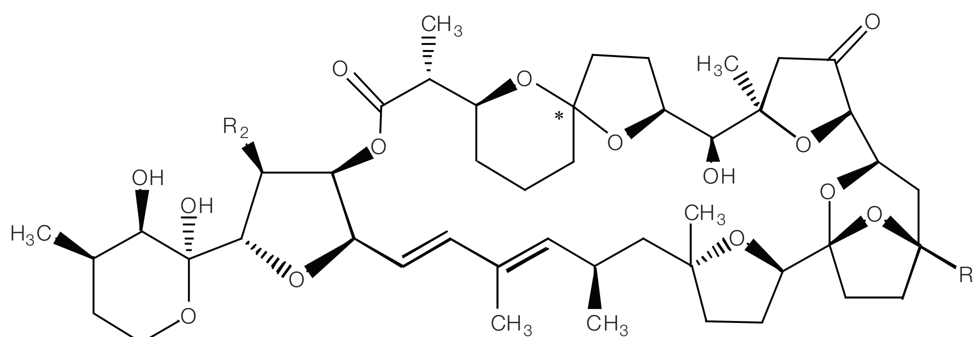
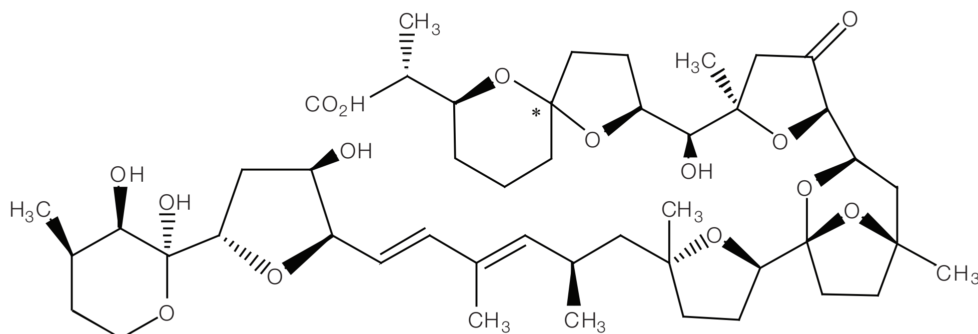


Figure 2. Structures of PTXs[7,66]

Toxin	R1	R2	*C-7
PTX-1	CH_2OH	H	*R*
PTX-2	CH_3	H	*R*
PTX-2b	CH_3	H	*S*
PTX-3	CHO	H	*R*
PTX-4	CH2OH	H	*S*
PTX-6	COOH	H	*R*
PTX-7	COOH	H	*S*
PTX-11	CH_3	OH	*R*
PTX-11b	CH_3	OH	*S*



Toxin	*C-7
PTX-2SA	*R*
7-epi-PTX2SA	*S*

64. PTXs exclusively arise from *Dinophysis spp.* which can also produce toxins from the OA group, and are therefore always accompanied by OA toxins[64]. This makes it difficult to assess the contribution of PTXs to human cases of DSP. It was previously suggested that PTX-2 seco acid (SA) and 7-epi-PTX-2 SA may have been responsible for outbreaks of human illness involving nausea, vomiting and diarrhoea in Australia in 1997 and 2000 following consumption of shellfish contaminated with these compounds[67]. However, the observed effects were later attributed to OA esters in the shellfish[64]. Mussels implicated in the recent DSP incident in the UK (paragraphs 43-45) also contained PTXs, and there is uncertainty as to whether these compounds may have contributed to the effects observed.

65. As noted in the OA group section, European legislation currently regulates for the presence of OA group toxins and PTXs together, but there have been recent proposals to regulate PTXs separately.

Toxicology

Toxicokinetics

66. The only data available on the toxicokinetics of PTXs are unpublished results from a PhD thesis summarised in the background document for the FAO/IOC/WHO evaluation of marine biotoxins[3]. Significant amounts of PTX-2 and PTX-2 SA were found in the gastrointestinal contents and faeces following oral administration, with only traces detected in tissue and urine. Following i.p. administration, PTX-2 and PTX-2 SA were detected in the blood and internal organs as well as in gastrointestinal contents and faeces. However, total recovery of toxins was reportedly low with both routes of administration.

Acute toxicity

67. Acute lethality data following i.p. administration of PTXs to mice are summarised in table 5. The minimum amount of PTX-2 reported to cause death in mice following p.o. administration is 25 μg/kg bw, with 1 of 4 mice dying[20]. However, the dose response in this study was unusual with no lethality observed in 4 mice given 100 μg/kg bw, while 1/5, 2/5, and 1/4 mice died following administration of 200, 300 and 400 μg/kg bw, respectively. In a subsequent study, no overt signs of toxicity were observed in mice following oral administration of PTX-2 or PTX-2 SA at doses up to 5000 μg/kg bw[68]. A recent study reported no signs of toxicity in mice given an oral dose of PTX-11 at 5000 μg/kg bw[66].

Table 5. Acute lethality of PTXs in mice following i.p. administration

Compound	Dose (μg/kg bw)
PTX-1	250[69]
PTX-2	230[69]
PTX-3	350[69]
PTX-4	770[69]
PTX-6	500[69]
PTX-7	>5000[70]
PTX-8	>5000[70]
PTX-9	>5000[70]
PTX-11	250[66]
PTX-2SA	No effects at 5000[71]
7-*epi*-PTX-2-SA	No effects at 5000[72]

68. Unlike OA group toxins, PTXs are not thought to inhibit protein phosphatases, and the potential for PTXs to induce diarrhoea has been a matter of some debate. In a study by Ishige *et al.* diarrhoea was observed in mice following oral administration of PTX-2 at doses of 1000 (1/5), 2000 (2/5) and 2500 (2/5) μg/kg bw, while at a lower dose of 250 μg/kg bw the small intestine was swollen and filled with fluid[52,73]. Vacuole formation was observed in the epithelial cells of the small intestine. In contrast, PTX-1 did not induce diarrhoea in suckling CD-1 mice following oral administration at doses up to 2 μg/mouse[21], or following i.p. administration to suckling BALB/c mice at doses from 150-1000 μg/kg bw, respectively[26]. In addition, no diarrhetic effects were observed in mice following oral administration of PTX-2 or PTX-2 SA at 5000 μg/kg bw[74], oral or i.p. dosing with PTX-11 at 5000 μg/kg bw or i.p. injection with PTX-2 SA[66] or 7-*epi*-PTX-2 SA at doses of 5000 μg/kg bw[72].

69. More recently however, a poster presented at the 12th International Conference on Harmful Algal Blooms reported that oral administration of PTX-2 resulted in intestinal fluid accumulation in mice at doses of 400 μg/kg bw and above[24]. Tissue damage was observed in the small intestine, characterised by vacuole formation in epithelial cells. No effects were observed at 300 μg/kg bw. Intestinal fluid accumulation was also observed in rats following administration of PTX-2 with a LOAEL of 300 or 400 μg/kg bw when administered in 2% lecithin water or in saline, respectively. Notably, no effect was observed when PTX-2 or OA were administered separately to mice at a concentration of 300 μg/kg bw or 50 μg/kg bw, respectively, whereas fluid accumulation was observed when the compounds were given together.

70. Reports of hepatotoxic effects of PTXs are also conflicting. Formation of non-fatty vacuoles, congestion and the appearance of granules were observed in mouse liver following i.p. administration of PTX-1 at doses of 150-1000 μg/kg bw[26]. Only slight injuries were seen in mice given 150 or 200 μg/kg bw in this study. In a later study, the same authors reported formation of non-fatty vacuoles in the liver and swelling of small intestinal villi in mice given PTX-1 or PTX-2 (375 μg/kg bw) by i.p. administration, but no adverse effects following oral administration of PTX-1 and PTX-2 at 750 μg/kg bw[23]. Ishige *et al.*, however, reported that oral administration of PTX-2 to mice at doses of 250-2000 μg/kg bw resulted in hyaline droplet formation and granular degeneration in hepatocytes[73]. Increased granularity in the liver and increased serum activities of alanine aminotransferase, aspartate aminotransferase and sorbitol dehydrogenase were reported in mice given an i.p. injection of 200 μg PTX-2/kg bw[75].

71. In contrast to the above studies, several other studies have found no signs of toxicity in the liver or other organs following i.p. administration of PTX-2 SA or 7-*epi*-PTX-2 SA or oral administration of PTX-2, PTX-2 SA or PTX-11 at 5000 μg/kg bw[71,72,66].

Previous Risk Assessments

72. The EC Working Group on Toxicology of DSP and AZP identified an oral LOAEL of 250 μg/kg bw for PTXs, based on the report of fluid accumulation and injuries to the small intestine and liver at this dose by Ishige *et al.* (see paragraphs 68 and 70). A safety factor of 1000 (to account for use of a LOAEL and inter- and intra-species extrapolation) was applied to this value to derive an ARfD of 0.25 μg/kg bw[52].

73. FAO/IOC/WHO (2004) considered that the database was not sufficient to establish an ARfD for PTXs. However, it was noted that estimated human exposures to PTXs, assuming a 60 kg bw, for Canada (0.6 μg/kg bw) and Norway (1.6 μg/kg bw) are more than 8300 and 3100 lower, respectively, than the oral dose of PTX-2 or PTX-2 SA at which no adverse effects were observed in studies by Miles et al (5000 μg/kg bw; see paragraphs 67, 68 and 71)[64].

74. The information recently generated relating to induction of intestinal fluid accumulation and intestinal tissue damage in mice following oral administration of PTX-2 (see paragraph 69) was made available to the CRLMB Working Group on Toxicology for their discussions in 2005. The Working Group were also informed that pathological changes were observed in the stomach, lungs, liver, kidneys and intestines at 1500 μg/kg bw in this research, although these data were not presented at the 2006 Harmful Algae conference. It was noted that this work indicated a no observable adverse effect level (NOAEL) of 300 μg/kg bw. A safety factor of 100 was applied to this value to derive an ARfD of 3 μg/kg bw[65]. At the CRLMB meeting, it was noted that the discrepancies in findings relating to diarrhoea are to be investigated further.

COT Evaluation

75. While noting the conflicting results reported for PTXs, the COT considered that the studies reporting adverse effects following oral administration should not be discounted.

76. The Committee considered that it was appropriate to take the lowest identified LOAEL of 250 μg/kg bw, and apply an uncertainty factor of 1000 to derive an ARfD of 0.25 μg/kg bw for PTXs. An uncertainty factor of 1000 was selected to account for extrapolation from a LOAEL to a NOAEL and to allow for differences between species and human variability.

77. In the *in vivo* study reported at the 2006 Conference on Harmful Algae, diarrhetic effects were reported following oral administration of PTX-2 and OA together at doses that did not produce adverse effects when administered separately. While the available data are limited, it was noted that it may be prudent to consider the potential for combined effects of these toxin groups given that they are known to co-occur in shellfish. However, a conservative approach incorporating a large uncertainty factor (1000) had been taken in advising on an ARfD for PTXs, while the ARfD for OA was based on human data which may be expected to include incidents of consumption of shellfish containing both OA toxins and PTXs. Indeed, shellfish associated with the 2006 DSP outbreak in the UK contained toxins from both groups. This provided some reassurance that the proposed ARfDs for PTXs and OA toxins would offer adequate protection for the consumer.

78. In view of the incomplete nature of the database for PTXs, the Committee recommended that the ARfD for these toxins should be reviewed when further data are available.

Azaspiracids

Background

79. Azaspiracids (AZAs) were first discovered in 1995 in Ireland, and have subsequently been reported in several other countries including the UK, Norway, France, Spain and Morocco. To date 11 different AZA congeners have been identified. The structures of AZAs-1 to -5 are shown in Figure 3.

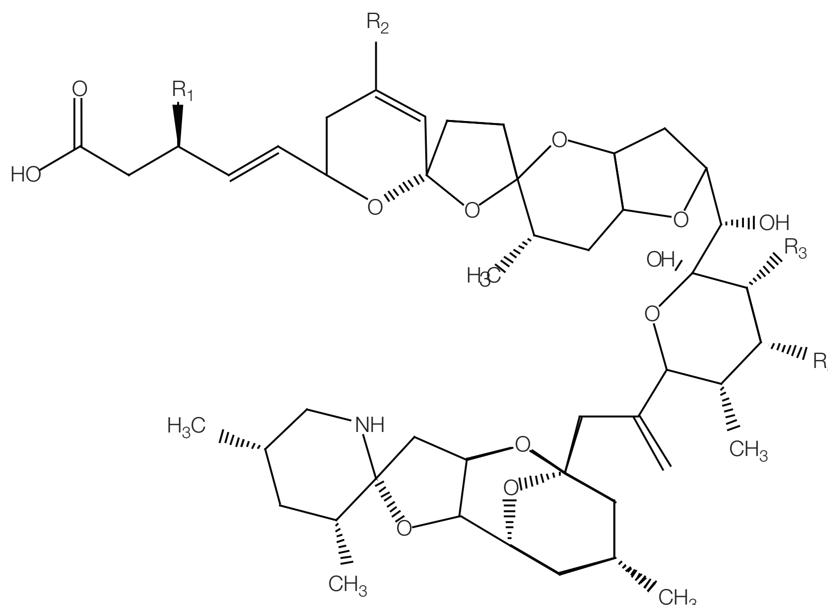


Figure 3. Structures of AZAs-1 to -5[76]

Toxin	R_1	R_2	R_3	R_4
AZA-1	H	H	CH_3	H
AZA-2	H	CH_3	CH_3	H
AZA-3	H	H	H	H
AZA-4	OH	H	H	H
AZA-5	H	H	H	OH

80. Cases of human illness following consumption of shellfish contaminated with AZAs have been reported, and symptoms of AZA poisoning (AZP) are similar to those of DSP, including nausea, vomiting, severe diarrhoea and stomach cramps[77].

81. Current legislation prescribes a maximum permitted level for AZAs in shellfish of 16 µg/100 g shellfish flesh.

Toxicology

Toxicokinetics

82. No data have been reported on the toxicokinetics of AZAs.

Acute Toxicity

83. Lethal doses of AZA-1, -2, -3, -4 and 5 in mice following i.p. injection are summarised in table 6.

Table 6. Acute lethality of AZAs in mice following i.p. administration

Compound	Dose (μg/kg bw)
AZA-1	200[78]
AZA-2	110[79]
AZA-3	140[79]
AZA-4	470[76]
AZA-5	<1000[76]

84. The number of mice that died within 24 hours of a single oral dose of AZA-1 was 2/2 at 500 μg/kg bw (8 weeks old), 3/6 at 600 μg/kg (5 weeks old) and 1/2 at 700 μg/kg (5 weeks old)[80]. In a later study, the lowest lethal oral dose of AZA-1 was reported to be 250 μg/kg bw in 5-month old mice[81].

85. Following oral administration of AZA-1 at 300 μg/kg bw, adverse effects were seen in the small intestine of mice, including congestion, pooled watery substances in the lumen and necrosis of the lamina propria[80]. More severe effects were seen at doses of 600 and 700 μg/kg bw. Fatty changes were observed in the liver at doses of 300 μg/kg bw and higher, and necrotic lymphocytes were seen in the thymus, spleen and Peyer's patches at 500 μg/kg bw and above.

Repeat dose toxicity studies

86. Ito *et al.*[81] administered 2 oral doses of 300-450 μg AZA-1/kg bw to male ICR mice. Animals were treated on days 0 and 3, and surviving mice were sacrificed between days 7 and 90 to observe recovery of organ injuries. Slow recoveries from injuries were found, including erosion and shortened villi persisting in the stomach and small intestine for more than 3 months, oedema, bleeding, and infiltration of cells in the alveolar wall of the lung for 56 days. Fatty changes in the liver persisted for 20 days, and necrosis of lymphocytes in the thymus and spleen for 10 days.

87. In the same study, the cumulative effects of sublethal doses of AZA-1 were assessed. Mice received doses of 1, 5, 20 or 50 μg/kg bw AZA-1/kg bw p.o. twice weekly up to 40 times. At the higher doses, many mice became so weak they were sacrificed and subjected to autopsy before completion of 40 injections (9/10 at 50 μg/kg bw and 3/10 at 20 μg/kg bw). All these mice showed interstitial pneumonia and shortened small intestinal villi in comparison with controls. Doses of 5 and 1 μg/kg bw did not cause death, even when administered 40 times. No signs of weakness or illness were observed in mice in these groups, but shortened intestinal villi were observed as with the animals given higher doses. Villi did not show full recovery 3 months after withdrawal of treatment. In the liver, focal necrosis, single cell necrosis, minor inflammation, mitosis and congestion were seen in a few mice, while 1/6 mice

administered 1 μg/kg bw had a hyperplastic nodule 3 months following the chronic AZA treatment. Lung tumours were observed in four mice; one from the 50 μg/kg bw group after 32 injections and three from the 20 μg/kg bw group 2 and 3 months following completion of treatment. Tumours were not observed in mice treated with lower doses or in the control mice. Hyperplasia of epithelial cells was also observed in the stomach of 6/10 mice administered 20 μg/kg bw.

