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

Preface

The COT has again discussed a wide range of toxicological issues in 2002. The Committee has provided advice on the important food safety issues of acrylamide in fried and baked foods and on mercury in fish and shellfish and urgent advice to the Food Standards Agency on dioxins in fish oil and kava kava. The Committee published its report on the Risk Assessment of Mixtures of Pesticides and Similar Substances and is in the final stages of completing a major report on Phytoestrogens and Health. It has also commented on the use of PAVA (Nonivamide) as an incapacitant spray, new toxicity data on breast implants, proposed a new tolerable daily intake for phenol and carried out a comprehensive review of the toxicology literature on the topical insect repellent diethyl-m-toluamide (DEET). Other issues on which the Committee has provided advice are detailed in this report.

In March 2002, the Food Standards Agency published its Report on the Review of Scientific Committees. The review looked at the role, methods of operation and effectiveness of the independent scientific committees that advise the Agency and made fifty recommendations covering a wide range of issues. The majority of these recommendations as they affect the Committee had already been implemented. The recommendation for holding Committee meetings in open session will be taken forward in 2003.

Professor Frank Woods retired as Chairman on 31 March having given 10 years of distinguished service to the Committee and I would like to thank him on behalf of the Committee for the outstanding contribution he has made. I have inherited an excellent Committee. My new colleagues are both knowledgeable and industrious and they have provided invaluable support to me as a 'new boy'.

Finally, I would like to add my sincere thanks and appreciation of the work of the administrative and scientific secretariats without whose excellent work the Committee would not be able to function.

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

Acrylamide in fried and baked food

1.1 Members were presented with a short paper that outlined the reasons for current concerns. These stemmed from the publication on 24 April 2002 of the results of a study carried out by the Swedish National Food Authority. The study found acrylamide at concentrations up to 2300 μg/kg (wet weight) in a range of fried, baked and processed carbohydrate based foods such as chips, crisps, biscuits, breakfast cereals and crispbreads. Analysis of foods had been carried out using a new and unvalidated methodology.

1.2 The Food Standards Agency subsequently commissioned a small survey at the Central Science Laboratory (CSL) in York. This survey involved the analysis of samples by two methods, one previously validated for determination of acrylamide in some food types, the other based on the new Swedish methodology. The methods gave similar results and the levels of acrylamide detected were similar to those in comparable foods in the Swedish study. Acrylamide was not detectable in raw or boiled potatoes, but the level in chips appeared to increase with the cooking time.

1.3 Members were informed that acrylamide was to be considered by the Scientific Committee on Food (SCF) in early July and at an expert consultation set up by the World Health Organisation (WHO) in late June. The WHO consultation included four UK delegates: Mr Steve Wearne, Head of Chemical Contaminants Division, Food Standards Agency; Professor Peter Farmer, Chair of COM and expert on haemoglobin adducts of acrylamide; Dr Laurence Castle, CSL, who conducted the UK survey; Dr Richard Cary, HSE, rapporteur for the risk assessment of acrylamide prepared under the Existing Substances Regulations.

1.4 The Food Standards Agency has issued a statement for consumers, and held a meeting with representatives of the food industry and interested parties to discuss the areas where further information is required. These included investigation of levels of acrylamide in other types of food, the chemistry of acrylamide formation and the conditions under which it forms, and the relevance of haemoglobin adducts to human health.

1.5 Members agreed that further studies were needed to determine the chemical mechanism of acrylamide formation in food and the relationship between cooking time and content. It was postulated that acrylamide could derive either from protein or lipid degradation products.

1.6 The possible use of cancer risk estimates, commonly used in the US, was also discussed. It was noted that current COC advice is that risk estimates are not acceptable but the available models are due to be reconsidered as part of the planned review of the COC guidelines on risk assessment.

1.7 The COT agreed that there was currently insufficient information to recommend changes to diet or cooking methods. Further research could include:

- possible parallels with the formation of polycyclic aromatic amines or heterocyclic amines;

- the possible effects of superheating such as may occur in the formation of crispbreads;
- investigation of acrylamide levels in other foods and following other cooking processes such as microwaving;
- investigation of the mechanism of acrylamide formation, including determination of the source of the amino group;
- further clarification of the adverse effects following different routes of administration in humans.

Balance of expertise within the Committee

1.8 The Office of Science and Technology's Code of Practice for Scientific Advisory Committees requires that a committee be given regular opportunity to review the balance of expertise within its membership. Members were asked to comment on whether the current balance of expertise was adequate to allow thorough evaluation of the toxicological issues within the Committee's remit. They were also asked to advise on the areas of expertise that should be sought in appointment of new Members to replace two long-standing members due to complete their term of office at the end of March 2003.

1.9 It was noted that although there is mathematical expertise with regard to statistics and epidemiology within the Committee, further expertise on experimental study design and analysis would be helpful. Members also considered that neurotoxicology is an increasingly important area, which the Committee currently lacks, and that expertise in both neurotoxicity and neurobehavioural effects would be beneficial.

DEET – review of toxicology literature on topical insect repellent diethyl-m-toluamide

1.10 *N-N*-diethyl-*m*-toluamide (DEET) is an insect repellent which is used to prevent nuisance bites from mosquitoes, ticks, biting flies and mites, and to lower disease transmission from these pests. It has been available world-wide for 40 years and has been reported to give the best duration of protection and broad-spectrum effectiveness. It is available in the UK in a variety of formulations and concentrations, including liquid, cream, lotion and stick products for direct application to skin and aerosol and pump-spray products for application to skin or to clothing.

1.11 The COT was asked to consider the safety of DEET as the Department of Health was considering a strategy to combat a potential outbreak of West Nile Virus in the UK, as there was evidence that some species of mosquito in the UK could transmit the virus.

1.12 The COT considered toxicological data from animal studies and the small number of human case reports of severe central nervous system (CNS) toxicity. The evaluation included confidential data submitted by the DEET Joint Venture Group. In accordance with the agreed procedures for dealing with confidential information, a draft statement was forwarded to the DEET Joint Venture Group prior to finalisation and release. The COT agreed to keep DEET under review.

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

Di-isopropylnaphthalene

1.14 Di-isopropylnaphthalene (DIPN) is a mixture of isomeric di-isopropylnaphthylenes. DIPN was first introduced in 1970 in Japan as a solvent for the carbonless copy paper market, eventually replacing the polychlorinated biphenyls (PCBs) which were formerly used for this purpose. DIPN is now manufactured by Rutgers Kureha Solvents GmbH (RKS) and supplied to the printing industry. Recycled paper used in paper and board manufacture may include carbonless and thermal copy papers from office waste, in which DIPN is used as the solvent for the ink system. The DIPN may not be completely removed by the treatment of the recycled fibres and may be present in the finished board and thus migrate into food. The Committee had considered toxicological data on DIPN on a number of occasions during 1998 and 2000 and had been unable to determine a no observed adverse effect level (NOAEL); therefore a Tolerable Daily Intake (TDI) could not be set.

1.15 In October 2002, the COT considered a new 90-day study on one of the isomers of DIPN, 2,6-DIPN, and an evaluation of the toxicological information on DIPN carried out by a consultant toxicologist, with a view to identifying a NOAEL and therefore establishing a TDI for DIPN. The COT considered that whilst the report by the consultant toxicologist was a balanced review, the 24-month toxicity/carcinogenicity study proposed in the report as identifying a NOAEL was inadequate for that purpose. Members agreed that, because of the poor survival, the study would not be considered acceptable as a chronic toxicity study by current standards, and therefore could not be used as the basis for establishing a TDI. The 90-day study on 2,6-DIPN could not be used in the setting of a TDI since there were effects seen at all dose levels, and because the isomer used in the study was not representative of the commercially available DIPN isomeric mix.

1.16 The COT concluded that the toxicological database on DIPN was insufficient to set a TDI and that more information was required. The COT recommended that the company would need to submit a well conducted 90-day study on the commercially available mixture of DIPN isomers before a TDI could be established. Members also reiterated their previous recommendation that levels of DIPN in recycled paper and board food packaging should be kept as low as reasonably practicable to minimise migration into food.

Dioxins in fish oil – urgent advice

1.17 The Food Standards Agency received results of a survey of concentrations of dioxins and polychlorinated biphenyls (PCBs) in certain fish oil supplements. The COT chairman was asked to provide advice on the health implications of the results, in order to support the Agency's decision on the appropriate action to be taken. The Chairman requested opinions from three members who had taken part in the recent discussions of the Tolerable Daily Intake (TDI) for dioxins and dioxin-like PCBs before reaching his conclusions.

1.18 Thirty-three samples of fish oil dietary supplements, representing 24 different products had been analysed for levels of dioxins and dioxin-like PCBs. The exposures to dioxins and dioxin-like PCBs were estimated from the recommended dosages for schoolchildren (stratified in 4 age groups; 5-6, 7-10, 11-14 & 14-18), adults and senior citizens. Since none of the sampled products were recommended for children under 5 years old, no estimates were made for toddlers and infants.