88. Brief details of a second, unpublished repeat-dose oral toxicity study are provided in a risk assessment of AZAs recently conducted by the Scientific Committee of the Food Safety Authority of Ireland (FSAI)[82] and in the background report for the FAO/IOC/WHO Expert Consultation[3]. In this study, groups of at least 10 mice were administered AZA-1 at doses of 5, 10 or 20 μg/kg bw once or twice weekly for 20 weeks. Surviving mice were then given doses ranging from 4-20 μg/kg bw for up to 1 year. The study also included 52 control mice. No tumours were observed among 66 mice sacrificed at 8 months, but 2 malignant lymphomas and 3 lung tumours were seen in the 20 remaining AZA-treated mice at 1 year. The dose at which all tumours occurred is not specified, although it is noted that three of the tumours again occurred at 20 μg/kg bw. The FSAI report notes that 9/126 (7%) mice treated in these two long-term studies developed tumours, compared with none of the control mice.

Reproductive toxicity

89. Microinjection of Japanese medaka finfish embryos with AZA-1 (≥ 40 pg AZA-1/egg) resulted in reduced somatic growth and yolk absorption within 4 days of exposure, as well as delayed onset of blood circulation and pigmentation[83]. Embryos had slower heart rates than controls for the 9 day *in ovo* period and reduced hatching success. These effects were dose-dependent, with failure to hatch occurring in approximately 50% of embryos exposed to ≥ 40 pg/egg, 90% of those exposed to 80-120 pg/egg, and all of the embryos injected with 120-160 pg/egg. Microinjection of a contaminated mussel extract containing AZA-1, -2 and –3, OA and DTX-2 resulted in similar responses.

Human data

90. In total, 5 AZP incidents have been reported in the Netherlands, Ireland, Italy, France and the UK since the identification of AZA in 1995, with all cases linked to the consumption of Irish shellfish prior to the introduction of a regulatory limit for AZAs in Europe in 2001[84]. Since the introduction of the regulatory limit, no cases of AZP have been reported despite evidence of two major incidents of AZA contamination of shellfish in this period.

Epidemiology data

91. No epidemiological details are available from the majority of these incidents, although limited information collected from an incident that occurred following consumption of mussels in Arranmore Island, Ireland in 1997[85] has been used in international risk assessments of AZAs.

92. Around 20-24 individuals are believed to have been affected in the Arranmore AZP incident, with 7-8 of these cases confirmed following consultation with a physician. Symptoms were reported as vomiting, diarrhoea and nausea, with all patients making a complete recovery after 2-5 days. There were no indications of any hepatotoxic effects and no individuals subsequently presented with illness that could be related to the initial poisoning. The lowest amount of mussels reported to have been consumed by an affected individual was 10-12.

Previous Risk Assessments

93. The Food Safety Authority of Ireland (FSAI) first performed a risk assessment of AZAs in shellfish in 2001[59]. This was largely based on the information obtained from the Arranmore incident, together with data on levels of AZAs present in the hepatopancreas of mussels collected from Arranmore in the months following the incident. Mussels were first collected 2 months after the incident and collections continued at regular intervals over the following 6 months. AZA levels were assessed by LC-MS.

94. Probabilistic modelling was applied to the data on AZA concentrations in mussels following the incident as well as data on variation in mussel tissue yields in order to estimate the likely AZA intake of the individual who became ill following consumption of 10-12 mussels. Evidence at this time suggested that AZA levels may be reduced by as much as 71% by cooking, and this information was also included in the assessment. Results of the modelling suggested that the AZA intake was likely to have been between 6.7 μg (5th percentile) and 24.9 μg (95th percentile).

95. The EC Working Group on Toxicology of DSP and AZP considered the FSAI risk assessment in the light of new data suggesting that AZA levels in shellfish are not reduced during cooking[52]. The range for the LOAEL was recalculated as being between 23 μg (5th percentile) and 86 μg (95th percentile). The Working Group applied a safety factor of 3 to these values to derive an ARfD within the range of 7.7 μg and 28.7 μg per person, or between 0.128 and 0.478 μg/kg bw assuming a 60 kg bw. The CRLMB Working Group on Toxicology also derived an ARfD of 0.128 μg/kg bw in its assessment of AZAs, based on the lower LOAEL of 23 μg/person[65].

96. FAO/IOC/WHO established a provisional ARfD for AZAs of 0.04 μg/kg bw, based on the lower LOAEL of 23 μg per person and a 60 kg bw, and applying a 10-fold safety factor to take into consideration the small number of people for whom data are available[64].

97. Most recently, the Scientific Committee of FSAI performed a re-evaluation of its 2001 risk assessment, in the light of relevant data published since 2001[82]. These data related to the tissue distribution of AZAs in mussels, the ratios of different AZAs in mussel tissue, and the influence of cooking on AZA concentrations within mussels.

98. Expert opinion on the relative proportions of AZAs in hepatopancreas versus whole flesh had been used in the 2001 FSAI risk assessment to calculate the likely concentration of AZA-1 in whole flesh, based on measurements in hepatopancreas. However, a recent publication reported a series of measurements of hepatopancreas:whole flesh ratios in 28 mussel samples collected in Ireland between 2001 and 2003[86]. These data were used to generate a cumulative distribution describing the variability of the measured ratios, which was then used to recalculate the range of estimates of AZA-1 levels in the whole flesh of mussels in the Arranmore incident. This indicated that AZA-1 levels within the mussels may have been higher than originally estimated, with an average estimate of 2 μg/g compared with the previous estimate of 1.3 μg/g.

99. The 2001 FSAI risk assessment used a single value for the proportion of AZA-2 and AZA-3 relative to AZA-1 likely to be present within contaminated mussels. Since this time, information from the 2005 Irish biotoxin monitoring programme has generated a range of 75 different proportions for AZA-2 and AZA-3 relative to AZA-1. These data were used in the 2006 risk assessment in order to provide what was considered to be a more accurate estimate of the total AZA concentration within the mussels associated with the Arranmore incident.

100. Recent data indicate that steaming of raw fresh mussels results in a 2-fold higher concentration of AZAs in the cooked flesh (whole flesh and hepatopancreas) compared with the uncooked flesh[86]. This was attributed to the loss of water/juice from the mussels. On this basis, it was considered appropriate to calculate mussel consumption by individuals during the Arranmore incident in terms of raw weight rather than having to account for the reduction in mussel meat weight during cooking (approximately 50%). However, it was acknowledged that a degree of uncertainty remains in this part of the exposure assessment due to a lack of knowledge on mussel meat weight in the Arranmore growing site in 1997.

101. The use of these new data in the probabilistic approach used by FSAI resulted in a substantially higher estimate of the AZA intake associated with AZP on Arranmore compared with previous assessments. The revised estimates of AZA intake associated with human illness were calculated to be between 50.1 μg (5th percentile) and 253.3 (95th percentile) per person.

102. The FSAI Scientific Committee applied a safety factor of 3 to the median AZA intake estimate associated with AZP (113.41 μg per person) to derive an ARfD of 0.63 μg/kg bw, assuming a 60 kg bw. This safety factor was applied to account for possible intra-species variation in the toxicodynamic effects of AZAs. It was considered that a further safety factor was not required for intra-species variation in toxicokinetics, due to an absence of clear evidence for metabolism resulting in a more toxic compound. It was also suggested that metabolic activation is unlikely as the toxicity of AZA is targeted to the gastrointestinal tract.

103. The FSAI Scientific Committee noted that the derived ARfD of 0.63 μg/kg bw is comparable to the maximum intake value of 0.67 μg/kg bw for a 60 kg individual consuming 250 g of mussels at the current regulatory limit of 16 μg/100 g shellfish flesh. It was considered that the validity of the proposed ARfD is supported by the absence of reported incidents of AZP since the introduction of the 16 μg/100 g shellfish flesh regulatory limit for AZAs, despite evidence that approximately 216,000 portions of oysters have been legally placed on the market with AZA levels between 10 and 16 μg/100 g shellfish flesh. It was suggested that this information could be viewed as crude evidence of a much wider epidemiological data set than that provided by the Arranmore incident alone, indicating that a larger safety number was not required to account for the small number of people involved in the Arranmore incident for whom epidemiological data are available.

COT Evaluation

104. The COT considered the epidemiological data from the Arranmore AZP incident in the light of the new data presented in the 2006 FSAI risk assessment.

105. Concerns were raised that the absence of information on levels of AZAs in mussels associated with illness represented a significant source of uncertainty in the risk assessment.

106. In addition, the Committee expressed concerns over the appropriateness of the safety factor of 3 applied in the FSAI assessment. This was based on toxicodynamic variability with an assumption of no toxicokinetic variation due to a lack of clear evidence for metabolism of AZA resulting in a more toxic compound. The potential for variation in elimination of AZAs did not appear to have been considered.

107. In addition, it was noted that the limited animal data on acute oral toxicity of AZAs indicated a LOAEL of 250 μg/kg bw, the lowest reported lethal oral dose of AZA-1 in an acute study. Application of an uncertainty factor of 1000 to this value, to account for differences between species, human variability and extrapolation from a lethal dose, would indicate an ARfD of 0.25 μg/kg bw – lower than the ARfD of 0.63 μg/kg bw proposed by FSAI. While repeat-dose toxicity studies with AZA-1 had identified a potential for adverse effects following repeated oral administration at relatively low levels, it was noted that regular exposure to AZAs over a prolonged period was unlikely to occur in humans given general patterns of shellfish consumption in the UK.

108. The absence of reported AZA poisonings since the introduction of the regulatory limit in 2001, despite evidence of two major incidents of AZA contamination of shellfish between 2001 and 2005, provided the COT with some reassurance that the ARfD proposed by FSAI was in practice sufficiently protective. However, it was noted that the absence of reported AZP incidents should not be taken as evidence that no such incidents have occurred in this time, and is not an adequate basis for risk assessment.

Yessotoxins

Background

109. Yessotoxins (YTXs) have been detected in microalgae and/or bivalve molluscs in Australia, Canada, Italy, Japan, New Zealand, Norway and the United Kingdom[64]. They have not been found to induce diarrhoea in animal studies, and there are no reports of human illness associated with these toxins. The structures of those YTXs for which most toxicological data are available are shown in Figure 4.

110. Current legislation prescribes a maximum permitted level for YTXs in shellfish of 100 μg/100 g shellfish flesh.

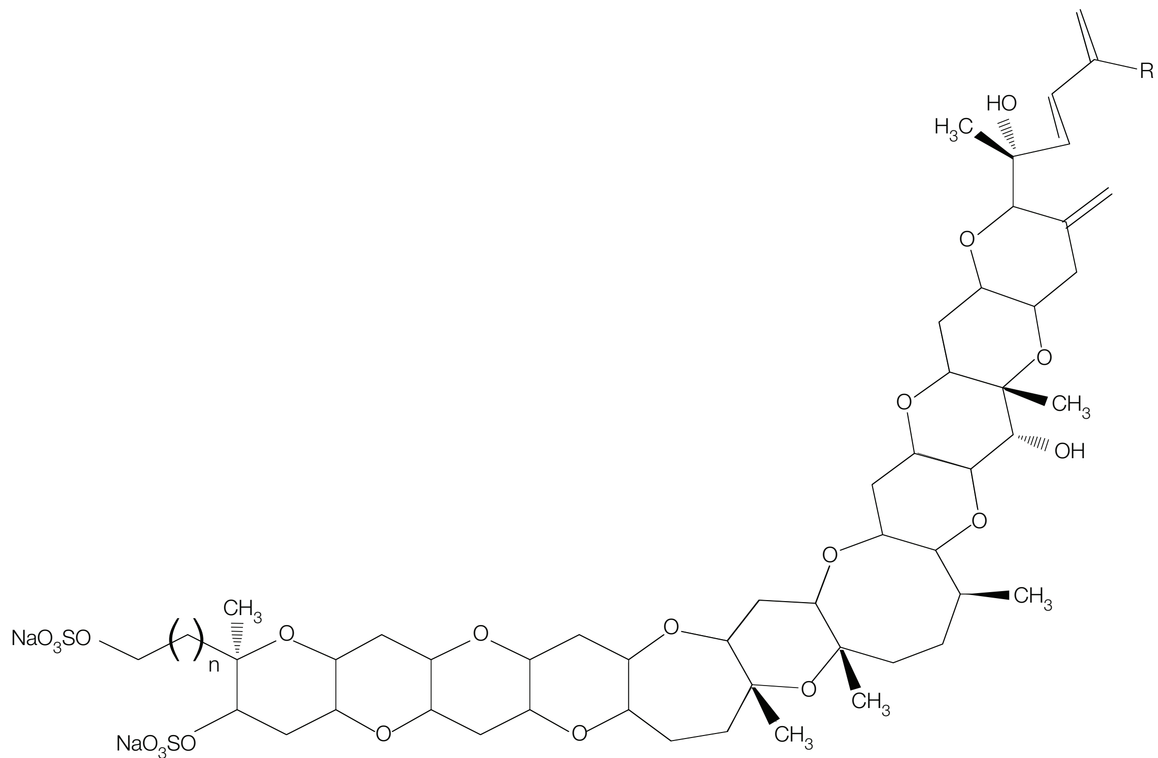


Figure 4. Structures of YTXs[7]

Toxin	R	n
YTX		1
45-hydroxyYTX	OH	1
homoYTX		2
45-hydroxyhomoYTX	OH	2

Toxicology

Toxicokinetics

111. No data have been published on the toxicokinetics of YTXs. However, an unpublished study reported in the background document for the FAO/IOC/WHO Expert Consultation found that the majority of an oral dose of YTX in mice was recoverable from the faeces, suggesting poor absorption from the gastrointestinal tract[3].

Acute toxicity

112. The acute lethality of YTX following i.p. injection in mice was originally reported as 100 μg/kg bw[69]. Subsequent studies have reported LD_{50}s of 286 μg/kg bw[87] and 512 μg/kg bw[18]. Ogino *et al.* reported that the minimum amount of YTX required to kill a mouse following i.p. injection was between 80-100 μg/kg bw[20], whereas in a later study death only occurred at doses ≥750 μg/kg bw[88]. The acute lethality of 45-hydroxyYTX has been reported as 100 or ca. 500 μg/kg bw[69,89].

113. The i.p. lethalities of homoYTX and 45-hydroxyhomoYTX were originally reported to be similar to those previously reported for YTX (100 μg/kg bw) and 45-hydroxyYTX (ca. 500 μg/kg bw)[90]. More recently, however, an LD_{50} of 444 μg/kg bw has been reported for homoYTX, while 750 μg/kg bw of 45-hydroxyhomoYTX did not cause death in this study[18].

114. No deaths have been observed following oral administration of YTX to mice at doses up to 10,000 μg/kg bw[88,18]. The FAO/IOC/WHO background document notes that unpublished research found no deaths at oral doses of 50,000 μg/kg bw. Oral administration of homoYTX and 45-hydroxyYTX at 1000 μg/kg bw also did not result in death[18].

115. Following i.p. administration in mice, a single dose of YTX at 300 μg/kg bw did not cause discernible changes in the liver, pancreas, lungs, adrenal glands, kidneys, spleen or thymus[87]. The intestines were also examined in this study, but the authors did not report whether or not adverse effects were observed. Electron microscopy (EM) showed severe cardiac damage including swelling and degeneration of the endothelium of capillaries, swelling of almost all cardiac muscle cells and rounding of mitochondria.

116. No changes were observed in the lung, thymus, liver, pancreas, kidney, adrenal gland, jejunum, colon and spleen of mice administered single i.p. doses of YTX up to 1000 μg/kg bw[88]. Slight intercellular oedema was detected by light microscopy in cardiac muscles of animals given 750 and 1000 μg/kg bw, while EM analysis of hearts of animals given 1000 μg/kg bw revealed swelling of myocardial muscle cells and separation of organelles that was most pronounced near the capillaries.

117. Histological examination of major organs and tissues including the liver, heart, lungs, kidney, spleen, thymus and brain did not show morphological changes in mice given a single i.p. injection of YTX (265-750 μg/kg bw), homoYTX (375-750 μg/kg bw) or 45-hydroxy-YTX (750 μg/kg bw)[18]. TUNEL staining was performed on heart tissue but no apoptosis was detected.

118. Examination of the cerebellar cortex of Swiss CD-1 mice given a lethal i.p. dose of YTX (420 μg/kg bw) indicated damage to the Purkinje cells[91]. Immunocytochemical analysis indicated an increased positivity for S100 protein, and a decreased response to calbindin D-28K, beta-tubulin and neurofilaments. In a subsequent study using both lethal (420 μg/kg bw) and sublethal (10 μg/kg bw) doses of YTX, no effects were detected in the cerebellar cortex at the sublethal dose[92]. In the cerebral cortex, no morpho-functional alterations were observed at either dose. Morphological changes were detected in the thymus at both doses, including apoptosis, predominantly of thymocytes and increased mitosis. Alterations in cytokine levels were also observed.

119. The only adverse effects of YTXs reported following oral administration are ultrastructural alterations in cardiac muscle cells, similar to those observed following i.p. administration. Ultrastructural alterations in cardiomyocytes have been observed by EM in mice in a number of studies following single oral administration of YTX at concentrations of ranging from 1000-10,000 μg/kg bw, and at 1000 μg/kg bw of homo-YTX and 45-OH-homo YTX[88,18]. In the earlier of these two studies, no adverse effects were detected in cardiac muscle cells by light microscopy at a dose of 1000 μg/kg bw, although animals receiving this dose were not examined by EM. Light microscopy only detected adverse effects in myocytes at doses of 7500 and 10,000 μg/kg bw[88]. No changes were detected in the hearts of mice treated with oral doses of 500 μg YTX/kg bw[87].

120. In a short-term study, administration of YTX (2000 μg/kg bw), homoYTX (1000 μg/kg bw) and 45-OH-YTX (1000 μg/kg bw) to female CD-1 mice daily for 7 days resulted in ultrastructural changes in cardiac muscle cells, detected by electron microscopy (EM). No signs of toxicity were observed in the other organs or tissues that were examined, including the brain, thymus and spleen[93].

121. Information presented at the 2004 International Conference on Molluscan Shellfish Safety by Espenes *et al.* relating to a further study of repeated oral exposure to YTX is described in the background document for the FAO/IOC/WHO Expert Consultation. In this study, NMRI mice were exposed to YTX seven times in 21 days by oral intubation, at doses of 1000, 2500 and 5000 μg/kg bw. Mice were killed 3 days following last treatment, and major organs including the myocardium, brain, thymus and spleen were studied by light microscopy. The myocardium was also examined by EM. No clinical signs were observed in any of the groups exposed to YTX, and there were no differences in body weight gain between treated mice and controls. No pathologic effects were observed by light microscopy. By EM, some vacuoles were observed in the myocardium of mice treated at the highest dose only. The authors suggested that the reason for the apparent conflict with previous studies showing ultrastructural changes in myocardium at lower doses may have been due to the 3-day delay between final dosing and sacrifice of the mice. They suggested that any damage occurring following treatment may have been repaired in this time[94].

122. In a recent study, atrophy and structural alteration of the thymus was observed in mice 24 hours following consumption of shellfish tissue contaminated with OA and YTX (estimated intakes of 18 and 1.4 μg/kg bw, respectively[27]. Histopathological changes were also observed in the spleen immediately following consumption of contaminated shellfish, but these effects were less marked 24 hours following consumption. However, similar effects were found with OA alone and it is therefore unclear whether YTX may have contributed to the effects observed.

Previous Risk Assessments

123. On the basis of the data available in 2001, the EC Working Group on Toxicology of DSP and AZP considered that findings of no adverse effects by light microscopy following a single oral administration of 1000 μg YTX/kg bw[88] represented a NOAEL[52]. The Working Group applied a safety factor of 100 to this to derive an ARfD of 10 μg/kg bw.

124. FAO/IOC/WHO (2004) concluded that the repeated administration study of Espenes *et al.* (see paragraph 121) indicated a NOAEL of 5000 μg/kg bw. A safety factor of 100 was applied to derive an ARfD of 50 μg YTX eq/kg bw.

125. The approach of FAO/IOC/WHO was also adopted by the CRLMB Working Group on Toxicology[65].

COT Evaluation

126. The Committee questioned the relevance of the observed alterations in cardiac myocytes, and of the apparent recovery from injury following treatment in the most recent study. Cardiac tissue does not readily regenerate following injury, and alterations were not observed by light microscopy at doses up to 5000 μg/kg bw. It was considered possible that the EM findings may have been artefactual, but the available evidence was insufficient to draw firm conclusions.

127. Despite the uncertainty over the significance of the reported alterations in cardiac muscle cells, it was considered that it would be conservative to use these data to establish an ARfD for YTXs. An uncertainty factor of 100 was applied to the NOAEL of 5000 μg/kg bw identified in the 21 day repeat-dose study, resulting in an ARfD of 50 μg/kg bw.

Conclusions

Okadaic Acid Group

128. We consider that human case reports should be used as a basis for risk assessment of OA group toxins, although we note the uncertainties relating to the amount of toxins consumed in many of these incidents.

129. We note that the totality of published epidemiology data for OA toxins indicates a LOAEL of around 1 μg/kg bw. While the limited information from the recent UK DSP incident may suggest a lower LOAEL of 0.7 μg/kg bw, the data from this incident are difficult to interpret as shellfish associated with the outbreak also contained PTXs, and there is also uncertainty with respect to which of two shellfish portion sizes sold at restaurants involved in the incident had been eaten by individuals who became ill. Had all individuals eaten the larger portion size, a LOAEL of 1.3 μg/kg bw would be indicated, in line with the previous epidemiology data.

130. The 2006 DSP outbreak indicated a reported response rate of up to 10%, suggesting that more than the most susceptible minority were affected at this dose. We therefore consider that an uncertainty factor of 3 would not be sufficient for extrapolation from a LOAEL to a NOAEL in this case. An uncertainty factor of 10 should be applied, resulting in an ARfD of 0.1 μg OA eq/kg bw.

131. We note that a portion size of 250 g is a reasonable estimate for high level consumption of shellfish in the UK[95]. We conclude that 2.4 μg OA eq/100 g shellfish meat would be the maximum concentration considered to be without appreciable health risk, assuming a 60 kg adult bodyweight.

132. We note that this concentration is lower than the current regulatory limit for OA group toxins together with PTXs of 16 μg/100 g shellfish meat. Furthermore, the MBA currently prescribed in EU legislation for detection of these toxins in shellfish monitoring programmes is not sufficiently sensitive to detect the presence of OA group toxins at this level.

Pectenotoxins

133. We consider that it is appropriate to use the lowest identified LOAEL in animal studies of 250 μg/kg bw as the basis for deriving an ARfD for PTXs. An uncertainty factor of 1000 should be applied to account for inter- and intra-species variation and extrapolation from a LOAEL to a NOAEL, resulting in an ARfD of 0.25 μg/kg bw.

134. However, we note the conflicting and incomplete nature of the database for PTXs, and recommend that the ARfD should be reviewed when further data become available.

135. On the basis of an ARfD of 0.25 μg/kg bw, a PTX concentration of 6 μg/100 g shellfish meat would be the maximum concentration considered to be without appreciable health risk, assuming a 60 kg bodyweight. As with the OA group toxins, we note that this concentration is lower than the current regulatory limit, and that the MBA is not sufficiently sensitive to detect the presence of PTXs at this level.

Azaspiracids

136. We consider that the limited epidemiology data from the 1997 Arranmore AZP incident currently provide the best available evidence for risk assessment of AZAs, although we note that there are considerable uncertainties in the information derived from this incident, particularly with respect to a lack of accurate information on the levels of AZAs in mussels associated with illness.

137. The ARfD of 0.63 μg/kg bw proposed by FSAI is comparable to the maximum intake of 0.67 μg/kg bw for a 60 kg individual at the current regulatory limit for AZAs of 16 μg/100 g shellfish meat. We conclude that the absence of reported AZA poisonings since the introduction of the regulatory limit in 2001, despite evidence of two major incidents of AZA contamination of shellfish between 2001 and 2005, provides some reassurance that the ARfD proposed by FSAI would in practice be sufficient for the protection of the health of the consumer. However, we note that the absence of reported AZP incidents should not be taken as evidence that no such incidents have occurred in this time, and is not an adequate basis for risk assessment in isolation.

Yessotoxins

138. The relevance of the observed alterations in cardiac myocytes following oral administration of YTXs in animal studies is unclear. However, we consider that it would be conservative to use these data to establish an ARfD for YTXs.

139. A NOAEL of 5000 μg/kg bw can be identified on the basis of apparent absence or recovery from injury three days following final treatment in the repeat-dose study by Espenes *et al.* (paragraph 121). Application of an uncertainty factor of 100 to account for inter- and intra-species variation results in an ARfD of 50 μg/kg bw.

140. We conclude that a YTX concentration of 1200 μg/100 g shellfish meat would be the maximum concentration considered to be without appreciable health risk, assuming a 60 kg adult bodyweight.

Analytical methods

141. When the COT last considered DSP toxins in 1994, it recommended that efforts be made to develop a more quantitative assay to replace the MBA in the UK shellfish monitoring programme for lipophilic biotoxins. We reiterate this recommendation and, furthermore, we note that alternative methods may be required in order to support detection of OA group toxins and PTXs in shellfish at the concentrations identified as necessary for protection of public health. As alternative methods become available, it will be important to give careful consideration to the most appropriate way to sum the concentrations of specific toxin analogues.

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December 2006

References

1 COT. (2006). Statement on risk assessment and monitoring of paralytic shellfish poisoning (PSP) toxins in support of public health. 1-28.

2 Aune, T. (2001). Risk assessment of toxins associated with DSP, PSP and ASP in seafood. De Koe, W. J., Samson, R. A., Van Egmond, H. P., Gilbert, J., and Sabino, M. Mycotoxins and Phycotoxins in Perspective at the Turn of the Millennium. Proceedings of the X International IUPAC Symposium on Mycotoxins and Phycotoxins (May 2000, Guarujá, Brazil). [16], 515-525.

3 FAO/IOC/WHO. (2004). Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs, Oslo, Norway, Sept 26-30 2004 – Background Document. *Oslo, Norway, Sept 26-30 2004.*

4 Takai, A.(1988). Okadaic acid. Protein phosphatase inhibition and muscle contractile effects. *J Muscle Res Cell Motil* 9: 563-5.

5 Garcia, C., Truan, D., Lagos, M., Santelices, J.P., Diaz, J.C., Lagos, N. (2005). Metabolic transformation of dinophysistoxin-3 into dinophysistoxin-1 causes human intoxication by consumption of O-acyl-derivatives dinophysistoxins contaminated shellfish. *J Toxicol Sci* 30: 287-296.

6 Torgersen, T., Aasen, J., Aune, T. (2005). Diarrhetic shellfish poisoning by okadaic acid esters from Brown crabs (Cancer pagurus) in Norway. *Toxicon* 46: 572-578.

7 Yasumoto, T. (2000). Historic considerations regarding seafood safety. Botana, L. M. Seafood and Freshwater Toxins. [1], 1-17. Marcel Dekker Inc.

8 Carmody, E.P., James, K.J., Kelly, S.S. (1996). Dinophysistoxin-2: the predominant diarrhoetic shellfish toxin in Ireland. *Toxicon* 34: 351-9.

9 Vale, P. and Sampayo, M. (2002). First confirmation of human diarrhoeic poisonings by okadaic acid esters after ingestion of razor clams (Solen marginatus) and green crabs (Carcinus maenas) in Aveiro lagoon, Portugal and detection of okadaic acid esters in phytoplankton. *Toxicon* 40: 989-96.

10 MAFF. (1994). Food surveillance paper no. 42: Natural occurring toxicants in food.

11 Matias, W.G., Traore, A., Creppy, E.E. (1999). Variations in the distribution of okadaic acid in organs and biological fluids of mice related to diarrhoeic syndrome. *Human and Experimental Toxicology* 18: 345-350.

12 Matias, W.G. and Creppy, E.E. (1996). Evidence for enterohepatic circulation of okadaic acid in mice. *Toxic Substance Mechanism* 15: 405-414.

13 Ito, E., Yasumoto, T., Takai, A., Imanishi, S., Harada, K. (2002). Investigation of the distribution and excretion of okadaic acid in mice using immunostaining method. *Toxicon* 40: 159-65.

14 Matias, W.G. and Creppy, E.E. (1996). Transplacental passage of okadaic acid. *Hum Exp Toxicol* 15: 226-30.

15 Yasumoto, T. and Murata, M. (1990). Polyether toxins involved in seafood poisoning. Hall, S. and Strichartz, G. Marine Toxins: Origin, structure and molecular pharmacology. 120-132. American Chemical Society.

16 Yasumoto, T., Fukui, M., Sasaki, K., Sugiyama, K. (1995). Determinations of marine toxins in foods.

17 Aune, T., Larsen, S., Aasen, J.A., Rehmann, N., Satake, M., Hess, P. (2007). Relative toxicity of dinophysistoxin-2 (DTX-2) compared with okadaic acid, based on acute intraperitoneal toxicity in mice. *Toxicon* 49: 1-7.

18 Tubaro, A., Sosa, S., Carbonatto, M., Altinier, G., Vita, F., Melato, M., Satake, M., Yasumoto, T. (2003). Oral and intraperitoneal acute toxicity studies of yessotoxin and homoyessotoxins in mice. *Toxicon* 41: 783-92.

19 Le Hegarat, L., Jacquin, A.G., Bazin, E., Fessard, V. (2006). Genotoxicity of the marine toxin okadaic acid, in human caco-2 cells and in mice gut cells. *Environmental Toxicology* 21: 55-64.

20 Ogino, H., Kumagai, M., Yasumoto, T. (1997). Toxicologic evaluation of yessotoxin. *Nat Toxins* 5: 255-9.

21 Hamano, Y., Kinoshita, Y., Yasumoto, T. (1986). Enteropathogenicity of diarrhetic shellfish toxins in intestinal models (Studies on diarrhetic shellfish toxins: I.). *Journal of the Food Hygienic Society of Japan* 27: 375-379.

22 Yanagi, T., Murata, M., Torigoe, K., Yasumoto, T. (1989). Biological activities of semisynthetic analogs of dinophysistoxin-3, the major diarrhetic shellfish toxin. *Agricultural and Biological Chemistry* 53: 525-530.

23 Terao, K., Ito, E., Ohkusu, M., Yasmumoto, A. (1993). A comparitive study of the effects of DSP-toxins on mice and rats. Toxic Phytoplankton Blooms in the sea. 581-586. New York, Elsevier.

24 Ito, E. (2006). Verification of diarrhetic activities of PTX-2 and okadaic acid *in vivo*. *12th International Conference on Harmful Algae*.

25 Ito, E. and Terao, K. (1994). Injury and recovery process of intestine caused by okadaic acid and related compounds. *Natural Toxins* 2: 371-377.

26 Terao, K., Ito, E., Yanagi, T., Yasumoto, T. (1986). Histopathological studies on experimental marine toxin poisoning: I. Ultrastructural changes in the small intestine and liver of suckling mice induced by dinophysistoxin 1 and pectenotoxin 1. *Toxicon* 24: 1141-1152.

27 Franchini, A., Marchesini, E., Poletti, R., Ottaviani, E. (2005). Swiss CD-1mice fed on mussels contaminated by okadaic acid and yessotoxins: effects on thymus and spleen. *Eur J Histochem* 49: 179-188.

28 Aune, T. and Yndestad, M. (1993). Diarrhetic shellfish poisoning. Falconer, I. R. Algal toxins in seafood and drinking water. [5], 87-104. Academic Press Ltd.

29 Aonuma, S., Ushijima, T., Nakayasu, M., Shima, H., Sugimura, T., Nagao, M. (1991). Mutation induction by okadaic acid, a protein phosphatase inhibitor, in CHL cells, but not in S. typhimurium. *Mutation Research Fundamental and Molecular Mechanisms of Mutagenesis* 250: 375-381.

30 Fessard, V., Grosse, Y., Pfohl, L.A., Puiseux, D.S. (1996). Okadaic acid treatment induces DNA adduct formation in BHK21 C13 fibroblasts and HESV keratinocytes. *Mutation Research* 361: 133-141.

31 Le Hegarat, L., Nesslany, F., Mourot, A., Marzin, D., Fessard, V. (2004). Lack of DNA damage induction by okadaic acid, a marine toxin, in the CHO-Hprt and the *in vitro* UDS assays. *Mutat Res* 564: 139-147.

32 Le Hegarat, L., Fessard, V., Poul, J.M., Dragacci, S., Sanders, P. (2004). Marine toxin okadaic acid induces aneuploidy in CHO-K1 cells in presence of rat liver postmitochondrial fraction, revealed by cytokinesis-block micronucleus assay coupled to FISH. *Environ Toxicol* 19: 123-128.

33 Hegarat, L.L., Orsiere, T., Botta, A., Fessard, V. (2005). Okadaic acid: Chromosomal non-disjunction analysis in human lymphocytes and study of aneugenic pathway in CHO-K1 cells. *Mutat Res* 578: 53-63.

34 Le Hegarat, L., Puech, L., Fessard, V., Poul, J.M., Dragacci, S. (2003). Aneugenic potential of okadaic acid revealed by the micronucleus assay combined with the FISH technique in CHO-K1 cells. *Mutagenesis* 18: 293-8.

35 Suganuma, M., Fujiki, H., Suguri, H., Yoshizawa, S., Hirota, M., Nakayasu, M., Ojika, M., Wakamatsu, K., Yamada, K., Sugimura, T. (1988). Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promoter. *Proc Natl Acad Sci U S A* 85: 1768-71.