1.19 Members agreed it was appropriate to consider the survey results in the light of the TDI of 2 pg TEQ/kg body weight (bw) per day, noting that, according to the 1997 Total Diet Study, average intake of dioxins and dioxin-like PCBs from the UK diet was 1.8 pg TEQ/kg bw/day. Some fish oil samples, if taken at the recommended dosage, would lead to a higher intake of dioxins than from dietary sources. The recommended adult doses of three separate samples exceeded the TDI from the intake of the oil alone. The problem was particularly evident with two of these samples, which exceeded twice the TDI (i.e. exceed 4.0 pg TEQ/kg bw/day) from intake of the oil alone in virtually all age groups. The recommended dosage of two further samples would also result in intakes in excess of the TDI in children of some age groups before taking into account the dietary contribution.

1.20 Overall, Members agreed that the levels of dioxin and dioxin-like PCBs in five of the oils, together with average dietary intake, provided a risk of excessive intakes in some or all groups when compared with the TDI of 2 pg TEQ/kg bw/day.

1.21 Samples of multiple batches of two fish oils were measured. These samples had relatively low TEQ content and estimated intakes for adults were similar to those from a single portion of salmon per week. However, there was a marked variation in the dioxin content of these oils between different batches. Some batches of these oils, when taken together with the average diet, would lead to intakes in the region of twice the TDI in children under the age of 11. Since children are the susceptible age group, consideration should be given to the recommended dosage and age range for use of these products. Members also noted that the recommended dosages had a marked influence on the intakes. No clear guidance was given on dosage for children for some of these products.

1.22 This assessment was incorporated in the published results of the study.

Enzyme submission – Chymosin preparation derived from GM *aspergillus niger var. awamori*

1.23 Chymosin is an enzyme preparation that had previously been granted clearance by the COT. In December 2000, the manufacturer sought clearance of a modified recovery and purification procedure for this enzyme preparation. The COT raised a number of questions relating to comparison of the product with the material used in the original toxicity studies and purity and specifications of the product. At that time, COT agreed to one year's temporary clearance, whilst further information was provided to answer these points.

1.24 The manufacturer had provided new information in response to the previous request from the COT. Two main concerns were identified: the lack of any direct comparison with the material used in the original toxicity studies and that data were only available for a limited number, rather than the anticipated maximum number, of production cycles. Members considered that the response from the manufacturer was inadequate and suggested that, if adequate comparison between the original and new material was not possible, toxicity testing on the new material was required. COT recommended a further temporary clearance of 2 years whilst new data are provided.

Enzyme submission – Newlase: analytical method to detect rhizoxin

1.25 Newlase is an immobilised enzyme preparation used in the manufacture of modified fats and oils. In 2000, the COT recommended Newlase be given a two-year temporary clearance pending submission of analytical data confirming that the mycotoxin rhizoxin was not present within the preparation.

1.26 The company provided results of rhizoxin analysis for 20 samples from production batches of Newlase, all of which were below the limit of detection. The COT considered that the analytical method was suitably sensitive to detect rhizoxin, but that 20 samples were not adequate to demonstrate the absence of rhizoxin. In addition, the COT did not consider sufficient information had been submitted relating to the variability of responses or on verification of analyses by using spiked samples. Spiking with rhizoxin standard should be used in all future analyses otherwise there was no certainty of the efficacy of the extraction.

1.27 The COT was unable to recommend full approval of the immobilised enzyme preparation Newlase in the absence of additional details of analyses conducted with spiked samples. The COT recommended a further 2-year temporary clearance of Newlase, during which time the company was requested to collate the further information requested.

Food additives and behaviour

1.28 In 2000 and 2001, the COT discussed the results of a research project entitled "Do food additives cause hyperactivity and behaviour problems in a geographically defined population of three-year-olds?". A COT statement was agreed in 2001 but not released at that time as the researchers had submitted their work for publication in the peer-reviewed scientific literature. However, the researchers had not been successful in publishing their work and in order to avoid further delay in providing information to consumers, the Food Standards Agency published a summary of the work on its website and made a copy of the full research report available to the public in its Library.

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

Food chemical exposure assessment

1.30 Dietary exposure assessment is an important part of the food chemical risk assessment framework. It involves consideration of the chemical levels in food and consumption patterns of those foods, but the actual approach taken depends on the purpose of the assessment and the quantity and quality of the available information. Food Standards Agency officials had drafted a Best Practice Guide for Food Chemical Exposure Assessment to support a consistent approach within the Agency, and to provide information to the COT. Members were asked to comment on the draft version of the Best Practice Guide.

1.31 Members considered that the document successfully highlighted the difficulties in conducting food intake assessments and the main sources of uncertainty. The use of 7-day diary methods for the collection of data should be encouraged, in preference to shorter recording periods, although it was recognised that for some foods 7 days would not be long enough. Data for infrequently eaten foods could be obtained from food frequency questionnaires. Biomarker studies were also encouraged, as although they are more complex to conduct, they increase confidence in the accuracy of dietary intakes.

1.32 Clarification of some aspects was requested. In respect of assessing exposure when a chemical is not detectable in some food groups, halving the limit of detection was considered to result in an arbitrary value, and reporting upper and lower bound estimates more accurately reflects the range within which the true value may lie. Clearer referencing of the age groups studied in the National Diet and Nutrition Survey (NDNS), other dietary surveys and dietary reference studies was required, together with discussion of whether the approaches taken in the different surveys should be harmonised. Reliable consumption data were not currently available for infants in the 6-18 month age range, which is a potentially vulnerable age group. High level consumption is commonly taken as the 97.5th percentile, representing a compromise between reliability of the consumption and exposure estimates, given the NDNS sample size, and adequate consumer protection. Further statistical clarification was required, with consideration of how the shape of the distribution could influence the choice of percentile that is used. The limitations of the data should be clearly stated, with respect to number of consumers and whether the samples analysed are representative of products consumed by different populations throughout the year. Limitations in the available consumption data for some ethnic groups were noted.

1.33 Members commented on the imprecision associated with the use of bodyweights in exposure assessment, and the increase in average weights reported in the most recent NDNS. At bodyweights greater than 60-70 kg there would be increased amounts of adipose tissue, which may act as a depot for some chemicals. It was suggested that it might be opportune to re-investigate the use of bodyweight and consider other approaches such as body surface area.

1.34 Members were advised that the Agency was undertaking a review of its dietary surveys programme and that the Committee's comments would be fed into this review at a workshop to be held in January 2003.

Future discussion items

1.35 Most of the Committee's work is based on the need for advice from Government Departments, particularly the Food Standards Agency and Department of Health, and thus tends to be reactive. Following discussions on the Code of Practice for Scientific Advisory Committees and the Government's Response to the Phillips Enquiry, Members had agreed they wished to have an annual agenda item to discuss topics that are likely to be of interest/concern in the future. A list of future topics is displayed on the COT website, allowing interested parties an opportunity to provide additional information.

1.36 Topics suggested included:

- the derivation of uncertainty factors in the context of human variability, exposures in childhood and *in utero;*
- variation in developmental effects in infants and children, with a view to re-examining whether the default uncertainty factor is sufficient;
- the use of physiologically based pharmacokinetic (PBPK) modelling;
- variability in susceptibility to adverse effects of food supplement preparations used in weight loss, particularly for obese individuals;
- genotype-environment interactions (it was noted that COC was in the process of producing a statement on this topic);
- interactions between environmental and dietary chemicals and drugs, possibly in collaboration with the Medicines Control Agency;
- interaction with Europe;
- chemoprotective factors in food;
- consideration of toxicogenetics to further the work of the joint COT/COC/COM meeting on proteomics and genomics held in October 2001, and provide the genetic basis for individual variation in susceptibility;
- Possible effects of chemicals on the male reproductive system.

Joint meeting of the COT/COC/COM

1.37 As reported in the 2001 Annual Report, the COT, COC and COM held a joint open meeting in October 2001 to discuss the use of genomics and proteomics in toxicology. Genomics and proteomics offer rapid screening methods for identifying specific gene sequences and studying gene and protein expression. Their use in toxicology is increasing and it was considered important that the committees were able to consider the technology and provide guidance on its applicability in toxicological risk assessment carried out by the three committees. In particular the committees sought to determine whether these techniques could be used in a regulatory framework.

1.38 The meeting provided a useful forum for discussion of the topic with brief presentations outlining the techniques and their applicability followed by a more general discussion among the participants. The participants agreed the techniques offered considerable potential for use in risk assessment especially in the area of hazard identification. However, the group concluded that at present it would be inappropriate to use these technologies as primary tools in regulatory toxicological risk assessment. In addition, the group noted that until good databases of relevant and validated information that can be analysed by reliable bioinformatic methods are available the techniques can only serve as adjuncts in regulatory risk assessment.

1.39 The joint COT/COC/COM statement is included at the end of this report. A full meeting report has been published (Mutagenesis, **18**: 311-317, 2003).

Kava kava – urgent advice

1.40 In July 2002, the Food Standards Agency requested urgent advice on the herbal preparation kava kava. This was produced by the Secretariat in consultation with a COT member and the COT Chairman.