36 Fujiki, H., Suganuma, M., Suguri, H., Yoshizawa, S., Takagi, K., Uda, N., Wakamatsu, K., Yamada, K., Murata, M., Yasumoto, T., et, a.l. (1988). Diarrhetic shellfish toxin, dinophysistoxin-1, is a potent tumor promoter on mouse skin. *Jpn J Cancer Res* 79: 1089-93.

37 Suganuma, M., Tatematsu, M., Yatsunami, J., Yoshizawa, S., Okabe, S., Uemura, D., Fujiki, H. (1992). An alternative theory of tissue specificity by tumor promotion of okadaic acid in glandular stomach of SD rats. *Carcinogenesis* 13: 1841-5.

38 Suganuma, M., Okabe, S., Sueoka, E., Nishiwaki, R., Komori, A., Uda, N., Isono, K., Fujiki, H. (1995). Tautomycin: an inhibitor of protein phosphatases 1 and 2A but not a tumor promoter on mouse skin and in rat glandular stomach. *J Cancer Res Clin Oncol* 121: 621-627.

39 Feng, G., Ohmori, Y., Chang, P.L. (2005). Production of chemokine CXCL1/KC by okadaic acid through the nuclear factor-{kappa}B pathway. *Carcinogenesis*

40 Yasumoto, T., Oshima, Y., Yamaguchi, M. (1978). Occurrence of a new type of toxic shellfish poisoning in the Tohoku district. *Bull Jpn Soc Sci Fish* 44: 1249-1255.

41 Kat, M.(1983). Diarrhetic mussel poisoning in the Netherlands related to the dinoflagellate Dinophysis acuminata. *Antonie Van Leeuwenhoek* 49: 417-27.

42 Kat, M. (1979). The occurrence of *Prorocentrum* species and coincidental gastrointestinal illness of mussel consumers. Anderson, D. M., White, A. W., and Baden, D. G. Toxic Dinoflagellates. 73-77. Amsterdam, Elsevier.

43 Underdal, B., Yndestad, M, Aune, T. (1985). DSP intoxication in Norway and Sweden, Autumn 1984- Spring 1984. Anderson, D. M., White, A. W., and Baden, D. G. Toxic Dinoflagellates, 489-494. Amsterdam, Elsevier.

44 Krogh, P., Edler, L., Granelli, E., Nyman, U. (1985). Outbreak of diarrhetic shellfish poisononing on the west coast of Sweden. Anderson, D. M., White, A. W., and Baden, D. G. Toxic Dinoflagellates, 501-504. Amsterdam, Elsevier.

45 De Schrijver, K., Maes, I., De Man, L., Michelet, J. (2002). An outbreak of diarrhoeic shellfish poisoning in Antwerp, Belgium. *Eurosurveillance* 7: 139-141.

46 Vale, P. and Sampayo, M.A. (1999). Esters of okadaic acid and dinophysistoxin-2 in Portuguese bivalves related to human poisonings. *Toxicon* 37: 1109-21.

47 Vale, P. and Sampayo, M.A. (2002). Esterification of DSP toxins by Portuguese bivalves from the Northwest coast determined by LC-MS—a widespread phenomenon. *Toxicon* 40: 33-42.

48 Scoging, A. and Bahl, M. (1998). Diarrhetic shellfish poisoning in the UK. *Lancet* 352: 117-

49 Quilliam, M., Gilgan, M., Pleasance, S., DeFrietas A, Douglas, D. (1993). Confirmation of an incident of diarrhetic shellfish poisoning in Eastern Canada. Smadya, T. and Shimizu, Y. 547-552. Amsterdam, Elsevier.

50 Lembeye, G., Yasumoto, T., Zhao, J., Fernadez, R. (1993). DSP Outbreak in Chilean Fjords. Smayda, T. J. and Shimizu, K. Toxic Pytoplankton Blooms in the Sea. 525-529. Amsterdam, Elsevier.

51 Wilson, N. and Sim, J. (1996). Review of the New Zealand marine biotoxin monitoring programme data: Report for the Public Health Group, Ministry of Health.

52 EU/SANCO. (2001). Report of the working group on toxicology of DSP and AZP, 21 to 23rd May 2001, Brussels.

53 Fernandez, M. L. and Cembella, A. D. (1995). Mammaliam Bioassays. Hallegraeff, G. M. and et.al. Manual on Harmful Marine Microalgae. [10, Part B], 213-228. UNESCO.

54 Yasumoto, T., Murata, M., Oshima, Y., Sano, M., Matsumoto, G. K., Clardy, J. (1984). Diarrhetic Shellfish Poisoning. Ragelis, Edward. ACS Symposium Series (no. 262): Seafood Toxins. American Chemical Society.

55 James, K. J., Bishop, A. G., Carmody, E. P., Kelly, S. S. (2000). Detection methods for okadaic acid and analogues. [11], 217-237.

56 Murata, M., Shimatani, M., Sugitani, H., Oshima, Y., Yasumoto, T. (1982). Isolation and structural elucidation of the causitive toxin of the diarrhoetic shellfish poisoning. *Bull Jpn Soc Sci Fish* 48: 549-552.

57 Aune, T. (2006). Personal Communication.

58 PHLS(1994). Diarrhetic shellfish poisoning associated with mussels. *Commun Dis Rep Wkly* 4: 1-1.

59 FSAI. (2001). Risk assessment of azaspiracids (AZAs) in shellfish.

60 Holland, B., Welch, A. A., Unwin, I. D., Buss, D. H., Paul, A. A., Southgate, D. A. T. (1991). McCance and Widdowson's The Composition of Foods. 5th. Cambridge, Royal Society of Chemistry.

61 FAO. (1989). FAO fisheries technical paper no. 309: Yield and nutritional value of the commercially more important fish species. Rome, FAO.

62 PHLS(1997). An outbreak of diarrhetic shellfish poisoning. *Commun Dis Rep Wkly* 7: 1-1.

63 Cordier, S., Monfort, C., Miossec, L., Richardson, S., Belin, C. (2000). Ecological analysis of digestive cancer mortality related to contamination by diarrhetic shellfish poisoning toxins along the coasts of France. *Environ Res* 84: 145-50.

64 FAO/IOC/WHO. (2004). Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs. Oslo, Norway, Sept 26-30 2004.

65 CRLMB. (2005). Report on working group meeting, Cesenatico, Italy, 24-25 october 2005.

66 Suzuki, T., Walter, J.A., LeBlanc, P., MacKinnon, S., Miles, C.O., Wilkins, A.L., Munday, R., Beuzenberg, V., Mackenzie, A.L., Jensen, D.J., Cooney, J.M., Quilliam, M.A. (2006). Identification of pectenotoxin-11 as 34S-hydroxypectenotoxin-2, a new pectenotoxin analogue in the toxic dinoflagellate Dinophysis acuta from New Zealand. *Chem Res Toxicol* 19: 310-318.

67 Burgess, V. and Shaw, G. (2001). Pectenotoxins: An issue for public health: A review of their comparative toxicology and metabolism. *Environment International* 27: 275-283.

68 Miles, C.O., Wilkins, A.L., Munday, R., Dines, M.H., Hawkes, A.D., Briggs, L.R., Sandvik, M., Jensen, D.J., Cooney, J.M., Holland, P.T., Quilliam, M.A., Mackenzie, A.L., Beuzenberg, V., Towers, N.R. (2004). Isolation of pectenotoxin-2 from Dinophysis acuta and its conversion to pectenotoxin-2 seco acid, and preliminary assessment of their acute toxicities. *Toxicon* 43: 1-9.

69 Yasumoto, T., Murata, Michio, Lee, Jong-Soo, Torigoe, Koichiro. (1989). Polyether toxins produced by Dinoflagellates. Natori, S., Hashimoto, K., and Ueno, Y. 10, p.375. Elsevier.

70 Sasaki, K., Wright, J.L., Yasumoto, T. (1998). Identification and Characterization of Pectenotoxin (PTX) 4 and PTX7 as Spiroketal Stereoisomers of Two Previously Reported Pectenotoxins. *J Org Chem* 63: 2475-2480.

71 Miles, C.O., Wilkins, A.L., Munday, R., Dines, M.H., Hawkes, A.D., Briggs, L.R., Sandvik, M., Jensen, D.J., Cooney, J.M., Holland, P.T., Quilliam, M.A., Mackenzie, A.L., Beuzenberg, V., Towers, N.R. (2004). Isolation of pectenotoxin-2 from Dinophysis acuta and its conversion to pectenotoxin-2 seco acid, and preliminary assessment of their acute toxicities. *Toxicon* 43: 1-9.

72 Miles, C.O., Wilkins, A.L., Munday, J.S., Munday, R., Hawkes, A.D., Jensen, D.J., Cooney, J.M., Beuzenberg, V. (2006). Production of 7-epi-pectenotoxin-2 seco acid and assessment of its acute toxicity to mice. *J Agric Food Chem* 54: 1530-1534.

73 Ishige, M., Satoh, N., Yasumoto, T. (1988). Pathological studies on the mice administered with the causative agent of diarrhetic shellfish poisoning (okadaic acid and Pectenotoxin-2). *Hokkaidoritsu Eisei Kenkyushoho* 38: 15–18-

74 Miles, C.O., Wilkins, A.L., Munday, R., Dines, M.H., Hawkes, A.D., Briggs, L.R., Sandvik, M., Jensen, D.J., Cooney, J.M., Holland, P.T., Quilliam, M.A., Mackenzie, A.L., Beuzenberg, V., Towers, N.R. (2004). Isolation of pectenotoxin-2 from Dinophysis acuta and its conversion to pectenotoxin-2 seco acid, and preliminary assessment of their acute toxicities. *Toxicon* 43: 1-9.

75 Mi, Y.Y. and Young, C.K. (1997). Toxicity and changes in hepatic metabolizing enzyme system induced by repeated administration of Pectenotoxin 2 isolated from marine sponges. *Korean Journal of Pharmacognosy* 28: 280-285.

76 Ofuji, K., Satake, M., McMahon, T., James, K.J., Naoki, H., Oshima, Y., Yasumoto, T. (2001). Structures of azaspiracid analogs, azaspiracid-4 and azaspiracid-5, causative toxins of azaspiracid poisoning in Europe. *Biosci Biotechnol Biochem* 65: 740-2.

77 McMahon, T. and Silke, J. (1996). Winter toxicity of unknown aetiology in mussels. *Harmful Aglae News* 14: 2-

78 Satake, M., Ofuji, K., Naoki, H., James, K., Furey, A., McMahon, T., Silk, J., Yasumoto, T. (1998). Azaspiracid, a new marine toxin having a unique spiro ring assemblies, isolated from Irish mussels. *J Am CHem Soc* 120: 9967-9968.

79 Ofuji, K., Satake, M., McMahon, T., Silke, J., James, K.J., Naoki, H., Oshima, Y., Yasumoto, T. (1999). Two analogs of azaspiracid isolated from mussels, Mytilus edulis, involved in human intoxication in Ireland. *Nat Toxins* 7: 99-102.

80 Ito, E., Satake, M., Ofuji, K., Kurita, N., McMahon, T., James, K., Yasumoto, T. (2000). Multiple organ damage caused by a new toxin azaspiracid, isolated from mussels produced in Ireland. *Toxicon* 38: 917-30.

81 Ito, E., Satake, M., Ofuji, K., Higashi, M., Harigaya, K., McMahon, T., Yasumoto, T. (2002). Chronic effects in mice caused by oral administration of sublethal doses of azaspiracid, a new marine toxin isolated from mussels. *Toxicon* 40: 193-203.

82 FSAI. (2006). Risk assessment of azaspiracids (AZAs) in shellfish. A report of the Scientific Committee of the Food Safety Authority of Ireland.

83 Colman, J.R., Twiner, M.J., Hess, P., McMahon, T., Satake, M., Yasumoto, T., Doucette, G.J., Ramsdell, J.S. (2005). Teratogenic effects of azaspiracid-1 identified by microinjection of Japanese medaka (Oryzias latipes) embryos. *Toxicon* 45: 881-890.

84 James, K.J., Fidalgo Saez, M.J., Furey, A., Lehane, M. (2004). Azaspiracid poisoning, the food-borne illness associated with shellfish consumption. *Food Addit Contam* 21: 879-892.

85 McMahon, T. and Silke, J. (1998). Re-occurrence of winter toxicity. *Harmful Algae News* 16: 12-12.

86 Hess, P., Nguyen, L., Aasen, J., Keogh, M., Kilcoyne, J., McCarron, P., Aune, T. (2005). Tissue distribution, effects of cooking and parameters affecting the extraction of azaspiracids from mussels, Mytilus edulis, prior to analysis by liquid chromatography coupled to mass spectrometry. *Toxicon* 46: 62-71.

87 Terao, K., Ito, E., Oarada, M., Murata, M., Yasumoto, T. (1990). Histopathological studies on experimental marine toxin poisoning: 5. The effects in mice of yessotoxin isolated from Patinopecten yessoensis and of a desulfated derivative. *Toxicon* 28: 1095-1104.

88 Aune, T., Sorby, R., Yasumoto, T., Ramstad, H., Landsverk, T. (2002). Comparison of oral and intraperitoneal toxicity of yessotoxin towards mice. *Toxicon* 40: 77-82.

89 Satake, M., Terasawa, K., Kadowaki, Y., Yasumoto, T. (1996). Relative configuration of yessotoxin and isolation of two new analogs from toxic scallops. *Tetrahedron Letters* 37: 5955-5958.

90 Satake, M., Tubaro, A., Lee Jong, S., Yasumoto, T. (1997). Two new analogs of yessotoxin, homoyessotoxin and 45-hydroxyhomoyessotoxin, isolated from mussels of the Adriatic sea. *Natural Toxins* 5: 107-110.

91 Franchini, A., Marchesini, E., Poletti, R., Ottaviani, E. (2004). Acute toxic effect of the algal yessotoxin on Purkinje cells from the cerebellum of Swiss CD1 mice. *Toxicon* 43: 347-352.

92 Franchini, A., Marchesini, E., Poletti, R., Ottaviani, E. (2004). Lethal and sub-lethal yessotoxin dose-induced morpho-functional alterations in intraperitoneal injected Swiss CD1 mice. *Toxicon* 44: 83-90.

93 Tubaro, A., Sosa, S., Altinier, G., Soranzo, M.R., Satake, M., Della, L.R., Yasumoto, T. (2004). Short-term oral toxicity of homoyessotoxins, yessotoxin and okadaic acid in mice. *Toxicon* 43: 439-445.

94 Espenes, A., Aasen, J., Hetland, D., Satake, M., Smith A, Eraker, N., and Aune, T.(2004 – In Press). Toxicity of yessotoxin in mice after repeated oral exposure. *Proceedings from ICMSS04, Galway, 2004.*

95 Henderson L, Gregory J, Swan G. (2002). National diet and nutrition survey: adults aged 19-64 years. *TSO* Volume 1: types and quantities of foods consumed.

Statement on uranium levels in water used to reconstitute infant formula

Background

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

2. The current advice from the Food Standards Agency is, in general, to avoid using natural mineral water to prepare infant feed as some brands contain high levels of minerals, which may be unsuitable for infants. New legislation is in preparation that will amend the Natural Mineral Water, Spring Water and Bottled Drinking Water Regulations 1999 to allow natural mineral waters sold in the UK to make claims for their suitability for infant feeding, provided that they meet specific criteria. It is intended that natural mineral waters that essentially meet the limits required for tap water would be considered acceptable. The legislation will protect consumers by indicating which natural mineral waters are suitable for the preparation of infant feed.

3. In 2005, work conducted by scientists in Germany[1] on the uranium content of various natural mineral waters raised concerns on the acceptability of using natural mineral water and other bottled waters for the preparation of infant feed.

4. The World Health Organization (WHO) established a Tolerable Daily Intake (TDI) of 0.6 µg/kg body weight (bw) per day and a guideline value for uranium in drinking water of 15 µg/L[2]. The Committee was asked to comment on the potential health implications for infants consuming formula milk made up with water containing uranium at this guideline level, to assist the Agency in developing advice on the suitability of using natural mineral water and other bottled waters to reconstitute infant formula.

Absorption, distribution, metabolism and excretion of uranium

5. The average gastrointestinal absorption of soluble uranium has been reported to be 1-2% in humans[3,4]. Data from laboratory animals indicate a comparable range of uranium uptakes. In general, uranium uptake increases with the solubility of the uranium compound and after fasting[4].

6. Uranium absorption has been reported to be higher in neonatal rats and pigs. For example, when given uranium by gavage two day old rats had uranium uptakes of 1-7%[5], while 30% of uranium administered on post natal day one was found in the skeleton of pigs, seven days later[6]. There are few data on the extent of uranium uptake in children.

7. Absorbed uranium tends to accumulate in the kidneys and, in particular, in the skeleton where the uranyl ion replaces calcium in hydroxyapatite.

8. Uranium is primarily excreted in the faeces, with approximately 1% excreted via the urine. The overall elimination half-life of uranium in humans has been estimated to be 180-360 days[2].

Toxicity

9. The critical toxicological effect of uranium is nephrotoxicity, with damage occurring principally to the proximal convoluted tubules[2]. Nephrotoxicity has been observed in acute, sub-acute, sub-chronic and chronic oral studies in rats, mice, rabbits and dogs. Nephritis has been reported to occur in humans following high level exposure to uranium[2]. There is some evidence that uranium inhibits both sodium transport-dependent and sodium transport-independent ATP use and also inhibits mitochondrial oxidative phosphorylation in the renal proximal tubules.

10. Renal toxicity was observed in early studies in rats, dogs and rabbits fed high doses of uranium compounds for periods of 30 days to 2 years discussed by the US Environmental Protection Agency[7]. The lowest dose tested was equivalent to 2.8 mg/kg bw/day uranium in a 30-day study in rabbits, in which "modest" renal damage was noted. In longer term studies in rats and dogs, the renal effects were generally identifiable within 30 days of the start of treatment[7].

11. More recent studies have been conducted in New Zealand white rabbits and Sprague-Dawley rats treated with uranium for a maximum of 91 days[8,9,10].

12. The sub-chronic study in rats[8] was used as the basis of the WHO TDI. Groups of 15 male and female rats were given drinking water containing 0.96, 4.8, 24, 120 or 600 mg/L uranyl nitrate hexahydrate. The doses received were equivalent to 0.06, 0.31, 1.52, 7.54 and 36.73 mg/kg bw/day uranium in male rats and 0.09, 0.42, 2.01, 9.98 and 53.56 mg/kg bw/day uranium in female rats. There were no treatment related differences in fluid or food consumption. No significant dose-related effects were found in a range of haematological and serum biochemical parameters. Urinalysis was not conducted.

13. Kidney weights were unaffected by uranium treatment. However, treatment related lesions were observed in both sexes at all doses. In males, nuclear vesiculation, cytoplasmic vacuolation and tubular dilation were observed at all dose levels. At doses of 0.31 mg/kg bw/day uranium, glomerular adhesions, apical displacement of the proximal tubular epithelial nuclei and cytoplasmic degranulation were also apparent. The authors considered these effects could result in permanent injury to basement membranes with loss of nephrons and reduced renal function. In females, nuclear vesiculation of the tubular epithelial nuclei, capsular sclerosis of glomeruli and reticulin sclerosis of the interstitial membranes was observed at all doses, and anisokaryosis in all but one of the mid-dose group. The authors considered the capsular sclerosis of the glomeruli and the reticulin sclerosis of the interstitial membranes in the females to be particularly important as although not severe effects, they were non-reversible and thus sustained exposure could lead to more damaged glomeruli and impaired renal function. There was no clear dose-response for the adverse pathological effects observed over a large (600-fold) dose range.

14. In a comparable study in rabbits, dose-dependent histopathological changes in the kidney were reported. The changes observed in the kidneys consisted of foci of cytoplasmic vacuolation in proximal renal tubular epithelium resting on normal basement membrane. This was accompanied by vesiculation and pyknosis of tubular nuclei, where the epithelium was injured prior to any changes in the basement membrane. However, the interpretation of the findings in this study was complicated by the occurrence of Pasteurella infection in some of the male rabbits. Urinalysis indicated few significant changes in the treated animals. In a subsequent reversibility study[10] the adverse kidney effects had not completely or consistently recovered after the 91-day recovery period in the top dose animals.

Epidemiology Studies

15. A number of studies of human populations have been conducted in areas of Canada where the drinking water contains naturally high levels of uranium. Although uranium intakes in these populations have not been linked to overt kidney disease, correlations have been shown between uranium exposure and various biomarkers of renal toxicity.

16. Clinical studies discussed by WHO[2] of 324 persons exposed to concentrations of uranium of up to 700 μg/L in drinking water showed a trend of increasing ,-2-microglobulin excretion. This suggested the presence of an early sub-clinical tubular defect with β-2 microglobulin being a useful marker of sub-clinical toxicity.

17. In a preliminary study[11], microalbuminuria (a marker for glomerular damage) was assessed in 100 people consuming drinking water containing up to 14.7 μg/L uranium. Linear regression analysis revealed a statistically significant association between 'uranium cumulative index' (based on the level of uranium, the level of consumption of the water and the length of time living at the current residence) and urinary albumin levels. However, most subjects had levels of urinary albumin within the normal range. The authors concluded that there was a relationship between uranium exposure and microalbuminura but that it was not clinically significant at the levels of exposure measured in the study.

18. Zamora and colleagues (1998)[12] measured indicators of kidney function in two groups consuming drinking water containing either <1 μg/L or 2-781 μg/L uranium. A correlation was found between uranium intake and urinary levels of glucose, alkaline phosphatase and ,-2-microglobulin. The authors concluded that at the levels of intake observed in the study (0.004-9 μg/kg bw) the chronic ingestion of uranium in drinking water affected kidney function at the proximal tubule.

19. A study by Kurttio *et al*., (2002)[13] measured a range of serum and urinary parameters (calcium, phosphate, glucose, albumin, creatinine and β-microglobulin) to assess renal function in 325 Finnish subjects exposed to high (>100 μg/L), medium (10-100 μg/L) or low (<10 μg/L) levels of uranium in well water. Urinary uranium levels were associated with increased fractional excretion of calcium, phosphate and glucose. Uranium concentration in drinking water and daily intake of uranium was associated with increased fractional excretion of calcium only. Uranium exposure was not associated with impairment of creatinine clearance or increase in urinary albumin, which are markers of renal injury. The authors concluded that uranium exposure was weakly associated with altered proximal tubule function without a clear threshold, this was taken to suggest that even low uranium levels can cause nephrotoxic effects. However, glomerular function was not affected, even in the high uranium exposure group. The authors considered that the safe concentration of uranium was within the range 2-30 μg/L.

Derivation of the WHO TDI and guideline value for drinking water

20. The WHO considered nephrotoxicity to be the most sensitive adverse effect, and derived a TDI for soluble uranium based on the lowest available lowest observed adverse effect level (LOAEL) of 0.06 mg/kg bw/day uranium from the male rats in the 91-day study[8]. A total uncertainty factor of 100 was applied, incorporating factors of 10 for inter-species variation and 10 for inter-individual variation. The resulting TDI was 0.6 μg/kg bw/day. An additional uncertainty factor for extrapolation from a LOAEL to a no observed adverse effect level (NOAEL) was not considered necessary because of the "minimal degree of severity" of the histopathological changes observed. Since the estimated half-life of uranium in the kidney was 15 days and there was no suggestion that the severity of the lesions would be exacerbated following continued exposure, an additional uncertainty factor was not required for extrapolation from sub-chronic to chronic exposure.

21. The WHO then established a provisional "guideline value" for uranium levels in drinking water. Following consideration of uranium levels in food, 80% of the TDI was allocated to intake from drinking water. Based on the assumption that a 60 kg adult consumes 2 L/day water, this resulted in a provisional guideline of 15 μg/L.

Uranium exposure in infants

22. Recent intake calculations have used a body weight of 4.5 kg and a consumption of 700 mL formula/day to represent the highest ratio between intake and bodyweight in infants[14,15] and these values have been used here to estimate potential infant exposures to uranium. Uranium exposure from food has not been taken into account as uranium levels are lower. Data from the 2001 Total Diet Study[16] suggest that at the highest (97.5th) levels of exposure, uranium in food provides 6-16% of the TDI for adults and toddlers respectively.

23. If formula milk was reconstituted with water containing 15 μg/L uranium, consumption of 700 mL/day would represent an intake of 10.5 μg or 2.3 μg/kg bw/day for a 4.5 kg infant compared to the TDI of 0.6 μg/kg bw/day, a 4-fold exceedance. As noted above, this is the highest calculated ratio and would change with body weight and milk consumption. At six months of age, other foods would be introduced to the diet and uranium exposure would be expected to decrease.

Discussion

24. The database for uranium toxicity is limited and further work would be desirable to assist in the risk assessment process. For example, there are few data available on uranium absorption which appears to vary between species. Limited data from laboratory animals suggest that uptake in neonatal animals is higher than in adults. There are no data on uranium uptake in human infants.

25. The study in male rats[8] used unconventional terminology, which is descriptive of morphology rather than diagnostic. The increase in irreversible capsular sclerosis and reticulin sclerosis in the females can be considered a clear adverse effect but was not dose-related and the severity of the lesions was not clearly graded. The authors did not consider the effects to be severe, suggesting they may be close to the NOAEL. The nuclear effects are of uncertain significance and are not reliable for use in setting the NOAEL. The WHO did not apply an uncertainty factor to extrapolate from a LOAEL to a NOAEL, suggesting that they considered the LOAEL to be a NOAEL.

26. The available data suggest that mild nephrotoxic effects associated with moderate levels of uranium exposure are reversible once exposure has ceased. This was demonstrated in a recovery study in rabbits by Gilman and colleagues[10]. As noted previously the half life of uranium is 15 days and the damage is not cumulative.

27. A number of epidemiological studies are available which examine the relationship between kidney function and uranium in drinking water. Some changes in urinary parameters and proximal tubule function are apparent at higher levels of uranium exposure but there is no evidence of effects on renal function. However, the epidemiological studies are of relatively small groups and do not specifically consider infants.

Conclusions

28. There are a number of limitations in the design and interpretation of the study by Gilman *et al* (1998a)[8] which was used by WHO to establish a TDI. However, despite these limitations, the TDI and accompanying guideline level of 15 μg/L for uranium in tap water would be expected to be protective of public health.

29. Reconstituting infant formula with water containing uranium at the WHO guideline value of 15 μg/L could lead to uranium intakes by infants up to six months of age exceeding the WHO TDI by about 4-fold.

30. It is possible that uranium absorption is higher in young infants, and the implications of a modest exceedance of the TDI are uncertain.

31. It is noted that the database on uranium toxicity is incomplete, however, on the basis of the available evidence, this potential exposure of formula fed infants does not raise specific concerns for health.

COT statement 2006/07
May 2006

References

1. BfR (2005) Uranium in mineral water: small quantities tolerated by adults, but water for baby food should be uranium-free: Statement No. 024/2005, Bundesinstitut fur Risikobewertung, 13 May 2005.

2. WHO (2005). Uranium in Drinking Water. Background Document for development of WHO guidelines for Drinking Water Quality. WHO/SDE/03.04/118.

3. Wrenn, M.E., Durbin, P.A., Howard, B., Lipsztein, J., Rundo, J., Still, E.T., Willis, D.I. (1985). Metabolism of Ingested U and Ra. Health Physics, 48, 601-633.

4. Leggett, R.W., Harrison, J.D. (1995) Fractional Absorption of Ingested Uranium In Humans, Health Physics, 68, 484-498.

5. Sullivan, M.F., Gorham, L.S. (1980). Absorption of Actinide Elements from the Gastrointestinal tract of Neonatal Animals. Health Physics, 38, 173-185.

6. Sullivan, M.F., Gorham, L.S. (1982). Further Studies on the Absorption of Actinide Elements from the Gastrointestinal tract of Neonatal Animals. Health Physics, 43, 509-519.

7. EPA (1989). Integrated Risk Information System (IRIS): Uranium, soluble salts: http://www.epa.gov/iris/subst/0421.htm. Last revised 1989.

8. Gilman, A.P., Villeneuve, D.C., Secours, V.E., Yagminas, A.P., Tracy, B.L., Quinn, J.M., Valli, V.E., Willes, R.J., Moss, A.M. (1998a). Uranyl Nitrate: 28-Day and 91 day Toxicity Studies in the Sprague-Dawley Rat. Toxicological Sciences, 41, 117-128.

9. Gilman, A.P., Villeneuve, D.C., Secours, V.E., Yagminas, A.P., Tracy, B.L., Quinn, J.M., Valli, V.E., Willes, R.J., Moss, A.M. (1998b). Uranyl Nitrate: 91-day Toxicity Studies in the Male New Zealand White Rabbit. Toxicological Sciences, 41, 138-151.

10. Gilman, A.P., Moss, A.M., Villeneuve, D.C., Secours, V.E., Yagminas, A.P., Tracy, B.L., Quinn, J.M., Long, G., Valli, V.E. (1998c). Uranyl Nitrate: 91-day Exposure and Recovery Studies in the Male New Zealand White Rabbit. Toxicological Sciences, 41, 138-151.

11. Mao. Y., Desmeules, M., Schaubel, D., Bérubé, D., Dyck, R., Brûlé,D., Thoma, B. (1995). Inorganic Components of Drinking Water and Microalbuminuria. Environmental Research, 71, 135-140

12. Zamora, M.L., Tracy, B.L., Zielinski, J.M., Meyerhof, D.P., Moss, M.A. (1998). Chronic Ingestion of Uranium in Drinking water: a Study of Kidney Bioeffects in Humans. Toxicological Sciences, 43, 68-77.

13. Kurttio, P., Auvinen, A., Salonen, L., Saha, H., Pekkanen, J. Mäkeläine, I., Väisäinen, S.B., Penttilä, I.M., Komulainen, H. (2002). Renal Effects of Uranium in Drinking Water. Environmental Health Perspectives, 110, 337-342.

14. SCF (2002). Opinion of the Scientific Committee on Food on bisphenol A. Scientific Committee on Food. Available at: http://europa.eu.int/comm/food/fs/sc/scf/out128_en.pdf.

15. EFSA (2005). Opinion of the AFC Panel related to semicarbazide in food. European Food Safety Authority. Available at: http://www.efsa.eu.int/science/afc/afc_opinions/1005_en.html.

16. FSA (2004). Food Safety Information Sheet 56/04. Uranium-238 in the 2001 Total Diet Study: http://www.food.gov.uk/science/surveillance/fsis2004branch/fsis5604

Statement on 2005 WHO Toxic Equivalency Factors for dioxins and dioxin-like compounds

Non-Technical Summary

1. Dioxins and dioxin-like chemicals are pollutants which accumulate in the food chain. It is generally acknowledged that their toxicity is mediated by the same mechanism of action. Hence, it is important that their toxic effects are evaluated together. Since their potency varies greatly, toxic equivalency factors (TEFs) have been developed in order to compare the various chemicals and assess the combined effect of mixtures of dioxins and dioxin-like chemicals. This statement relates to a recent World Health Organisation (WHO) review of the most up to date scientific information that compares the potency of these chemicals. Re-evaluation of the TEF values has resulted in small reductions in the estimated exposure of the UK population to the total activity of dioxins and dioxin-like chemicals. The COT agrees with the scientific rationale for the re-evaluated TEF values and concludes that they should be used in future UK assessments of dietary exposure to dioxins and dioxin-like compounds.

Introduction

2. Dioxins and dioxin-like chemicals are persistent organic pollutants that are resistant to metabolism and subject to bioaccumulation. Most, if not all, of their toxic and biological effects are mediated by the aryl hydrocarbon receptor (AhR). Many different congeners are released into the environment by industrial activity and, since these chemicals share a common mechanism, risk assessment should reflect the mixture rather than the isolated chemical. Experiments using mixtures of congeners are consistent with an additive model and, as a result of this generally accepted additivity, the toxic equivalency concept was developed in the 1980s.

3. The WHO has, on a number of occasions, convened Expert Panels to discuss toxic equivalency factor (TEF) values. This is because the Expert Panel has stated that the TEF concept should be thought of as an interim methodology, which should be subject to periodic review as new scientific information becomes available[1]. The Expert Panel initially set TEF values at a meeting in 1993 and re-evaluated them at a subsequent meeting in 1997. These re-evaluated TEFs were published in 1998 and endorsed by the COT in the same year[2]. In 2001, the COT undertook an extensive review of dioxins and dioxin-like chemicals[3], which resulted in the adoption of a Tolerable Daily Intake (TDI) of 2 pg WHO-TEQ/kg bw/day*.

4. In 2004 the European Food Safety Authority (EFSA) organised a scientific colloquium to discuss the risk assessment of dioxins and dioxin-like chemicals. This colloquium highlighted some differences in approaches to the risk assessment of these compounds and concluded that it was timely to review the TEF scheme. The WHO-IPCS Expert Panel was reconvened in June 2005 to perform the next periodic re-evaluation of the TEF values and to discuss and develop the TEF concept. A report of this meeting will be published in due course[4].

* The total toxic equivalent (TEQ) is defined as the sum of the products of the concentration of each congener, multiplied by the toxic equivalency factor (TEF).

5. The 2005 WHO-IPCS re-evaluation was based on a recently published relative effect potency (REP) database[1], which was constructed using refined inclusion/exclusion criteria. Of the REP values from the previous database used in the 1997 TEF reassessment, 47% met the more stringent criteria. These 381 REP values were combined with 253 REP values from new studies, forming the 2005 REP database[1]. Unweighted REP values from this database were used as a starting point for the TEF re-evaluation. When the 1997 TEF value for the congener differed from the 75th percentile of the *in vivo* REP distribution in the 2005 database, a more extensive review of the data was performed. During this review, expert judgement was used to assess individual studies and derive an appropriate TEF value based on studies that were most relevant to human exposure.