1.41 Kava kava is a herbal ingredient derived from the plant *Piper methysticum*, which is a member of the pepper family native to many Pacific Ocean islands. Kava kava root is a common constituent in modern herbal preparations. Kava kava products are available as tablets, capsules, tinctures and drops. Some kava kava products are sold as food such as teas. Since 2000 there have been a series of reports of serious hepatotoxicity in kava kava users including deaths and several cases requiring liver transplants. These reports occurred mainly in Germany but also in Switzerland, the US and UK. In December 2001, the Medicines Control Agency (MCA) and the Food Standards Agency advised against consumption of kava kava and urged the voluntary withdrawal of medicinal and food products from the market, pending a definitive safety assessment.

1.42 In July 2002, the Committee on Safety of Medicines (CSM) assessed the 68 detailed case reports of kava kava associated liver toxicity. The CSM concluded that kava kava could cause serious liver toxicity, although the precise mechanism by which such toxicity occurs is not understood and there are no clear predictors of this toxicity. The CSM considered that the risks of kava kava products outweighed the benefits and therefore, they advised that the licensing authorisation for kava kava products be withdrawn and the use of kava kava in unlicensed medicines be prohibited. The MCA announced the CSM conclusions and their regulatory proposals on 18 July.

1.43 There is a paucity of toxicological data on kava kava or its component lactones and the available data are inadequate to identify a no observed adverse effect level. The CSM has considerable experience in assessing evidence from case reports of adverse reactions. Whilst acknowledging the uncertainties in their analysis, the CSM concluded that kava kava is associated with hepatotoxicity. This toxicity is relatively rare but there are currently no data which permit identification of the mechanism of toxicity nor any patient or product characteristics that might increase the risk.

1.44 The risk assessment, as agreed by the COT chairman, concluded:

- *It is not possible to conclude that the hepatotoxicity is dose dependent nor can we exclude an immune mediated toxicity for which dose would not be relevant.*
- *There is evidence to suggest that hepatotoxicity can occur in users of the traditional kava kava preparations as well as in users of kava kava supplements.*
- *There are no data to compare the effects of extraction in hot liquid on the composition of the kava kava. Thus we are not able to determine if kava kava containing tea-bags result in exposures comparable to traditional or solvent extracted kava kava preparations.*
- *In the absence of a clear understanding of the hepatotoxicity, including its mechanism and relevant patient and exposure characteristics, it is not possible to exclude hepatotoxicity arising from food uses of kava kava. The information provided since December 2001 provides further evidence that consumption of kava kava may cause hepatotoxicity.*

Phenol: tolerable daily intake (oral)

1.45 The Environment Agency provides guidance on the health risks from contaminated land. The guidance recommends land-use-specific Soil Guideline Values (SGVs) for a range of chemical contaminants. Phenol has been identified as a soil contaminant of possible concern. In order to derive a SGV for phenol, the COT was asked to recommend an appropriate NOAEL and TDI for oral exposure to phenol.

1.46 The COT noted the opinion of the COM (2000) that, by the oral route, there is potential for a threshold of activity for the mutagenicity of phenol. The data on the toxicity of ingested phenol were considered sufficient to identify a NOAEL. The COT identified the critical study, an enhanced two-generation reproductive and developmental toxicity study in rats (Ryan *et al*. International Journal of Toxicology, 20: 121-142, 2001), in which the overall NOAEL was 70 mg/kg bw/day. The COT considered that although several studies had reported abnormal findings in animals at lower doses, these results were not consistent with the absence of comparable or related findings in other well-conducted studies at higher doses and longer periods of exposure to ingested phenol.

1.47 In determining a TDI for phenol, the COT considered that the use of the standard uncertainty factors of 10 for extrapolation from rodent data, and 10 for variability within the human population, would be appropriate. This resulted in a TDI of 0.7 mg/kg bw/day for ingested phenol.

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

PIP hydrogel implants

1.49 During 2000, the COT had considered a 90-day study on this hydrogel following a request from the Medical Devices Agency (MDA). The COT concluded that the findings observed in this limited and inadequate study could not be discounted and made recommendations for additional studies. The full conclusions were given in COT Statement 2000/09. The manufacturer voluntarily withdrew the implant from sale in the UK while additional information was obtained to address these and other concerns.

1.50 The manufacturer had recently submitted new data to MDA. This included a new 90-day study and other information on the degradation of the hydrogel. The formulation of the hydrogel differed from the earlier studies, reflecting a change in the marketed implant. The rationale for the new buffered formulation was unclear.

1.51 The COT concluded that there were concerns over the design of the study and interpretation of its findings. The results of the new rat study did not support the safety of either the new or old formulation and the effects seen in the new study could be indicative of a toxic response. The new studies provided no additional information which would allow them to provide any further advice on the extent or significance of toxicological risks. The COT restated its previous conclusion that there was a need for a properly conducted study with longer-term follow-up and with more detailed reporting compatible with current guidelines for chronic toxicity tests.

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

Polycyclic aromatic hydrocarbons in the 2000 Total Diet Study

1.53 The COT was presented with the results of analyses for polycyclic aromatic hydrocarbons (PAHs) in the food samples collected for a Total Diet Study (TDS) in 2000. Members were informed that dietary intakes had been estimated for the three compounds identified by the COC in 1996 as being of greatest concern: benz(a)anthracene (BaA), benzo(a)pyrene (BaP) and dibenz(a,h)anthracene (DBahA). These estimates had been made using an upper bound approach i.e. where levels in a sample are below the limit of detection, the compound is assumed to be present at the limit of detection. Members noted that whilst this approach is likely to over-estimate the intake it represented a suitably precautionary estimate for non-threshold effects.

1.54 Intake estimates had been derived from the 1979 TDS, for comparison with the 2000 TDS. Selection of food samples for the TDS is updated regularly based on purchasing patterns, but the available food consumption data do not allow for changes in dietary habits to be fully taken into account. Direct comparison with data from the 1979 TDS was also not possible as the 1979 intakes used a lower bound approach (levels below limit of detection regarded as zero). Members suggested that the effects of these different approaches on exposure estimates should be made clear in the published data.

1.55 The following conclusions were agreed:

- *Because some PAHs are genotoxic carcinogens, it is not possible to establish a level of intake that is without possible risk, nor to set a Tolerable Daily Intake. Exposure should be as low as reasonably practicable.*

- *We note that estimates of dietary exposure to PAHs derived from PAH concentrations in food groups in the 2000 TDS are up to 5-fold lower than the estimates based on the 1979 TDS. One of the most potent PAHs, DBahA, was not detectable in any of the food groups.*

- *Overall, we conclude that, although the estimated intakes do not allow for changing dietary habits, the data indicate that dietary exposure to PAHs has decreased over the past twenty years, and it is therefore likely that any associated risk of cancer will also have decreased.*

Polychlorinated biphenyls: effects on play behaviour

1.56 The COT commented on a paper by Vreugdenhil and colleagues (Environmental Health Perspectives, 110: A593-A598, 2002), describing studies on the effects of polychlorinated biphenyl (PCB) exposure on play activity in a cohort of 207 mother infant pairs. This was the latest publication from one of the two series of Dutch studies. Members had previously discussed the Dutch studies on the effects of exposure to dioxins and dioxin-like PCBs on development during their evaluation of dioxins in 2001. The study looked at possible effects of perinatal exposure to PCBs and dioxins on play behaviour in Dutch children at school age.

1.57 Any associations observed in the study represented recall from a single point in time and did not provide information on effects throughout development. The effects reported in boys and girls were inconsistent and it was difficult to define a plausible biological mechanism to account for these observations, except for possible perturbation of hormone balance. Overall, the COT concluded that this was an interesting study, but it did not provide sufficient evidence that PCBs could disturb sex hormones in humans at background levels of exposure.

Survey of mercury in fish and shellfish

1.58 In May 2002 the Food Standards Agency received the final report of a survey of the total mercury levels in imported fish and shellfish and UK farmed fish and their products. This survey reported high levels of mercury in some species of predatory fish (shark, swordfish and marlin) and moderately high levels in fresh tuna. In an initial response, the Agency released a precautionary interim statement following consultation with the COT Chairman, pending full review of the data by the COT. This statement advised the general population to restrict consumption of shark, swordfish and marlin to no more than one portion a week of any of these fish. It also gave precautionary advice to pregnant women, women intending to become pregnant and children to avoid consumption of these three species of fish.

1.59 The COT was asked to consider the most appropriate safety guidelines to use in assessing the health implications of mercury in fish, which is assumed to be predominantly methylmercury, and to consider possible health concerns associated with the estimated mercury intakes and blood level data provided.

1.60 The COT noted the uncertainties in the derivation of the Provisional Tolerable Weekly Intake (PTWI) by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and the differences in the major epidemiological studies of populations with high dietary exposure to mercury as a result of fish consumption. COT concluded that JECFA PTWI of 3.3 μg/kg bw/week for methylmercury was sufficiently protective for the general population. However COT recognised that it may not be sufficiently protective for women who are pregnant, or who may become pregnant within the following year, or for breast-feeding mothers, due to the potential risk of neurotoxicity to the developing fetus or neonate. The Committee therefore considered that the United States Environmental Protection Agency (EPA) reference dose of 0.1 μg/kg bw/day would be more appropriate for these risk groups.

1.61 The COT considered that dietary exposure to mercury resulting from average and high-level consumption of fish for which consumption data were available was not likely to be associated with adverse effects to the developing fetus. The COT also commented on the number of portions of shark, swordfish, marlin and tuna that would not be expected to result in adverse effects on the developing fetus or infant.

1.62 The COT made recommendations for future research and agreed that its conclusions should be reviewed following the JECFA evaluation of methylmercury in 2003. The COT statement is included at the end of this report.

PAVA (Nonivamide): use as an incapacitant spray

1.63 The COT was asked by the Home Office to advise on the health effects of pelargonyl vanillylamide (PAVA or Nonivamide) when used as the active ingredient of a chemical incapacitant spray. PAVA spray was being used by Sussex Police as an alternative to CS spray. PAVA is a synthetic equivalent to capsaicin, the active ingredient of pepper, and is also used as a food flavour and in human medicine for topical application as a rubifacient.

1.64 The COT reviewed the available data, and sought advice on the mutagenicity data from the COM. The COT considered that it was not possible to make a complete assessment of the likely health effects that could arise from the use of PAVA due to the limited toxicological data available. However, the COT recognised that exposures to the PAVA from the incapacitant spray would be low and for a short period.

1.65 Concern was expressed about the gaps in the data of relevance to users of the spray. Overall, the COT recommended further monitoring of the experience in use of PAVA spray, including the police officers using the spray, with particular attention being given to eye irritancy in those wearing contact lenses and to effects in those with asthma or hay fever and in women who may be pregnant.

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

Working Group on the risk assessment of mixtures of pesticides and similar substances

1.67 This COT Working Group was established in December 2000 to

- Assess the potential for multiple residues of pesticides and veterinary medicines in food to modify individual toxicity of chemicals in humans – the so-called "cocktail" effect;
- Evaluate what assumptions can be made about the toxicity of pesticides in combination;
- Consider the potential impact of combined exposure to pesticides and veterinary medicines by different routes;
- Formulate advice on the standard risk assessment procedures applicable to the safety evaluation of individual pesticides and veterinary medicines in the light of the above considerations.

1.68 Eight Working Group meetings and 2 open consultation meetings were held. A draft report was issued for public consultation on 15 February 2002. As part of the consultation process, an open meeting was held at the De Vere, Dunton Hall, Norwich on 28 February. An amended draft of the report, taking into account comments arising from the consultation was considered by the Working Group and submitted to the full COT for consideration at its meeting of 23 April 2002. The final report was issued in September 2002 and is available on the COT website at http://www.food.gov.uk/multimedia/pdfs/report(indexed).pdf or from the secretariat.

Ongoing work

Consideration of fluoride in the 1997 Total Diet Study

1.69 In 2000 the COT considered the results of the 1997 Total Diet Study. At the time, consideration of any potential effects of dietary fluoride was deferred until the Expert Group on Vitamins and Minerals (EVM) had reviewed fluoride. The COT considered the draft EVM review of fluoride in 2001, but considered that it would be more appropriate to wait until the EVM had finalised its view.

1.70 The EVM subsequently agreed that fluoride was not within its remit and has thus not completed a risk assessment for fluoride. The COT therefore considered the available data on fluoride and health outcomes and data on intakes from the 1997 Total Diet Study and from non-dietary sources. The COT will complete its discussions and issue a statement in 2003.

Survey of total and inorganic arsenic in food: results of the 1999 Total Diet Study

1.71 In 2002 the Food Standards Agency completed a survey of the total and inorganic arsenic levels in samples from the 1999 Total Diet Survey (TDS), which was carried out between 1999 and 2002. The COT was asked to comment on the survey and assess if the levels of arsenic in the diet posed a risk to human health.

1.72 The COT will complete its discussions and issue a statement in 2003.

Iodine in milk

1.73 The Committee has previously reviewed dietary exposure to iodine from cows' milk. In 1999, COT recommended that there was a need for further information on the different chemical forms of iodine in cows' milk.

1.74 In 2002, the COT was asked to consider the results of a study conducted by the Central Sciences Laboratory (CSL) investigating the different chemical forms of iodine in cows' milk. The results of the CSL study were inconclusive and COT has proposed to have a more substantive discussion of this topic at a future meeting.

1.75 COT requested that the current toxicological information on individual iodine species, information on the use of iodophors in milk production and details of the iodine content of animal feed be provided to inform this discussion.

Report on phytoestrogens and health

1.76 During 2002, the COT considered the draft report of the Working Group on 'Phytoestrogens and Health'. The draft report was issued for public consultation from October to December 2002. The final draft Phytoestrogen and Health report is due to be reconsidered by COT and subsequently finalised in early 2003.

Proposal to hold meetings in open session

1.77 Over the past few years the COT had considered the Phillips Report into BSE, the May Review into the way in which risk is handled by scientific advisory committees dealing with food safety and the *Code of Practice for Scientific Advisory Committees*, published by the Office of Science and Technology. The Board of the Food Standards Agency had requested a review into the role, methods of operation and effectiveness of the independent scientific committees that advise the Agency on food issues. The report was published in 2002 and made a total of 50 recommendations covering a wide range of issues, including increasing the openness of the work of the committees and conducting more business in public.

1.78 The current working practices of the COT address most of the recommendations on openness. The exception is the recommendation to conduct as much business as possible in open session, which has so far only applied to selected meetings on specific scientific issues. Resource implications mean that regular large open meetings are not feasible, but providing a few places for observers within the Agency's meeting rooms would be appropriate to the expected interest and resources. Some items would need to be discussed as reserved business such as dealing with commercially confidential data or pre-publication research data.

1.79 Openness was seen as a very important means of regaining public trust in the evaluation of evidence for the protection of human health. However it is important to ensure that the presence of observers is not inhibitory to the Committee's discussions.

1.80 Subject to discussions of a detailed protocol, the COT agreed in principle to move to open meetings during 2003.

Statements of the COT

Statement on a further toxicity study in the rat of hydrogel filler for breast implants

Statement on a review of toxicology literature on the topical insect repellent diethyl-m-toluamide (DEET)

Statement on a research project investigating the effect of food additives on behaviour

Statement on the use of PAVA (Nonivamide) as an incapacitant spray

Statement on phenol: tolerable daily intake (oral)

Statement on a survey of mercury in fish and shellfish

Statement on a further toxicity study in the rat of hydrogel filler for breast implants

Introduction

1. During 2000, because of concerns raised by clinicians about the safety of the fillers used in breast implants, the Medical Devices Agency (MDA) had decided to review the safety data on all breast implant fillers available in the UK. These included a hydrogel pre-filled breast implant manufactured by Poly Implant Prostheses. In September 2000, at the request of the MDA, the Committee considered a submission questioning the significance of the findings in a 90-day toxicity study in rats implanted with this hydrogel. We concluded:

    i) the conclusion of the study, namely that there were no pathological findings in the organs examined, was not supported by the limited experimental results provided, which were considered to be imprecise and inadequate.

    ii) the findings from the study could not be discounted. The Committee was not able to exclude the possibility that the reported lesions [in lymph nodes, liver and kidney] were indicative of a toxic or immunologically-mediated response.

    iii) further testing should be undertaken involving the administration of single doses of the filler gel with longer-term follow-up and with more detailed reporting compatible with current guidelines for chronic toxicity tests.

2. The product was voluntarily withdrawn from the UK market in December 2000 and an MDA Device Alert was issued to advise plastic surgeons and implanted women. MDA indicated that further advice on the safety of these implants would be provided as soon as it became available.

The implant

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

The rat toxicity study

4. The manufacturer had provided results from a new toxicity study to address the concerns raised by the original study. Groups of five female rats were injected once in each flank subcutaneously with 1.2cm^3 of the modified gel filler material or with saline as a control. Groups of dosed and control rats were killed after 4 weeks and 12 weeks. Limited observations were made during life and at necropsy. In addition to the organs examined histopathologically in the earlier study (injection sites, liver, lungs, kidney, thymus, spleen and "aortic" lymph nodes), mesenteric and axillary lymph nodes, brain, adrenals, ovaries and mammary glands were also investigated. In a number of cases lymph nodes from only 4 animals per group were examined histologically, with no explanation or identification of the animals omitted.

5. In the groups of rats that were killed at 4 and 12 weeks no abnormal clinical signs or differences in body weight were reported for either treated or control animals. However, in the treated animals residues of the gel and tissue damage were observed at the injection site. The histopathological changes in lymph nodes, livers and, to a lesser extent, the kidneys of the treated animals noted in the earlier study were not reproduced to the same extent in this study. There was reduction in adrenal weight in treated animals but the significance of these alterations in adrenal weight had not been investigated further by looking at the pituitary. Pulmonary vasculitis was observed in 60% of the treated group at 12 weeks but was not observed in the controls. The report described these findings as not being of toxicological significance.

6. The Committee considered that, despite having been carried out in 2001 to address its earlier concerns, the study was unsatisfactory in its design, execution and reporting. No explanation was provided for the discrepancies between the previous and new studies in the incidence of the liver and kidney lesions. The effects seen in the lungs and adrenals in the new rat study could be indicative of a toxic response. These changes and those previously observed in the liver and kidney require further study, including investigation of the reversibility of any changes observed.

Degradation of the filling material

7. Limited data were provided on the potential for *in vivo* degradation of the filling materials (both buffered and unbuffered). A substantial proportion of the dosed material was not recovered and the fate of this material had not been ascertained. The Committee considered that the potential degradation of the hydrogel had not been adequately addressed.