6. This re-evaluation uses half order of magnitude increments on a logarithmic scale (0.03, 0.1, 0.3 etc). TEF values represent 'order of magnitude estimates', therefore, a degree of uncertainty is implicit. The Expert Panel considered that these increments would be useful in the future so that the uncertainty of TEF values can be better described. Previous evaluations used increments of 0.01, 0.05, 0.1 etc.

Expert Panel Re-Evaluation

7. The re-evaluated TEF values are shown in Table 1. Detailed explanations of how the individual TEF values were determined have been reported by van den Berg et al.[4].

8. The TEF values for OCDD and OCDF were increased from 0.0001 to 0.0003 in light of a new subchronic toxicity study[5,6] and other *in vitro* data.

9. The TEF value for 1,2,3,7,8-PeCDF was reduced from 0.05 to 0.03 in line with the new half log increments. The rationale for this reduction was explained by van den Berg *et al.*[4]*:*

   "The WHO 1998 TEF was set at 0.05 which is within the 50th and 75th percentile of the REP distribution of eight in vivo studies. A new study[5] *found a REP of 0.01 for effects on hepatic vitamin A reduction, but another study reported a REP of 0.045 for cleft palate*[7]*. The majority of the vivo studies report a REP value below 0.1 but many relevant studies have REPs above 0.01. Therefore the Expert Panel decided that the 2005 TEF should become 0.03."*

10. The TEF value for 2,3,4,7,8-PeCDF was reduced from 0.5 to 0.3, also to be in line with the new half log increments. Rationale for reduction from van den Berg *et al.*[4] :

    "The WHO 1998 TEF was set at 0.5 which is well above the 75th percentile of the REP distribution of eight in vivo studies. Results from the long term US National Toxicology Programme (NTP) study in female Sprague Dawley rats using many different endpoints had become available. The REPs for neoplastic endpoints from the NTP study are around 0.2 to 0.3, while non-neoplastic endpoints have REPs that range from 0.7 to 1.1[8] *An older subchronic study pointed towards a REP of 0.4*[9]*. More recent studies using hepatic vitamin A reduction and immunological effects as endpoints also point toward a TEF below 0.5*[5,10]*. In view of this new information the consensus of the Expert Panel was to change the WHO 2005 TEF to 0.3."*

11. PCB 81 was increased from 0.0001 to 0.0003 on the basis of *in vitro* studies which indicate that PCB 81 is more potent than PCB 77. However, the Expert Panel expressed a low confidence in this assessment due to the absence of a reliable REP for PCB 81. PCB 169 was increased from 0.01 to 0.03 because the 1998 TEF was close to the median of the *in vivo* REP distribution and it was considered appropriate to raise the TEF to between the 50th and 75th percentile.

12. The 1998 TEF values for the mono-ortho substituted PCBs ranged from 0.00001 to 0.0005. The wide variation in REPs is illustrated in Figure 3 of van den Berg *et al.*[4]. In view of potential contamination of mono-ortho substituted PCB samples with more potent congeners, the Expert Panel expressed low confidence in the higher REP values within this group. The most environmentally relevant mono-ortho PCBs are 27, 105, 118, and 156 and it was decided to use the medians of the REP distribution range of these PCB congeners as a guide. This resulted in a recommended TEF of 0.00003 for these mono-ortho PCBs. A differentiation for all other remaining mono-ortho PCBs was considered unfeasible by the Expert Panel due to the lack of sufficient experimental data. Consequently a TEF of 0.00003 was recommended for all mono-ortho PCBs.

Table 1. Summary of WHO 1998 and WHO 2005 TEF values

Compound	1998 WHO-TEFs	2005 WHO-TEFs
chlorinated dibenzo-p-dioxins		
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	1	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.0001	**0.0003**
chlorinated dibenzofurans		
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDF	0.05	**0.03**
2,3,4,7,8-PeCDF	0.5	**0.3**
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,6,7,8,9-HpCDF	0.01	0.01
OCDF	0.0001	**0.0003**
non-ortho substituted PCBs		
3,3',4,4'-tetraCB (PCB 77)	0.0001	0.0001
3,4,4',5-tetraCB (PCB 81)	0.0001	**0.0003**
3,3',4,4',5-pentaCB (PCB 126)	0.1	0.1
3,3',4,4',5,5'-hexaCB (PCB 169)	0.01	**0.03**
mono-ortho substituted PCBs		
2,3,3',4,4'-pentaCB (PCB 105)	0.0001	**0.00003**
2,3,4,4',5-pentaCB (PCB 114)	0.0005	**0.00003**
2,3',4,4',5-pentaCB (PCB 118)	0.0001	**0.00003**
2',3,4,4',5-pentaCB (PCB 123)	0.0001	**0.00003**
2,3,3',4,4',5-hexaCB (PCB 156)	0.0005	**0.00003**
2,3,3',4,4',5'-hexaCB (PCB 157)	0.0005	**0.00003**
2,3',4,4',5,5'-hexaCB (PCB 167)	0.00001	**0.00003**
2,3,3',4,4',5,5'-heptaCB (PCB 189)	0.0001	**0.00003**

Bold values indicate a change in TEF value.

Abbreviations: T/Pe/Hx/Hp/OCDD, Tetr/Penta/Hexa/Hepta/Octa chlorodibenzodioxin;
T/Pe/Hx/Hp/OCDF, Tetra/Penta /Hexa/Hepta/Octa chlorodibenzofuran;
(P)CB, (Poly)chlorinated biphenyl.

Recalculation of Total Dietary Intakes

13. Previously 1998 WHO TEF values were used to estimate the dietary intakes of UK toddlers, school children, adults and senior citizens, and this was reported in the Food Survey Information Sheet (FSIS) 38/03[11]. These dietary intakes have been recalculated using the 2005 TEF values. Table 2 summarises the estimated upper bound dietary intakes of all age groups of dioxins and dioxin-like PCBs in 2001. Recalculation using the 2005 TEF values has resulted in reductions in estimated dietary intakes for the majority of age groups and occasionally no change, when compared to the 1998 TEF values. For comparison, the estimated intakes based on 1998 TEF values have been included in brackets.

Table 2. Summary of estimated upper bound dietary intakes of all age groups of dioxins and dioxin-like PCBs in 2001 calculated using 2005 WHO-TEFs (1998 WHO-TEFs in brackets)

Age group	Average Dietary Intakes (pg WHO-TEQ/kg bw/day)			High Level Dietary Intakes (pg WHO-TEQ/kg bw/day)		
	Dioxins	PCBs	Dioxins +PCBs	Dioxins	PCBs	Dioxins +PCBs
Senior citizens *						
living at home	0.3 (0.3)	0.3 (0.4)	0.6 (0.7)	0.6 (0.7)	0.6 (0.8)	1.1 (1.4)
in old peoples' homes	0.4 (0.4)	0.4 (0.5)	0.7 (0.9)	0.6 (0.8)	0.7 (0.9)	1.3 (1.6)
Adults *	0.3 (0.4)	0.3 (0.5)	0.7 (0.9)	0.6 (0.7)	0.8 (1.0)	1.4 (1.7)
Schoolchildren *						
4-6 years	0.7 (0.9)	0.7 (0.9)	1.4 (1.8)	1.4 (1.7)	1.4 (1.8)	2.8 (3.4)
7-10 years	0.6 (0.7)	0.5 (0.7)	1.1 (1.4)	1.0 (1.2)	1.0 (1.4)	2.0 (2.5)
11-14 years	0.4 (0.4)	0.3 (0.5)	0.7 (0.9)	0.8 (0.9)	0.7 (1.0)	1.5 (1.9)
15-18 years	0.3 (0.3)	0.3 (0.4)	0.6 (0.7)	0.5 (0.6)	0.5 (0.7)	1.0 (1.3)
Toddlers *						
1.5-2.5 years	1.0 (1.1)	0.9 (1.1)	1.9 (2.2)	2.1 (2.5)	2.1 (2.5)	4.2 (4.8)
2.5-3.5 years	0.8 (0.9)	0.7 (1.0)	1.6 (1.9)	1.7 (1.9)	1.7 (2.1)	3.5 (4.0)
3.5-4.5 years	0.8 (0.8)	0.7 (0.9)	1.4 (1.7)	1.5 (1.7)	1.4 (1.8)	2.9 (3.4)
Population average**	0.3 (0.3)	0.2 (0.4)	0.5 (0.7)			

Notes: Combined dietary intakes of dioxins and dioxin-like PCBs may not equal the sum of the separate intakes due to rounding.
* Consumer dietary intakes estimated using food consumption data from the National Diet and Nutrition Survey Programme (NDNS).
** Estimated using food consumption data from the National Food Survey. This method cannot estimate high level intakes.

14. The UK TDI of 2 pg WHO-TEQ/kg bw/day is derived from data relating to 2,3,7,8-TCDD, a potent dioxin congener and point of reference for the TEF values of other congeners. Particularly, this TDI was established based on a study showing effects of 2,3,7,8-TCDD on the developing male reproductive system, mediated via the maternal body burden[12]. Therefore, since the TDI was set based on 2,3,7,8-TCDD which has a TEF of 1, the TDI is unaffected by the re-evaluation of individual TEF values.

15. Table 3, recalculated from Table 7 of FSIS 38/03[11], summarises the percentage of consumers of different age groups who were estimated to exceed the UK TDI for dioxins and dioxin-like PCBs from the whole diet in 2001. Recalculation using the 2005 TEF values resulted in reductions in the percentage of consumers estimated to exceed the TDI. 2,3,4,7,8-PeCDF constituted approximately 10% of the average adult consumer TEQ for dioxins and dioxin-like PCBs. Therefore, reduction of the TEF for this congener from 0.5 to 0.3 was responsible for the majority of the reduction in calculated dietary exposure.

Table 3. Percentage of consumers of different age groups who are estimated to exceed the UK TDI for dioxins and dioxin-like PCBs from the whole diet in 2001

Age group	1998 WHO-TEFs	2005 WHO-TEFs
Senior citizens		
living at home	0.1	0.0
in old peoples' homes	0.0	0.0
Adults	1.1	0.03
Schoolchildren		
4-6 years	35.0	14.0
7-10 years	10.0	3.0
11-14 years	1.7	0.0
15-18 years	0.0	0.0
all children	10.0	3.8
Toddlers		
1.5-2.5 years	48.0	34.0
2.5-3.5 years	35.0	23.0
3.5-4.5 years	28.0	16.0
all toddlers	37.0	25.0

16. These recalculations continue to show that an appreciable number of toddlers exceed the UK TDI, with the highest estimated exposures predominantly in the younger age group. The skewed distribution of intakes of individual toddlers is shown in Figure 1. This graph shows that, whilst there are a few outliers, the majority of toddlers have intakes close to the TDI. The TDI (2 pg WHO-TEQ/kg bw/day) lies on a steep gradient of the toddler distribution curve; hence, a small decrease in calculated TEQ can result in a large reduction in the percentage exceeding the TDI.

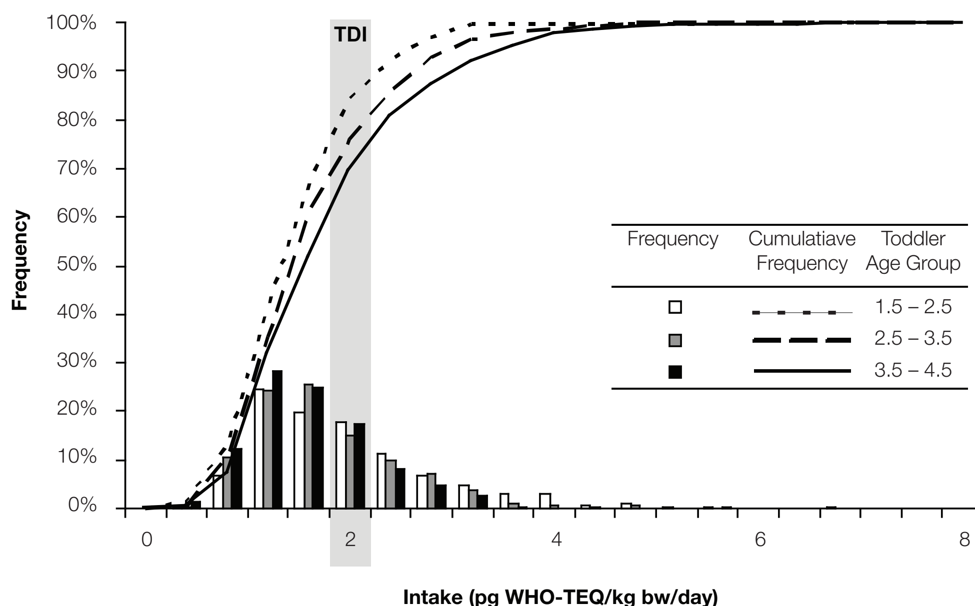

Figure 1. Toddlers' Dietary Intake of Dioxins and Dioxin-like Compounds Calculated using the 2005 TEF values

17. The NDNS programme does not gather consumption data for the 0 – 1.5 year age group; however, several surveys have been conducted by the Food Standards Agency, in order to assess the potential dietary exposure of this group. Surveys analysing infant formula[13] and baby food[14] indicate that these sources are unlikely to result in a dietary intake which exceeds the TDI. However, analysis of a small set of human breast milk samples indicated that infant dietary intakes, when recalculated using 2005 TEF values, are likely to be in the region of 35 pg WHO-TEQ/kg bw/day at 2 months, falling to 8 pg WHO-TEQ/kg bw/day at 10 months[15].

18. The health implications of exceeding the TDI at an early age are not clear. Previously, the Committee considered that, in view of the fact that the TDI was set based on effects on the developing male reproductive system mediated by maternal body burden, there was uncertainty with respect to whether similar effects would arise from post-natal exposure. However, the Committee concluded that there was no basis for assuming that the young infant is at increased risk[15]. Furthermore, recent publications suggest that the half lives of dioxin and certain other furan congeners in young children are considerably shorter than in adults[16,17]. Estimated exposures for all age groups have substantially declined since 1982[11] and it is anticipated that exposures will continue to decline in the future, due to the environmental controls already in place and those planned.

Development of the TEF Concept

19. The WHO Expert Panel noted that recent *in vivo* mixtures studies continue to demonstrate additivity, a tenet of the TEF concept. The Panel stated that PCB 126 could be used as a reference compound in rat studies with a REP of 0.1, but that further work is required to confirm that PCB 126 is suitable for use as a reference compound in mouse studies. It was considered that more research was also required for REP values in human systems to establish whether TEFs based on rodent species are also valid for humans.

20. The 'Ideal REP study design' was discussed and general guidelines suggested for both *in vivo* and *in vitro* studies. These are reported in van den Berg *et al.*[4]. The Panel recognised that criteria for weighted REP values, based on study type (*in vivo* versus *in vitro*, chronic versus acute, etc.), would be of value to future assessments.

21. The Panel noted that the current approach does not describe the range of REP values, and may reflect a bias in the judgement of the Expert Panel. Probabilistic methodology would require weighting factors to be applied to REPs determined in different types of study. Distribution of REPs would be expressed in terms of maximum and minimum values and would better describe the level of uncertainty. However, the Panel was concerned that varying degrees of conservatism might alter how these ranges are interpreted by national authorities.

22. Emerging evidence suggests that relative potency of several dioxins and dioxin-like compounds may differ when calculated based on administered dose versus tissue concentration (body burden). The possibility of using 'systemic TEFs', based on body burden, was raised by the Expert Panel. It was considered that, whilst from a biological and toxicological point of view, the use of systemic TEFs is recommended, at present the data are insufficient to develop this concept. The need to determine whether *in vitro* TEFs can be used as surrogates for systemic TEFs was highlighted. The Panel envisaged using systemic TEFs alongside intake TEFs for ingestion situations.

23. Concern was also expressed at the use of TEF values for abiotic matrices since TEF values have been developed primarily for calculating dietary exposure, with the greatest weight being placed on data from oral intake studies. The Expert Panel emphasised that, whilst calculating TEQ values may be useful for comparing abiotic matrices, factors such as fate, transport and bioavailability from each matrix should be specifically considered as part of the risk assessment.

Other Compounds for Potential Inclusion in the TEF Scheme

24. The Expert Panel considered the polybrominated dibenzo-p-dioxins (PBDDs) and dibenzofurans (PBDFs) should be given high priority for inclusion in the TEF scheme. A better human exposure analysis and more REP studies are required. Based on the AhR mechanism of action, inclusion of certain congeners of polybrominated biphenyls (PBBs) was also considered appropriate. However, further human exposure analysis should identify the possible relevance of PBBs to the total TEQ.

25. To address this exposure data requirement the FSA has surveyed samples from the 2003 and 2004 Total Diet Studies and related them to food consumption data[18]. In this analysis, TEF values for the chlorinated congeners were applied to brominated congeners based on advice provided by the committee[19]. This assumes equivalent potency and a similar structure activity relationship. This study estimated a dietary intake of <0.4 pg TEQ/kg bw/day for brominated dioxins and dioxin-like PBBs. On the basis of this information, the COT considered this intake did not raise additional toxicological concerns[20].

26. The Expert Panel noted that early *in vitro* studies suggest the mixed halogenated dibenzo-p-dioxins (PXCDDs) and dibenzofurans (PXCDFs) follow the same structure-activity rules as the PCDDs and PCDFs. It was felt that these should definitely be considered for inclusion in the scheme.

27. The possibility of contamination with more potent congeners requires attention before polychlorinated and brominated naphthalenes (PCNs and PBNs) can be considered for inclusion in the TEF scheme. Similar contamination issues also affect hexachlorobenzene (HCB). In addition confirmation of the dioxin-like properties of HCB are required before this compound can be considered for inclusion.

28. There is a need for more *in vivo* and *in vitro* information on PCB 37 (3,4,4'-TCB) in order to consider inclusion in the TEF scheme.

29. The Expert Panel considered that pure polybrominated diphenylethers (PBDEs) do not have AhR agonist properties and should not be included in the TEF scheme.

30. It was noted that non dioxin-like AhR ligands may modulate the effect of dioxin congeners and the potential impact of these compounds on the risk of toxicity posed by exposure to a particular level of TEQs should be further investigated.

Committee Discussion

31. Members reiterated that, in some instances, TEF values for individual congeners are based on a limited dataset. Where more data are available for an individual congener, there is commonly a large spread of REP values, which are based on a range of different toxicological endpoints. Owing to this inherent variability, the TEF values are, at best, order of magnitude estimates. It was also considered necessary to stress that, whilst TEFs are generally calculated based on administered dose, the toxicological endpoint used to derive the TDI was based upon maternal TCDD body burden. The TDI is expressed in terms of amount of TCDD (and hence TEQ) that would need to be ingested to achieve the 'tolerable body burden'. However, the amount of TEQ ingested on a daily basis is unlikely to directly reflect the total body burden of dioxins and dioxin-like compounds due to differences in the bioavailability and biological half-life of the various congeners.

32. Members highlighted that the TEF principle assumes that the toxicity of these compounds is mediated by a common aryl hydrocarbon receptor (AhR) mediated mechanism. It was noted that the possibility of non-AhR mediated toxicity should be considered if TEF values were substantially lowered.

33. Concern was expressed that an appreciable number of toddlers exceed the TDI and that exposure is likely to be higher in breast fed babies. Previously, the COT noted that intake is highest during breast feeding and that concentrations of dioxins in breast milk have decreased in recent years, in line with decreases in dietary exposure. Continuing controls on emissions to the environment are expected to further reduce dietary intake in the future.

Conclusions

34. We agree with the scientific rationale for the re-evaluated TEF values; although we concur with the opinion of the WHO Expert Panel, that this should be thought of as an 'interim' methodology, until a more suitable method of estimating risk from dioxins and dioxin-like compounds can be found.

35. We conclude that the revision of the TEFs does not raise additional concerns regarding exposure to dioxins and dioxin-like compounds, and that they should be used in future UK assessments of dietary exposure.

COT statement 2006/13
December 2006

References

1. Haws, L.C., Su, S.H., Harris, M., Devito, M.J., Walker, N.J., Farland, W.H., Finley, B., Birnbaum, L.S. (2006). Development of a refined database of mammalian relative potency estimates for dioxin-like compounds. *Toxicol Sci* 89: 4-30.

2. COT (1998). Toxic Equivalency Factors for Dioxin Analogues. *COT/COC/COM Annual Report*, 18.

3. COT (2001). Statement on the Tolerable Daily Intake for dioxins and dioxin-like polychlorinated biphenyls. *COT/COC/COM Annual Report*, 61-90.

4. van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E. (2006). The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds. *Toxicol Sci*

5. Fattore, E., Trossvik, C., Hakansson, H. (2000). Relative potency values derived from hepatic vitamin A reduction in male and female Sprague-Dawley rats following subchronic dietary exposure to individual polychlorinated dibenzo-p-dioxin and dibenzofuran congeners and a mixture thereof. *Toxicol Appl Pharmacol* 165: 184-194.

6. Wermelinger, M., Poiger, H., Schlatter, C. (1990). Results of a 9-month feeding study with OCDD and OCDF in rats. *Organohalogen Compounds* 1: 221-224.

7. Takagi, A., Hirose, A., Hirabayashi, Y., Kaneko, T., Ema, M., Kanno, J. (2003). Assessment of the cleft palate induction by seven PCDD/Fcongeners in the mouse fetus. *Organohalogen Compounds* 64: 336-338.

8. Walker, N.J., Crockett, P.W., Nyska, A., Brix, A.E., Jokinen, M.P., Sells, D.M., Hailey, J.R., Easterling, M., Haseman, J.K., Yin, M., Wyde, M.E., Bucher, J.R., Portier, C.J. (2005). Dose-additive carcinogenicity of a defined mixture of "dioxin-like compounds". *Environ Health Perspect* 113: 43-48.

9. Pluess, N., Poiger, H., Hohbach, C., Schlatter, C. (1998). Subchronic toxicity of some chlorinated dibenzofurans (PCDFs) and a mixture of PCDFs and chlorinated dibenzodioxins (PCDDs) in rats. *Chemosphere* 17: 973-984.

10. Johnson, C.W., Williams, W.C., Copeland, C.B., Devito, M.J., Smialowicz, R.J. (2000). Sensitivity of the SRBC PFC assay versus ELISA for detection of immunosuppression by TCDD and TCDD-like congeners. *Toxicology* 156: 1-11.

11. FSA (2003). Food Survey Information Sheet 38/03: Dioxins and Dioxin-like PCBs in the UK Diet: 2001 Total Diet Study Samples. 1-30.

12. Faqi, A.S., Dalsenter, P.R., Merker, H.J., Chahoud, I. (1998). Reproductive toxicity and tissue concentrations of low doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin in male offspring rats exposed throughout pregnancy and lactation. *Toxicol Appl Pharmacol* 150: 383-392.

13. FSA (2004). Food Survey Information Sheet 49/04: Dioxins and dioxin-like PCBs in infant formulae. 1-84.

14. FSA (2004). Food Survey Information Sheet 60/04: Dioxins and dioxin-like PCBs in baby food. 1-30.

15. COT (2004). Statement on the toxicological evaluation of chemical analyses carried out as part of a pilot study for a breast milk archive. *COT/COC/COM Annual Report* , 71-79.

16. Kerger, B.D., Leung, H.W., Scott, P., Paustenbach, D.J., Needham, L.L., Patterson, D.G., Jr., Gerthoux, P.M., Mocarelli, P. (2006). Age- and concentration-dependent elimination half-life of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Seveso children. *Environ Health Perspect* 114: 1596-1602.

17. Leung, H.W., Kerger, B.D., Paustenbach, D.J. (2006). Elimination half-lives of selected polychlorinated dibenzodioxins and dibenzofurans in breast-fed human infants. *J Toxicol Environ Health A* 69: 437-443.

18. FSA (2006). Food Survey Information Sheet 10/06: Brominated Chemicals: UK Dietary Intakes. 1-30.

19. COT (2005). Minutes of Item 8: Preliminary discussion on combination of brominated organic contaminants for toxicological evaluation – TOX/2005/36. 9-10. Meeting Minutes.

20. COT (2006). Statement on Organic Chlorinated and Brominated Contaminants in Shellfish, Farmed and Wild Fish. 1-20.

Joint Statements of the COT and COC

Statement on Royal Commission on Environmental Pollution: crop spraying and the health of residents and bystanders

Introduction

Background to RCEP report

1. Defra announced a public consultation on the need for buffer zones between agricultural applications of pesticides and residences in July 2003. This followed discussion in the Advisory Committee on Pesticides (ACP), taking account of some stakeholders concerns regarding risks to the health of rural residents as a result of crop spraying. Members of the ACP gave the following advice to Ministers

   'Members had concluded that on the basis of the information currently available the risk assessment for bystanders used at present provided adequate protection, even if spray is applied to the edge of a field. .... Nonetheless, the Committee recognised that many people may consider it socially unacceptable to spray right to the boundary of a neighbour's property. If Ministers agree, they may wish to consider options to restrict this practice.'
   (http://www.pesticides.gov.uk/acp.asp?id=586)

2. The specific conditions of use for individual pesticide products are supplemented by guidance on best practice contained in the statutory Code of Practice for the Safe Use of Pesticides on Farms and Holdings (the "Green Code"). Although failure to follow the Code's guidance is not in itself an offence, it may be used in evidence against the user if prosecuted for breach of the law. The consultation document asked for views on the risk assessment process, the Green Code and the need for buffer zones to prevent exposure. The consultation was based on the ACP's advice that the current regulatory system is adequate to protect human health but that there may be an issue of "social acceptability" in spraying right up to the boundary of someone's property. A series of options was presented which were i) Do Nothing (i.e. risk assessment process satisfactory), or ii) Introduce buffer zones at varying distances e.g. should these be 6 m, 10 m, 100 m, 300 m. An estimate of the amount of land which would be excluded from agricultural use by each proposed buffer zone was calculated. The outcome of the consultation was one of the highest number of responses to a Defra consultation in recent years (763 replies) but the responses appeared to separate into two distinct types of reply. Farmers/Growers and representative organisations opted for the status quo (i.e. no buffer zone). Pesticide stakeholder groups and the general public (most of whom were described by Defra as being loosely tied to the campaigns led by Stakeholder groups) opted for a buffer zone. Some of the replies claimed that there were significant public health issues and chronic ill health attributed to pesticides. Defra concluded that it was not possible to make an accurate judgement of public opinion as a whole.

3. The Defra minister (Rt Hon Alun Michael) announced (16 June 2004) that he would not introduce mandatory buffer zones but had asked the RCEP to examine the evidence on which the current system is based and the reasons for people's concerns. Mr Michael in placing this request was mindful of the advice from his chief scientist Professor Howard Dalton who had been asked to review the procedures used by the Pesticides Safety Directorate (PSD) for evaluating bystander risk. Professor Dalton had subsequently confirmed he was satisfied with the procedures used.

4. The RCEP announced its review on 4 August 2004. The remit set by the Commission was; “The Commission will examine the scientific evidence on which Defra has based its decision on bystander exposure and its policy on access to information on crop spraying. The Commission will also consider wider issues related to the handling and communication of risk and uncertainty, as well as public involvement, values and perceptions in this context “.

Overview of RCEP report

5. The RCEP published its report on the 22 September 2005.[1] The RCEP introduced their report in chapter 1 by noting that the subject of the review was complex and controversial. Individuals had concerns about potential exposure to pesticides arising because they occupy properties adjacent to farmland or because they have (or have had) access to such land, for example when using footpaths. The RCEP noted the official response that there was no scientific case for taking additional measures, such as the introduction of no-spray buffer zones, to protect members of the public who may be in the vicinity of a sprayed area; however, those who considered themselves to have been adversely affected had not been reassured by this response. The RCEP held an open meeting on the 25 September 2004. A set of questions regarding health aspects, exposure and modelling, legal aspects, policy and other aspects with regard to pesticides (e.g the scale of bystander exposure) arose from this meeting. The health related questions agreed for the RCEP study are reproduced below;

   “What are the biological effects of bystander exposure to pesticides (what is the knowledge base)? What are the limits of toxicology and epidemiology in cases of bystander exposure to pesticides? How plausible is it that pesticides cause the health problems reported? What systems are in place to respond to and record bystander exposure and how well do they work (e.g GPs, The Pesticide Incident Appraisal Panel, (PIAP)?

6. The remaining sections of chapter 1 provide background information on the health effects reported to the RCEP, the definitions of bystander and resident, the potential scale of bystander and resident exposure to agricultural spraying, the level of concern in other countries, the approach taken to gathering evidence and the structure of the RCEP report.

7. Chapter 2 of the RCEP report reviewed pesticide spraying and health and has been the predominant focus of the current COT and COC review. The recommendations given in chapter 6 of the RCEP report relating to health aspects were also considered in detail. Chapters 3 (Exposure), 4 (Legal liability) and 5 (Governance of agricultural pesticides) have not been reviewed in detail by COT and COC.

8. The COT and COC acknowledged that the subject of crop spraying and potential for ill health had generated considerable public concern. The COT and COC are scientific advisory committees which can be requested by Government Departments and Agencies to provide advice on the evidence presented to them. The Committees’ remit was restricted to a review of the contents of the RCEP report as written. The Committees were not, on this occasion, asked to undertake an independent review of pesticide safety and use. The Committees agreed that their remit referred to the scientific aspects of the RCEP report in relation to health and did not include wider aspects outlined by the RCEP in their report.

9. The COT and COC appreciated that the subject of crop spraying and potential for ill health is a sensitive and important public issue and gave due regard to this when considering the RCEP report. The COT did express some broad reservations about the way in which the RCEP presented their evidence and the manner in which its findings were expressed.

Background to the COT and COC review.

10. The COT and COC were asked by Defra and the ACP to comment on the RCEP report.. Members of the COM were alerted to the report and asked to provide any comments to the secretariat (none were received) The COT and COC considered the report along with a number of published papers[2,3], some information from the DH report published in 1996 on guidance for medical practitioners[4] and an example copy of a report from the Pesticides Incidents Appraisal Panel (PIAP).[5] The Committees based their consideration on paragraphs 2.1-2.69 of chapter 2 dealing with pesticides and health and provided only limited general comments on monitoring and reporting of health effects (paragraphs 2.70-2.107) where expertise resided predominantly in the regulatory authorities (PSD and the Health and Safety Executive (HSE)). [Throughout this statement the term "bystander" has been applied as stated in the RCEP report to include other groups such as residents.]

Advice requested from COT and COC.

11. The COT and COC were asked to

    i) Consider, based on members expertise and the evidence presented in the RCEP report, whether the conclusions and recommendations reached in respect of health related topics are appropriate (see paras 6.20-6.29 of the report)

    ii) Derive COT/COC conclusions in relation to the health related questions posed by the RCEP on the basis of the evidence reviewed and members' expertise, and to consider whether these concur with those reached with RCEP.

    iii) Consider whether any further work by COT/COC/COM should be undertaken with respect to bystander pesticide risk assessment and report any suggestions for further work to the ACP.

12. The COT discussed the report on the 14 February 2006 and the COC discussed the report on the 2 March 2006. The COT considered a draft working paper at its meeting of the 28 March 2006. Both committees considered that chapter 2 of the RCEP report (Pesticide spraying and health) was the most relevant section for discussion. The following summary of COT and COC conclusions follows the structure of this chapter. Members agreed that as the COT and COC had no experience of post market monitoring systems for pesticides they only provided general comments on that section of chapter 2.

COT comments on chapter 2 of the RCEP report

[Readers may wish to access the RCEP report for content on these sections:
http://www.rcep.org.uk/cropspraying.htm]

Paragraphs 2.1-2.15: Introduction, health effects attributed to pesticides, acute effects, chronic health effects

13. Members agreed with an RCEP conclusion of this section that no firm conclusion could be drawn that pesticide exposure was causing ill health experienced by bystanders and residents. The COT commented that level of exposure was critical and it was agreed that exposure of bystanders and residents would be significantly lower than for operators, even taking into account use of personal protective equipment by operators. The COT considered identification of adverse effects in operators to be a useful model for bystanders and therefore there was reassurance regarding the potential for adverse effects in bystanders.

14. It was considered that the only possible factor which could explain a difference between exposed bystanders and operators in the incidence of chronic ill health was a particular susceptibility in some bystanders . It was noted that although operators could be considered not to represent the full heterogeneity of the general population, the systemic acceptable operator exposure level (AOEL), which was used in risk assessment for both operators and bystanders, incorporated an uncertainty factor sufficient to account for inter-individual variation in the general population and was an appropriate approach for risk assessment of bystanders and residents. The COT noted that if a bystander did accidentally get exposed to a high exposure to certain pesticides then some acute adverse effects might occur.

15. Given the heterogeneity of bystanders and their low level of exposure compared to occupationally exposed groups, it was considered that there was little merit in undertaking epidemiological studies in bystanders as a group, and that a more appropriate approach would be to investigate genetic and phenotypic characteristics in individuals with self-reported chronic ill health as compared with equally exposed but symptom free bystanders. Such an approach would be required to identify the causes of ill health, and if there was any increased susceptibility in some individuals. The importance of appropriate controls was emphasised.

Paragraphs 2.16-2.19: Mechanisms of action of pesticides and possible targets in humans

16. The COT considered that the details presented in this section were relatively limited and observed that there were many classes of pesticides other than organophosphates and pyrethroids which had not been considered here. The COT agreed that the classical dose-response relationship was appropriate for the toxicological assessment of all pesticides evaluated to date.

Paragraphs 2.20-2.26: Epidemiology

[See also comments from COC on cancer epidemiology paras 22 and 23 of this statement].