Conclusions

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

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

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

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

March 2002

COT Statement 2002/01

Review of the toxicology literature on the topical insect repellent diethyl-m-toluamide (DEET)

Introduction

1. We have been asked by the Department of Health (DH) to review the available toxicology data on the insect repellent diethyl-m-toluamide commonly know as DEET. The Chief Medical Officer Professor Sir Liam Donaldson (Chief Medical officer for England) recently published a report "Getting Ahead of the Curve" which outlines a strategy for combating infectious diseases.[1]" Chapter 2 of the report (www.doh.gov.uk/cmo/publications.htm) presents a discussion of factors which might be responsible for new emerging diseases. These include changes in environment and land use, global travel and trade and climate change. The strategy gives examples of various diseases such as West Nile Fever transmitted to humans by mosquitoes. This disease is caused by infection with West Nile virus following bites from a number of species of mosquito, some of which can be found in the U.K (e.g. *Culex pipens*). It is a potentially fatal illness and it is therefore important to have a strategy to combat transmission and infection. Developing a strategy to combat mosquito transmitted illnesses involves a number of elements such as source reduction (i.e. removing habitats and use of pesticides to control mosquito larvae and adult mosquitoes). It is also important to have good communications with the public regarding any strategy which should include information for U.K. citizens travelling abroad. The topical application of insect repellents can be used as an aid in reducing mosquito bites and hence help to prevent the spread of vector borne illnesses such as West Nile Fever.

Background to insect repellents

2. 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. *N*, *N*-diethyl-*m*-toluamide (DEET) seems to be most effective and is the best studied insect repellent currently available to the general public. It has been reported by the United States Environmental Protection Agency (EPA) to effectively control mosquitoes, biting flies, biting midges, black flies, chiggers (mites), deerflies, fleas, gnats, horse flies, no see-ums, sand flies, small flying insects, stable flies and ticks.[2] It is also used in certain countries as a prophylactic agent against several insect borne diseases, such as Lyme Disease, Human Granulocyte Ehrlichiosis, Encephalitis, Malaria, Dengue Fever, Yellow Fever, West Nile Fever and Rocky Mountain Spotted Fever. Products containing DEET are also recommended for use by travellers going abroad for use in the prevention of insect borne diseases. 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 the prevention of infection with West Nile virus (www.cdc.gov/ncidod/dvbid/westnile/).[2] The Committee was aware of recent published research which provided evidence to suggest that DEET based insect repellents were the most effective products available.[3]

3. 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 of DEET in these products varies according to formulation type between 10%-95%. There is considerable experience in the use of DEET containing products in the U.S.A. where the EPA estimated (based on 1990 data) in its 1998 Reregistration review of DEET insect repellent products that approximately 30% of the US population uses DEET annually as an insect repellent (about 27% of adult males, 31% of adult females and 34% of children). There are thus millions of people of all ages using DEET containing products in the U.S.A. every year. There are no data on usage patterns in the UK but as noted above a wide range of products are freely available over the counter or via the internet.

Regulatory control of insect repellents

4. There is no regulatory approval scheme for topically applied insect repellents within the UK. Topically applied insect repellents are regulated under the Biocides Products Directive (BPD)(98/8/EC introduced 14 May 2000) enacted in UK legislation by the Biocide Products Regulations 2001 (which came into force on 6 April 2001). Topically applied insect repellents 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). The regulatory control of such a large number of products and categories is extremely complex. A centralised review scheme for existing biocide products is being set up by the European Commission but it is unlikely that insect repellents would be reviewed for several years and most likely after 2006. The UK Competent Authority is the HSE (Biocides and Pesticides Authorisation Unit). The HSE has established an independent advisory committee the Biocides Consultative Committee to provide advice with regard to regulatory reviews of biocides prepared under the EU Review Regulations.

5. The available products would also have to conform to labelling requirements as established by the Chemicals (Hazards Information and Packaging for Supply) Regulations 2000 (CHIP) which enact EU Directives on Dangerous Substances and Preparations (76/548/EEC).

Rationale for review

6. The Department of Health (DH) has been considering 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. This is predominantly concerned with surveillance strategies, source control (reducing breeding habitats) and consideration of appropriate pest control measures.

7. The DH was aware of a number of published reports concerning severe CNS toxicity in a small number of children following exposure to DEET. Information relating to 14 individuals had been reviewed by the US EPA in 1998.[2] The COT was asked to review the available literature and provide advice. The Committee considered a review of the available literature at its meeting of 26 February 2002 and a number of additional published studies on the potential neurotoxic effects of DEET at its

meetings on 11 April 2002 and 18 June 2002. The Committee heard independent expert neurotoxicology and neuropathology advice at the April and June 2002 meetings. Additional information (on toxicokinetics and evaluation of neurotoxicity) was provided by the DEET Joint Venture Group (Washington, U.S.A.) at the June and 23 July 2002 meetings.

8. Many of the DEET products on sale in the UK have specific labelling regarding age restrictions (e.g. do not use on children below age 10, 6, 3 or 2 years). There is no apparent consistency in the labelling advice on current products. This labelling does not arise from any regulatory review but reflects decisions reached by individual manufacturers in accordance with their own product liability risk assessment. The labelling advice issued by the EPA in 1998 did not refer to any age restrictions on use. More recently specific age limits have been introduced in Canada.[4] The Committee was therefore also asked whether it would be prudent for the DH to consider a similar approach to that recommended by the EPA or whether the recommended age restrictions as applied by some manufacturers in the UK on the use of DEET products were appropriate.

Exposure Assessment

9. It is not possible to undertake an assessment of the likely dermal exposure following use of topically applied insect repellents containing DEET available within the UK. Exposures would depend on types of product used (e.g. aerosol, pump sprays, lotions), the type of formulation and concentration of DEET, the recommended use pattern as outlined on the product label (including recommendations for multiple applications within one day), and the duration of product use (e.g. application on one day or daily application over several days/weeks). As noted in paragraph 3 above, no data are available on the use patterns of DEET topical insect repellent products in the UK. Such information may be obtained when the formal regulatory review under the Biocides Product Directives (98/8/EC) is initiated. It is noted that the US EPA and Health Canada used different approaches to estimate exposures in their reviews.[3,4] The EPA estimated dermal exposures to be 9.7 mg/kg bw/day in female adults, 12.1 mg/kg/day in adult males, 21.1 mg/kg bw/day in teenagers (aged 13-17 y) and 37.6 mg/kg bw/day in children (under 12 y). It is not known whether these values are mean or median estimates (no data on range was available).[3] Health Canada based its assessment on a study of 540 individuals (including adults and children) where the amount of DEET used was estimated by measuring product weight before and after use. This approach gave rise to estimates of 3.7 g/product/day for all product types and 2.3 g/product/day for formulations containing 100% DEET.[4] The DEET Joint Venture Group estimated the 95th percentile exposure in adults to be 3g DEET/person for females and 4g DEET/person for males. In the human volunteer dermal kinetics experiments this equated to dermal exposures of 57.6-65.8 mg/kg bw/day for females and 54.4-60.9 mg/kg bw/day for males (n= 3 for each group).

Summary of Toxicology

Metabolism studies in animals and humans

10. A review of all the available toxicological literature was prepared by the DH Toxicology Unit at Imperial College of Science Technology and Medicine which considered the EPA review document and published literature up to October 2002. This paper is published on the DH COT Website (www.doh.gov.uk/cotnonfood/index.htm). Thus a brief summary of the relevant data is given in this statement.

11. The dermal penetration and absorption of DEET have been extensively studied using both *in vitro* and *in vivo* methods in experimental animals. The percentage absorbed was dependent on the solvent used, concentration of DEET, method used to determine absorption (e.g. recovery in urine following dermal application or comparison of kinetics following intravenous or dermal application) and extent of occlusion used. It is difficult to compare these results. In rats dermal absorption values of up to 80% have been reported.[5]

12. A number of studies using human volunteers and *in vitro* methods with human skin samples are available. These studies confirm the influence of solvent and formulation on absorption of DEET. An investigation undertaken in human volunteers was submitted to the EPA as part of the reregistration review.[6] Allowing for the extent of recovery, estimates of absorption of 4-14% for a 15% ethanolic solution of DEET and 3-8% for 100% DEET were recorded in this study. The Committee agreed that this study had been conducted to acceptable standards. The Committee noted that data from studies in animals suggested that dermal absorption of DEET could vary between different formulations.

13. The Committee reviewed the data from toxicokinetic studies using single and repeated dermal administration of undiluted DEET to human volunteers (4 applications at 50 mg/kg bw) and rats (five applications at 1000 mg/kg bw) and single oral administration to rats (200 mg/kg by gavage) and dogs (75 mg/kg bw by administration in gelatin capsules).[7-11] These studies had been designed to provide plasma time course data for DEET at the 95th percentile of human use, the oral NOAELs reported for neurotoxicity in rats and dogs and the limit dose for dermal application in rats. The results showed that oral administration at the doses used resulted in a rapid peak plasma level; 15-45 minutes in rats and 30 minutes in dogs. A much slower absorption was evident following dermal administration with peak plasma levels reported at 8 hours in humans and 4-8 hours in rats. However the human plasma level was reduced after showering at 8 hours and while the Committee accepted the rationale that this prevented exposure overnight, members noted that this may have limited assessment of peak plasma levels. There were no apparent differences between the plasma time course for DEET following single or repeated dermal administration in humans or rats. Quantitative comparison of the peak plasma levels of DEET showed that levels were 33x higher in dogs and 16-34 x higher in rats given oral doses compared to dermal administration to humans.[12].