17. The COT agreed that a limitation of epidemiological studies in relation to pesticides was the imprecise exposure assessment, with often a complete lack of quantitation. Thus the Institute for Environment and Health review of studies on Parkinson's disease[6] had insufficient data available to identify individual pesticides; the best descriptor available was groups such as herbicides. Where associations were found it was not possible to relate them to dose. A key difficulty was the retrospective evaluation of exposure using self-reported questionnaires. Improvements in study design were required in this respect.

18. The COT observed the comparison in the RCEP report of the air quality standard for nitrogen dioxide and the relevant occupational standard. It was noted that the AOEL used in pesticide risk assessment incorporated an uncertainty factor sufficient to account for variation in susceptibility as might be seen in the overall population.

Paragraphs 2.27-2.34: Review of epidemiological studies

19. The COT considered that the review of epidemiological studies had been limited and that a more substantive review of the literature should be undertaken. Members noted that the RCEP did not come to any conclusion as to whether pesticide exposure was causing ill health. It was suggested that one possible way forward would be to consider para-occupational exposure, e.g. spouses and children of farmers who might have exposures above that of bystanders. It was noted that the American Farm Survey of Occupation might be one useful source, but a literature review should identify other relevant research projects (http://www.aghealth.org/). It was noted that such data did not necessarily enable cause and effect to be established.

20. The COT was aware of the difficulties in undertaking such work relating to many sources of exposure to pesticides and the many different types of pesticides in addition to potential exposures resulting from spray activities. Thus, for example, preliminary information from an investigation of people attending GP surgeries showed that 45% of them had used some form of pesticide in the domestic environment in the week before consultation.[7] However, it was noted that the background rate of exposure was not known.

21. It was noted that the RCEP report referred to clusters of ill health, but that clusters were not evidence of causation, and that a hypothesis of a minority of bystanders with heightened susceptibility was unlikely to fit with an area-based cluster pattern of ill health.

COC comments on epidemiology

22. The COC agreed that the RCEP had not had time to undertake a rigorous evaluation of all the available epidemiological literature. COC Members commented that the RCEP report had not clearly distinguished between hypothesis generating studies (such as geographical studies of clusters e.g. as undertaken by the Small Area Health Statistics Unit (SAHSU) http://www.sahsu.org/) and analytical studies which could be used to define dose-response relationships for pesticides associated with cancer and were of importance in the assessment of causality. The COC recalled that the main problems identified with regard to the Ontario review[8] were the selection of data used in it which had not considered available negative data as well as studies reporting positive associations for cancer, the selective interpretation of results and the lack of good exposure data in most studies. This last problem could not be remedied in any future review of such publications.

23. The COC agreed with the RCEP that better exposure definition in cancer epidemiology studies was a high priority for further research. The COC agreed that further evaluation of para-occupational studies would be valuable but that using status such as married to farmer as a proxy for para-occupational exposure limited the value of such studies with regard to identifying association with pesticide exposures. The COC agreed that appropriate biomonitoring studies (e.g. using biomarkers of exposure or of biological effect) would be helpful with regard to population studies of cancer. The Committee recognised the difficulties in associating current exposures with those that might be causal in cancer.

Paragraphs 2.35-2.39: Multisystem disease (chronic fatigue syndrome, multiple chemical sensitivity

24. The COT considered that there were two schools of thought with regard to multiple chemical sensitivity (MCS), that it was either psychological in nature or organic. Either cause could indicate a particular susceptibility in some individuals. It was noted that the literature indicated that two important factors in the reporting of ill health by bystanders were odour, which may not reflect exposure to an active ingredient, and the involuntary nature of exposure. These suggested that additional factors may be important in the condition.

25. The differences between multiple chemical sensitivity and chronic fatigue syndrome are unclear. This is, in part, a reflection of uncertainty in the aetiology of these conditions There were a number of similarities in reported symptoms, however not everyone with chronic fatigue syndrome reported sensitivity to chemicals.

26. The COT noted that a number of papers have been identified in the literature since the COT's last consideration of multiple chemical sensitivity in 2000, and agreed that these could be reviewed. This work might also involve reviewing chronic fatigue syndrome.

27. It was agreed that a fundamental research programme into multi-system disease involving research councils and the Department of Health as recommended by the RCEP was not warranted. With respect to chronic fatigue syndrome it was considered that there could be merit in investigating individuals with chronic fatigue syndrome who believe their condition is due to prior infection in comparison to those who believe it is due to chemical exposure. The COT commented that investigations using brain imaging techniques needed to incorporate appropriate controls. It was noted that even symptoms without an established physical basis could give rise to changes observable on functional brain imaging.

Paragraphs 2.40-2.53: Toxicology

28. The COT reviewed the two references cited in the RCEP on animal models which reflected some aspects of chronic fatigue syndrome.[2,3] The COT concluded, on the basis of this evidence and members' experience of toxicological test development, that there was no rationale for developing animal models to test for poorly-defined end effects such as multiple chemical sensitivity without a clear mechanistic basis for undertaking such work. One difficulty was the possibility of a psychological component in conditions such as chronic fatigue syndrome and multiple chemical sensitivity. Another was that the majority of the symptoms reported are subjective. Members had difficulty in identifying the value of *in vitro* techniques to investigate such complex multi-functional ill-health effects with poorly defined causation.

Paragraphs 2.54-2.64: Monitoring

29. The COT and COC noted that the majority of currently approved pesticides are eliminated quickly (e.g. within a day or two) once absorbed, and therefore biomarkers reflect exposure over the preceding days.[9] Levels of any biomarkers may relate more to time of exposure rather than dose, making calibration difficult. The Committee heard information on the studies instigated by PSD relating to permethrin and chlorpyrifos and agreed the proposed research would fulfil the COT suggestions for biomarker-related exposure assessment. Members noted the difficulties in undertaking such research. These include sampling, storage, analysis and obtaining ethical consent for participation. In addition, it was questioned whether the biomarkers which are currently available would be sufficiently sensitive to detect exposure in bystanders. Members noted that if biomonitoring was routinely used in data packages for pesticides there would be scope for comparing data with that derived from toxicological evaluation in animal studies.

30. The RCEP report had advocated large-scale studies along the same lines as the National Health and Nutrition Examination Survey (NHANES http://www.cdc.gov/exposurereport/) in the US. The COT considered that such programmes of work provided large numbers of results which were difficult to interpret. The COC noted that large studies such as Biobank (noted in 2.62 of the RCEP report) and EPIC (European Prospective Investigation into Cancer and Nutrition) would only be of value to measure chronic rather than acute exposures. Members agreed that it was important to consider potential biomonitoring studies, but considered that small scale focused prospective studies using pesticides for which there was good knowledge of kinetics in humans would be more informative for non-cancer endpoints. Such studies would form the basis for extrapolating to potential bystander exposure to other pesticide active ingredients.

Paragraphs 2.65-2.69: Recommendations: human health

31 The Committees considered the recommendations for human health presented in the RCEP report (reproduced in italics below).

i) Regarding 2.65 of the RCEP report; *Based on the conclusions from our visits and our understanding of the biological mechanisms with which pesticides interact, it is plausible that there could be a link between resident and bystander pesticide exposure and chronic ill health. We found that we are not able to rule out this possibility. We recommend that a more precautionary approach is taken with passive exposure to pesticides. The existing uncertainties indicate an urgent need for research to investigate the size and nature of the problem and any underlying mechanisms that link pesticide spraying to ill health* .The committees did not consider that there was a basis to support the recommendation that there was an urgent need for research. The Committees agreed that recommendations relating to additional precaution in risk assessment above the already precautionary approach used did not have a scientific basis and this was an issue of policy regarding pesticide approvals.

ii) Regarding paragraph 2.66 of the RCEP report; *We recommend that a comprehensive systematic review of the literature in this field be conducted that takes account of, and avoids, the shortcomings of the Ontario study* The COT agreed that an epidemiological review of para-occupational exposure to pesticides should be undertaken. The COT agreed a review of the literature on chronic fatigue syndrome and multiple chemical sensitivity should be undertaken. COC members doubted that a comprehensive systematic review would be valuable given the deficiencies in exposure measures in published studies. The COC agreed that geographical studies of cancer incidence linked to potential exposure (possibly to include appropriate biomonitoring data) should be considered

iii) Regarding paragraph 2.67 of the RCEP report; *We recommend that an imaginative systematic approach is taken to apply both well validated as well as novel clinical investigative methods to those with chronic symptoms linked to pesticide spraying such as magnetic resonance spectroscopy (MRS) and gene and protein profiling* The COT agreed that specialist investigations should be aimed at all potential causes of chronic illness such as chronic fatigue syndrome and multiple chemical sensitivity, not just the proposed hypothesis relating to bystander exposure to pesticides.

iv) Regarding paragraph 2.68 of the RCEP report; *We recommend that the Health Protection Agency and related organizations within the devolved administrations in Scotland and Wales collect population data on pesticides, their metabolites, and biomarkers of effects that would provide a sound basis for exposure assessment and could also be used to establish a national database for monitoring.* The COC considered that appropriate population biomonitoring could be of value in interpreting any studies of cancer and the potential association with exposure to pesticides. The COC noted the role of HPA in co-ordinating such work in the U.K. The COT concluded that targeted biomonitoring work was more preferable to gain an estimate of potential bystander exposure.

v) Regarding paragraph 2.69 of the RCEP report; *We recommend that the private sector and universities be encouraged to develop new animal models that better reflect the chronic disorders experienced by residents and bystanders exposed to pesticide spraying.*The COT considered that there was currently no clear rationale for developing animal models to test for poorly-defined end effects such as multiple chemical sensitivity without some mechanistic basis for undertaking such work. The COT considered that there was little value in using *in vitro* techniques to investigate such chronic ill health effects. (The Committees noted that all pesticides are tested for potential carcinogenicity in rodents)

Paragraphs 2.70-2.96: Health effects, monitoring and reporting

32. The Committees had not previously considered health effects monitoring and reporting of pesticides and therefore did not consider the conclusions and recommendations on this section of the RCEP report in any detail. The Committees were aware that expertise and experience of health monitoring scheme for pesticides was available in the relevant regulatory authorities (namely PSD and HSE). The COT made a generic comment that suggestions made in the RCEP report for further involvement of primary care would be difficult to undertake and that the RCEP had not considered the diversity of ways in which primary care is delivered. Members considered that most general practitioners would not have the time to consider the causes of the mainly ill defined symptoms individuals may present with.

33. The COT considered that the advice published by DH in 1996 regarding advice to general practitioners with suspected ill health attributed to pesticides was potentially unhelpful as it did not allow for all causes of illness to be investigated.

COT/COC conclusions

34. The Committees agreed the following overall conclusions with regard to the questions posed:

    i) *Consider, based on members expertise and the evidence presented in the RCEP report whether, the conclusions and recommendations reached in respect of health related topics are appropriate (see paras 2.65-2.69 reproduced in para 31 (and 6.20-6.29) of the RCEP report)*

    The COT and COC did not concur with the recommendation in paragraphs 2.65-2.67 and 2.69 of the RCEP report for the reasons outlined above in para 31of this statement, but did concur with the suggestion made in para 2.68 of the RCEP report. The COT and COC agreed that it was important that a number of areas of further work were undertaken. These are given in para iii) below.

    ii) Derive COT/COC conclusions in relation to the health related questions posed by the RCEP (see para 5) on the basis of the evidence reviewed and member's expertise, and to consider whether these concur with those reached with RCEP.

    The COT and COC concluded that the available evidence did not convince members that there was a high degree of urgency for further research. The Committees agreed that there was no scientific basis for an additional precautionary approach to the risk assessment of pesticides.

    iii) Consider whether any further work by COT/COC/COM should be undertaken with respect to bystander pesticide risk assessment and report any suggestions for further work to the ACP.

    The COT agreed that an epidemiological review of para-occupational exposure to pesticides should be undertaken. The COT agreed a review of the literature on chronic fatigue syndrome and multiple chemical sensitivity should be undertaken. The COC agreed that geographical studies of cancer incidence linked to potential exposure (possibly to include appropriate population biomonitoring data) should be considered. The COT agreed that in the first instance a targeted approach to biomonitoring research would be more informative to gain an estimate of bystander exposure.

COT statement 2006/05
COC statement 2006/S1
April 2006

References

1. Royal Commission on Environmental Pollution (2005). Crop spraying and the health of residents and bystanders. September 2005. http://www.rcep.org.uk/cropspraying.htm

2. Chao CC et al (1992). Immunologically mediated fatigue: A murine model. Clinical Immunology and immunopathology, 64, 161-165.

3. Overstreet D and Djuric V (2001). A genetic rat model of cholinergic hypersensitivity: implications for chemical intolerance, chronic fatigue and asthma. Annals of the New York Academy of Sciences, 933, 92-102.

4. Department of Health (1996). Pesticide poisoning. Second edition. Notes for the guidance of medical practitioners. (published DH)

5. HSE (2005). Pesticides incidents Report. Field Operations Directorate Investigations. 1 April 2004-31 March 2005. (published HSE).

6. Brown TP; et al. (2006) Pesticides and Parkinson's disease – is there a link? Environ Health Perspect. 114: 156-164.

7. Rushton L. Personal Communication to COT 14 February 2006.

8. Ontario College of family Physicians (2004). Pesticides Literature Review. http://www.ocfp.on.ca/English/OCFP/Communications/CurrentIssues/Pesticides/

9. Marrs TC and Ballantyne B (2004) Pesticide Toxicology and International Regulation. Edited by Timothy C. Marrs and Bryan Ballantyne Copyright 2004 John Wiley & Sons, Ltd.TISBN: 0-471-49644-8. ... (References to specific pesticides can be obtained through the WHO International Programme on Chemical Safety (IPCS) at http://www.inchem.org/.)

2006 Membership of the Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment

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Declaration of members' interests during the period of this report

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor I Hughes (Chair)	BP Amoco	Shares	Archives of Disease in Childhood	Associate Editor
	BP Amoco	Daughter is an employee of this Company	Academy of Medical Sciences	Fellow
			Society for Endocrinology	Member
			Royal College of Paediatrics and Child Health	Fellow; Senior Examiner; Regional Academic Advisor
			Medical Research Council	Member of Advisory Board
			Pfizer	Funds received from all these sources for Departmental research and education in medicine and health related topics
			Aventis	
			NovoNordisk	
			Diabetes UK	
			Wellcome Trust	
			Juvenile Diabetes Research Fund	
Dr D Bell	Alliance & Leicester	Shares	FSA	Research Contract
	BAA		AstraZeneca	BSRC CASE studentship
	BG			
	Centrica			
	HBOS Plc			
	International Power			
	RT Group			
	Rolls Royce			
	Scottish Power Thus			
	United Facilities			
	National Grid Transco			

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor A Boobis OBE	Bank Santander	Shares	GlaxoSmithKline	Support by Industry
	Scottish Power		FSA	Research Contract
	Centrica		Department of Health	Research Contract
	BG Group		ILSI HESI	Unpaid member of Board of Trustees
	Halifax			
	Barclays		Elsevier	Editor-in-Chief; Food and Chemical Toxicology
	National Grid			
	Transco		JMPR JECFA (vet drugs) EFSA PPR	Member
	BT Group			
	Astellas Pharma	Consultancy		
Dr P Carthew	Unilever	Employee	NONE	NONE
Dr R Dearman	Syngenta CTL	Salary	NONE	NONE
	AstraZeneca	Shareholder		
Dr J Hinson	GlaxoSmithKline	Shareholder	Society for Endocrinology	Council member and Education Advisor
			Journal of Endocrinology	Member of the editorial board
			Current Opinions in Endocrinology and Diabetes	
Dr P Jackson	British Heart Foundation	Lecture Fees	Medtronic AVE	Research Grant
	Mitchell & Butler	Share Ownership		
	Intercontinental Hotels			
	Marks & Spencer			
Professor J Lunec	NONE	NONE	Scilucent LLC USA	Funding research group to investigate toxicology of soya-bean oil implants
Professor D Ray	Medical Research Council	Employer	Consortium of: Bayer, DuPont, FMC Syngenta & Valent	Research Grant
	ZLB Behring Switzerland	Consultancy		
	Bayer AG (Germany)			

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor I Rowland	Alpro Foundation	Consultancy	ILSI Europe	Partner in EC funded project
	Unilever			
	Glanbia			
	Clasado			
	Woolwich	Shares	Cerestar (Belgium)	Funded Research
	Halifax		Geest	
			Vitacress	
			Yakult UK	
			Nicobrand	CAST PhD studentship
			Scottish Crops Research	
Dr L Rushton	Transport & General Workers Union	Consultancy – completed	European Silica	Ongoing Cohort Study Contract to IEH – completed
	Friends Provident	Shares	International Manganese Institute	Contract to IEH to prepare criteria document – completed
	Northern Rock		American Chemistry Council	Contract to IEH for systematic review and meta analysis – completed
	Unilever	Consultancy – advice on design of an epidemiological survey relating to dermatitis	CONCAWE	Contract to Imperial College for research study: pooled analysis and update of case-control studies of benzene and leukaemia – ongoing

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