14. The evidence from metabolism studies in rats following oral dosing[5] and from human volunteers following topical application of DEET[6] suggested that absorbed DEET was metabolised and excreted in a similar manner in rats and humans. The predominant pathways of metabolism appear to involve oxidation of the methyl group on the aromatic ring and N-deethylation of the amide moiety. The available studies in animals which used dermal administration also showed that absorbed DEET was excreted predominantly via the urine.

15. In one recently published study, results from *in vitro* metabolism experiments using liver microsomes from humans, rats and mice provided confirmatory evidence that the route of metabolism is qualitatively similar in humans and rodents. Additional *in vitro* experiments using individual human cytochrome P450 isozymes established that CYP2B6 is the principal cytochrome P450 involved in metabolism of DEET by ring hydroxylation. Studies using phenotyped human liver microsomes showed that individuals with the highest levels of CYP2B6, 3A4, 2C19 and 2A6 had the greatest potential to metabolise DEET. These data, in part, may help to explain inter-individual differences in response to DEET.[13]

Toxicology: Studies in animals

[A table of No Observed Adverse Effect Levels derived from the available toxicology studies is given on page 30]

Acute toxicity, irritancy, sensitisation

16. DEET is of low acute oral, dermal and inhalation toxicity in studies in adult animals designed to quantify mortality. The Committee noted that there was evidence in rats to show that young animals and particularly females were more sensitive than adult animals to the acute lethal effects of DEET.[14]

Acute neurotoxicity

17. The predominant signs of toxicity in rats given oral doses of 2.5-4 g/kg bw DEET, which resulted in lethality between 50 minutes up to 24 hours post dose, were CNS depression with myoclonic twitching seen in some animals (triggered by auditory or tactile stimuli). Recovery in surviving animals was slow. There was evidence of histological changes in animals given lethal doses in the CNS predominantly consisting of vacuolation of myelin sheaths of fibres in the cerebella roof nuclei and cytoplasmic clefts which were diffusely distributed throughout the brain.[14]

Table. Summary of NOAEL values from animal toxicity studies

Study	Route	Species	NOAEL	Basis for NOAEL
90-day range finding study[2]	Oral/dietary	Hamster	61mg/kg/day	Reduced body weight and food consumption at 305mg/kg/day
2 year chronic/ carcinogenicity study[23]	Oral/dietary	Rat	100mg/kg/day	Reduced body weight, food consumption and increased cholesterol levels in female rats dosed at 400mg/kg/day
1 year subchronic toxicity study[23]	Oral/capsule	Dog	100mg/kg/day	Reduced body weights, food consumption and cholesterol levels together with increased ptyalism incidence in dogs dosed at 400mg/kg/day
78-week carcinogenicity study[23]	Oral/dietary	Mouse	500mg/kg/day	Value is systemic toxicity NOAEL based on reduced body weights and food consumption in mice dosed at 1000mg/kg/day
2 generation reproduction study[23]	Oral/dietary	Rat	250mg/kg/day	Value is reproductive toxicity NOAEL. No developmental effects at highest dose tested (i.e. 250mg/kg/day)
Developmental toxicity study[22]	Oral/gavage	Rat	250mg/kg/day	Value is maternal toxicity NOAEL. Reduced body weights and food consumption. Increase in clinical signs (e.g. hypoactivity, ataxia), mortality and mean liver weight seen in rats dosed at 750mg/kg/day
Developmental toxicity study[22]	Oral/gavage	Rabbit	325mg/kg/day	No maternal or developmental toxicity at the highest tolerated dose (i.e. 325mg/kg/day)
Acute neurotoxicity studies in rats[15, 16]	Oral/gavage (single dose)	Rat	200 mg/kg	Decrease in motor activity seen in two studies at 500 mg/kg
Neurotoxicity studies in in dogs (bolus dose in gelatine capsules on 5 successive days) [12]	Oral (gelatin capsule)	Dog	75 mg/kg bw/day for 5 days	>125 mg/kg bw abnormal head movements, ataxia/ptosis at 175 mg/kg bw, convulsions at 225 mg/kg bw.
Subchronic dermal neurotoxicity study[19-21]	Dermal	Rat	Not identified	Doses of 4 mg/kg bw/day and above affected performance in sensorimotor tests. Doses of 40 mg/kg bw/day and above resulted in histological changes in the CNS
Multigenerational exposure neurotoxicity study[15]	Oral/dietary	Rat	Approximately 95 mg/kg bw/day	Increase in motor activity at 200 mg/kg bw/day in males, 275 mg/kg bw/day in females

18. The Committee considered that the finding of decreased vertical motor activity in the functional observational battery in adult rats given an oral dose of DEET of 500 mg/kg bw by gavage was consistent with a neurotoxic effect.[15] Reduced motor activity in rats following a single oral dose of 500 mg/kg bw (in mineral oil) was also reported in a separate published study.[16] Evidence for reduced completion of reinforcement learning schedules in rats has been documented in a study where rats were given a single oral dose of 500 mg/kg bw.[17] The NOAEL reported in these studies was 200 mg/kg bw. The Committee noted that there were no sensorimotor function tests in young animals and questioned whether the NOAEL would be lower in such animals.

Irritancy/skin sensitisation

19. DEET is a mild skin irritant in rabbits and is not a skin sensitiser in guinea pigs. Technical grade DEET has given some evidence for eye irritation in rabbits. It is difficult to assess the data from the available study[18] in comparison to the criteria for classification under the Dangerous Substances Directive (67/548/EEC), but the evidence of reversal by 168 h suggests that DEET did not induce serious damage to eyes but should be regarded as an eye irritant. The Committee considered it would be prudent to assume such a classification for all formulated products unless appropriate data are available to suggest otherwise. DEET would not be classified as a skin irritant.

Subacute/subchronic toxicity

20. The Committee considered that a number of effects seen at high doses in the available toxicology studies were not relevant to risk assessment. These included effects on body weight gain associated with reduced food intake.[2,22,23] It is not clear whether the effect on body weight/food consumption is due to palatability or an effect on the treated animal. The effects on kidney histology (hyaline formation) seen in rats[2] in 90 day studies at 400 mg/kg bw/day were not seen in subchronic studies in other species or in a 2 year study in rats[23] and are therefore not considered relevant. The effects on spermatogenesis seen in hamsters at 624 mg/kg/day were not found in other species.[2]

Subchronic neurotoxicity

21. The Committee considered published studies which had been specifically designed to investigate the potential neurotoxicity following repeated doses. The Committee commented that there was a clear difference between the results obtained following dietary administration to rats or dermal application (in 70% ethanol) to the shaved skin of rats. These differences required an explanation.

22. The Committee considered the results of a multigeneration reproduction study in which DEET was administered to rats via the diet continuously over two generations and then for a further 9 months at 5000 ppm (reported to be equivalent to 200 mg/kg bw/day in males and 275 mg/kg bw/day in females).[15] The Committee noted the finding of a transient increase in motor activity (in the first 5-15 minutes of the functional observational battery) in F_2 40 week old rats but no histological changes.[15] The Committee considered that the methods used for fixation of tissues by *in situ* perfusion and histology were adequate and noted that a morphometric evaluation of the CNS had not been undertaken

in this study. Although the effects were not accompanied by any histological changes in the CNS, the Committee felt that the effects on motor activity could not be discounted. The NOAEL from this study was 2000 ppm (approximately 95 mg/kg bw/day). The Committee also noted that limited details of studies in dogs had been published, which showed evidence of neurotoxicity at doses (using gelatin capsules) of 125 mg/kg bw and above. The NOAEL was reported to be 75 mg/kg bw.[12]

23. Three dermal studies have been published from a single laboratory and were considered at the COT meetings in April and June 2002. The main findings from each of these studies are outlined below.

Abdel-Rahman et al Experimental Neurology, vol 172, 153-171, 2001[19]

24. Groups of five male rats received dermal doses of DEET at 40 mg/kg bw/day for 7 days per week for 60 days (in 70% ethanol). 24 h after the last dose the animals were anaesthetised with pentobarbital, and perfused through the heart with saline followed by 4% paraformaldehyde and 0.15% glutaraldehyde in Tris buffer. The brains were removed, postfixed and embedded in paraffin. Four micrometre coronal sections were cut through different brain regions and sections stained with haematoxylin and eosin. The authors undertook morphometric evaluation of the density of healthy (surviving) and dying neurons and immunohistochemistry with microtubule associated protein 2 (MAP-2) to assess neurodegeneration. The authors also undertook immunohistochemistry with glial fibrillary acidic protein (GFAP) to assess upregulation of GFAP-immunopositive structures.

25. A significant reduction in the density of healthy (or surviving neurons) was reported in the motor cerebral cortex, the dentate gyrus, the CA1 and CA3 subfields of the hippocampus and the cerebellum. A significant number of degenerating neurons (eosinophilic) were documented in these brain regions. The neuron loss in the motor cortex and the CA1 subfield was corroborated by a significant decrease in MAP-2. Analysis of GFAP indicated a significant hypertrophy of astrocytes in hippocampus and cerebellum of all treated groups.[19]

Abou-Donia MB et al, Journal of Toxicology and Environmental Health part A vol 62, 523-541, 2001[20]

26. Dermal doses of 4, 40 or 400 mg/kg DEET (97.7% pure) in 70% ethanol were given to rats for 7 days per week for 60 days. Ethanol was used as a control. Neurobehavioural testing (including sensorimotor testing for reflexes, response to stimuli (vibrissae touching), grip time, beam walking and inclined plane test) were undertaken at days 30, 45 and 60 of treatment. Blood Brain Barrier (BBB) permeability was assessed 24 h after the last exposure by tail vein injection of ^{3}H-hexamethonium iodide determination of plasma and tissue radioactivity levels.

27. Significantly reduced BBB permeability was reported in the brainstem at 40 and 400 mg/kg bw/day. A significant effect on beam walking, beam walking time and grip strength was reported at all doses including 4 mg/kg bw/day at all time points. The effects on beam walking time, however, did not show an increasing response with duration of dosing.[15]

Abou-Donia MB et al., Toxicological Science vol 60, 30, 5-314, 2001[16]

28. Male rats were treated with DEET (40 mg/kg bw/day in 70% ethanol) for 45 days. A number of combination experiments, involving DEET, pyridostigmine and permethrin, were also undertaken. Sensorimotor testing was undertaken on days 30 and 45. The authors also undertook to measure brain cholinesterase and plasma cholinesterase using the Ellman method and to measure ligand binding to nicotinic and muscarinic acetyl choline receptors in brain regions.

29. No statistically significant effects on beam score or walk time were reported, although an increase in beam walk time may have been documented in the graphical representation of results in the paper. A significant reduction in inclined plane performance and grip time was documented in DEET treated rats at days 30 and 45. A significant increase in ligand binding for m2 muscarinic acetyl choline receptor was reported.[16]

Studies on reproduction

30. There was no evidence of toxicologically significant effects on reproduction in rats, or on development in rats and rabbits.[2,22] Evidence of overt maternal toxicity was documented in a rat developmental study at doses of 750 mg/kg bw/day but no effects on development were reported.[22]

Mutagenicity

31. DEET does not have any structural alerts to suggest mutagenic potential and this is supported by negative results in a bacterial mutation assay in *Salmonella typhimurium*. Negative results have also been reported in assays to investigate clastogenic potential in CHO cells and Unscheduled DNA Synthesis (UDS) in primary rat hepatocytes.[6] The Committee on Mutagenicity (COM) has asked to see full reports of the latter two studies before finalising its conclusions. The reports have been received and will be assessed at the COM February 2003 meeting. **"[Added to paragraph 31 – April 2003]....** The COM concluded (at its February 2003 meeting) that there were limitations in all of the submitted studies. In particular, the COM considered that the exogenous metabolising fraction used in the in-vitro chromosome aberration assay had not produced satisfactory results with cylophosphamide. However, overall the results of the Ames test, an in-vitro cytogenetics assay in CHO cells and an in-vitro UDS assay in hepatocytes were negative. This information together with the information previously reviewed by the Committee (lack of structural alerts with DEET, negative Ames tests and negative carcinogenicity studies) suggest there is no concern with regard to the mutagenicity of DEET."

Carcinogenicity

32. The Committee on Carcinogenicity concluded that the available information in the study published by Schoenig *et al*[23] suggested that the chronic toxicity/carcinogenicity studies undertaken in mice and rats had been adequately conducted. There was no evidence of induction of tumours by DEET in either of these studies. Thus DEET was not carcinogenic in experimental animals.

Risk assessment based on animal studies

33. There is no evidence for any treatment related mutagenicity, carcinogenicity or effects on reproduction. There is evidence for mild neurotoxic effects in rats in a number of neurobehavioural tests after both single oral doses and upon subchronic exposures by oral and dermal routes of exposure. Effects on motor activity were noted in acute and subchronic studies in rats at doses of 500 mg/kg bw/day. The NOAEL in these oral studies was 200 mg/kg bw/day. There is also evidence for neurotoxic effects in dogs given an oral bolus dose of 125 mg/kg bw. The NOAEL in this 5 day study in dogs was 75 mg/kg bw/day.

34. The Committee noted that the dermal route of application and duration of the studies from Abou Donia's laboratory were relevant to the risk assessment of the use of DEET as an insect repellent. The studies were considered to be internally consistent. A number of design/methodological problems were evident in the neurotoxicity studies. The use of 70% ethanol as a solvent may have affected the dermal absorption of DEET.

35. The Committee heard from an independent expert neuropathologist and a neurotoxicologist. Members agreed that their evidence supported the view that the *in situ* perfusion had been adequately undertaken but a number of the lesions might be either due to treatment with DEET or represent background variation in histology. Members agreed that the morphometric methods were adequate but did not represent the most modern approach to measuring neuronal cell loss. Members felt that the approach to evaluating quantitative neuronal loss in the Purkinje cell layer was acceptable. Members discussed the lack of evidence for a range of neuropathological findings which might have been expected if neurons were at different stages of degeneration leading up to cell death and removal of cellular debris. The reason for this was unclear, although it was noted that the evidence for glial proliferation could represent a cellular response to neuronal loss. Members felt that on balance given the expert independent neuropathology advice, that the evidence of lesions in the cerebellum, and in particular the Purkinje cell layer, was convincing in respect of treatment with DEET.

36. Members discussed the suggestion that an independent peer review of the pathology would resolve all the difficulties in evaluation of the Abdel Rahman study.[19] It was felt that such an approach would not overcome any methodological/design problems in the original study nor the observations of neurobehavioural effects. Overall the Committee concluded that there was a need for independent verification of the neuropathology findings with DEET. Any repeat experiment would need to consider both single and repeat dose studies, the practical aspects of undertaking repeated dose dermal studies up to 60 days and the need for additional toxicokinetic data.

37. Members were aware that evidence for neurotoxicity and/or CNS neuropathological effects had been documented by Abou Donia and colleagues using permethrin and pyridostigmine and combinations of these chemicals with DEET. It was difficult to resolve why these structurally and toxicologically dissimilar chemicals should produce similar patterns of neurotoxicity. Reduced scores in the beam walking test and grip strength test were reported in rats given dermal doses of 4 mg/kg DEET bw/day after 30, 45 and 60 days of treatment. In addition there were histological changes in the CNS in animals treated at 40 mg/kg DEET bw/day for 60 days. It is not known whether the effects on sensorimotor testing noted after 30 and 45 days dermal dosing with DEET were associated with any histological lesions. In contrast repeated dermal application studies undertaken for the DEET Joint Venture Group did not report any signs of neurotoxicity at dose levels of up to 1000 mg/kg DEET bw/day (5 days/week for 13 weeks). However this study did not include any specific neurotoxicity tests. 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.

38. Overall, the Committee considered that there were considerable uncertainties regarding the studies published by Abou-Donia and colleagues. The Committee concluded that, in view of the 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.

39. The Committee agreed that neurotoxicity had been demonstrated in oral studies in rats and dogs and that the NOAEL in dogs of an oral dose of 75 mg/kg bw and mean dermal absorption values of 5.6% or 8.4% could be used for risk assessment. Margins of safety compared to dermal doses (as reported above in paragraph 9) were small and unacceptable.

40. The Committee was aware that the DEET Joint Venture Group (DJV) had proposed[12] that 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 3g DEET/day in adult females and 4g 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 16-34 x 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. Overall it was agreed that a reduced uncertainty factor (from the traditional value of 100) was appropriate but it was not possible to specify what the uncertainty factor should be. Members did not agree with the rationale proposed for an uncertainty factor of 10.[12] The most appropriate value would be between 10 and 100. Members agreed that no definite conclusions could be drawn regarding the margins of safety documented in this paper.[12]

Toxicology: Evidence from human case reports: EPA evaluation 1998

41. With regard to the case reports of severe CNS toxicity, these were subject to a detailed assessment by the EPA during its reregistration review. The EPA evaluated the data on 14 individuals. This assessment was based on six individuals where information had been published prior to 1989, five individuals reported by Oranski in 1989[37] and information on a further three individuals published after 1989. The EPA undertook a calculation of the likely incidence of severe CNS toxicity in children and concluded that the risk was minimal (1 in 100 million applications of DEET).

Toxicology: Evidence from human case reports: COT evaluation (2002)

42. An overview of all the published case reports detailing adverse effects documented in individuals exposed to DEET is presented in the DH Toxicology Unit paper (doh.gov.uk/cotnonfood/index.htm). Twenty-five published reports were found.[24-49] These predominantly came from the U.S.A. The effects reported have to be considered in the context of the large proportion of the US population regularly using DEET containing insect repellents.

43. Eight published reports concerned effects on the skin.[24-31] Six of the reports concerned individual cases where moderate to severe skin irritation were reported.[24,25,28-31] Rarely there is evidence that urticarial reactions to DEET maybe allergic in nature (i.e. due to specific immunological responses).[24,30,31] The two other reports concerned US military personnel.[6,27] Evidence of severe skin reactions was reported in some individuals stationed in South Vietnam during late the 1960s, who used insect repellent preparations containing 75% DEET. [26]Further evidence of severe skin reactions was reported in 10 US military personnel who used a preparation containing 50% DEET in the 1980s. [27] It is uncertain what role co-formulants played in these reactions. The findings from these reports have not been reproduced on such a scale in reports concerning use of insect repellents by non-military personnel in the U.S.A. and are not consistent with the large number of people using DEET products in the U.S.A. every year. The evidence suggests that under normal conditions of use DEET rarely causes skin irritation. However, in cases where exposure levels are high, or where exposure is prolonged then skin reactions may occur. In rare instances urticarial reactions to DEET may be allergic in nature.

44. Eleven published reports concerned CNS toxicity. In total there was evidence of CNS toxicity in 18 individuals predominantly females (for details see DH Toxicology Unit paper at http://www.doh.gov.uk/pdfs/reviewofdeet.pdf).[32-42] Fifteen of these individuals were aged between 1-8 years and the remaining three individuals were aged 14, 16 and 29y. Signs of severe CNS toxicity in these individuals included seizures, unconsciousness/coma, tremors, convulsions, depressed reflexes and ataxia. There was evidence of deliberate ingestion in five individuals.[32,39,42] All of these individuals recovered. There were 14 individuals where dermal exposure predominated.[32-38,40,41] Three deaths occurred, of which two might be associated with exposure to DEET.[32-34] The third individual who died showed evidence of Reye's syndrome at post mortem and thus symptoms were unlikely to be associated with DEET exposure.[33] All of the remaining 14 individuals recovered mostly within a few days of admission to hospital. Recovery in two individuals was reported to take three weeks and three months respectively.[37-40]

45. The available information on dermal exposure varies between the different reports and is difficult to assess. For some of these individuals dermal exposure was described as prolonged (daily applications for 1-3 months[32,28]), frequent[34] and/or high.[35,36] However for others,[38,40,41] the exposures reported varied from a single exposure to repeated exposures over a few days. The Committee agreed that there was evidence to suggest that exposure to DEET had resulted in severe CNS toxicity in few individuals, predominantly in children indicating a possible susceptibility.

46. The Committee noted that the small number of reported cases of severe CNS toxicity had to be considered in the context of the large numbers of people using DEET containing products and thus the risk of such effects was extremely remote. However, the Committee was concerned regarding the possibility of publication bias and that reports of cases with less severe neurotoxicity would not be published. It was possible that signs of neurotoxicity that were not clinically overt would not be identified by reviews of case reports. Members noted that the human data were virtually all from case reports and that there had been no published epidemiological studies of DEET exposure and adverse effects and agreed that such investigations should be considered.

47. Eight published reports referred to a range of adverse effects.[39,43-49] Three referred to psychosis as the predominant clinical effect,[43-45] two referred to adverse effect on reproduction[41,44] and four to a range of other clinical effects.[39,46-48] It is difficult to draw any conclusions regarding these reports in view of the lack of information on exposures, the complex nature of symptoms reported and the possibility of other causes of the symptoms reported. Given the lack of effects of DEET on reproduction and development in experimental animals, it is unlikely that the two reports of adverse effects on reproduction in humans are related to exposure to DEET.[46,49]

48. A recent review of reports to the US American Association of Poison Control Centers between 1993 and 1997 has been published.[50] The authors evaluated information pertaining to 20,764 exposures involving insect repellents containing DEET. Nearly 70% of cases reported no symptoms related to exposure to DEET. It was reported that 26 subjects experienced major effects (13 were adults). Evidence of neurotoxicity was documented in four out of the 13 adults and 6 out of the remaining 13 individuals (three infants, seven children and three teenagers). Two individuals (a male aged 26 y and a female aged 34 y died). There was little information documented regarding these two individuals. Both had applied DEET topically. There was a report of seizure in the 26 year old male but it is possible that aspiration of food contents may have been the cause of death as this was identified at autopsy. The Committee noted that the outcome of this descriptive analysis of routine data collection was consistent with the evaluation of the published case reports. In order to follow-up this evaluation of the published literature the Department of Health undertook an examination of data provided by the six UK National Poisons Information Service (NPIS) centres. Data were sought for a four year period 1 January 1997-31 December 2001 Information on over 1 million calls to the NPIS was available. There were reports on 153 individuals exposed to DEET. Overall there was evidence to demonstrate potential for localised effects (skin/eye irritation) following accidental exposure to DEET in a small number of cases. There were no reports of severe CNS toxicity in children. A single case of deliberate ingestion leading to coma was reported in one case involving an adult male.

Labelling

49. The Committee was also asked to provide advice on two different approaches to labelling DEET products, namely with or without specified age restrictions. The Committee noted that it did not usually provide advice on risk management but would consider risk management options. It was noted that in 1998 the EPA finalised a number of label recommendations for DEET products, but this did not include any specific age restrictions on use.[2] In the UK manufacturers have applied their own age-restrictions on use of DEET containing insect repellents. The Committee was aware that no regulatory review of insect repellents under the Biocides Product Directive was likely until after 2006, and therefore considered that it would be appropriate to suggest that industry seek to attain a consistent approach to labelling of DEET products through voluntary action. The Committee noted that a recent Regulatory Review had been completed in Canada (dated 15 April 2002) which had concluded that there was need for concentration restrictions, reapplication limits/day and age specific limitations on use.[3] Members felt this approach was acceptable but noted that the Canadian review did not include the most recent animal neurotoxicology studies in its risk assessment.

COT conclusions

50. The Committee agreed the following conclusions. In reaching these conclusions, the Committee was aware that DEET is an efficacious insect repellent and would be important in any strategy to reduce the transmission of diseases where insects are vectors.

Exposure

i) We note that there is a lack of data on exposures in the UK and suggest that industry should make appropriate information publicly available.

Animal toxicity data

ii) DEET is neurotoxic in experimental animals at high oral dose levels which result in mortality. There is evidence of neuropathological lesions in the CNS of animals given lethal doses.

iii) Evidence for neurotoxicity and neuropathological lesions have recently been documented following repeated dermal application of DEET to rats at comparatively low dose levels. The Committee concluded that, in view of the methodological problems with these studies, and difficulties in assessing the results that additional studies to verify the results obtained represented the most appropriate course of action to take.

iv) A risk assessment can be undertaken based on peak plasma levels of DEET in experimental animals at the NOAEL and in humans at the 95th percentile of exposure. It is not possible on current data to determine the appropriate Uncertainty Factor to use in risk assessment but this is likely to be between 10 and 100. The margins of safety using this approach varied between 16-34x. It is not possible to draw any definite conclusions on the significance of these values.

Evidence in humans

v) There are a small number of published reports of severe CNS toxicity which concern 18 individuals (children aged below 16, and one adult aged 29) where exposure to DEET containing insect repellents was followed by severe CNS toxicity. Three of these individuals died. Most of these reports document prolonged, frequent and/or high exposures to DEET but in a few cases exposures were apparently brief and could be reasonably expected to be relevant to normal use of DEET containing insect repellents. These neurotoxic effects were almost exclusively seen in children aged below 16 y indicating the latter may represent a more susceptible group. The Committee noted that the small number of reported cases had to be considered in the context of the very large numbers of people using DEET containing products. Thus the risk of such severe effects was considered to be extremely remote.

vi) A review of reports received by the six UK National Poisons Information Service (NPIS) centres for a four year period 1 January 1997-31 December 2001 was considered. The Committee was reassured that no reports of severe CNS toxicity similar to those reported in the published literature were found.

vii) We suggest that it would be prudent for the Department of Health to undertake further monitoring of data such as the Home Accident Surveillance Scheme, the Hospital Episode Statistics, mortality statistics and information from the National Poisons Information Service Centres for any reports concerning DEET occurring during 2002.

viii) There are no published epidemiological studies of DEET exposure and adverse effects available. The Committee agreed that such investigations should be considered.

Labelling

ix) In the UK, manufacturers have applied their own age-restrictions on use of DEET containing products. The Committee noted that risk management was beyond its terms of reference but agreed to comment on the options adopted by Regulatory Authorities in the U.S.A. and Canada. The Committee was aware that a regulatory review of insect repellents under the Biocide Products Directive was unlikely to take place for several years. The Committee agreed that it would be appropriate to suggest that industry seek to attain a consistent approach to labelling of DEET products through voluntary action.

Review

x) The Committee agreed to keep DEET under review.

Summary of Recommendations

xi) The following recommendations were agreed.

- Information on exposure should be made publicly available.
- Additional studies in animals are required to verify the neuropathological and neurobehavioural 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.

November 2002

COT statement 2002/05

References

1. Department of Health (2002). Getting ahead of the curve. A strategy for combating infectious diseases (including other aspects of health protection). A report by the Chief Medical Officer. Crown copyright.

2. EPA. (1998). United States Environmental Protection Agency. Reregistration Eligibility Decision (RED) DEET. EPA 738-R-98-010.

3. Fradin MS and Day JF (2002). Comparative efficacy of insect repellents against mosquito bites. New England Journal of Medicine, **347**, (1), 13-18.

4. Health Canada (2002). Re-evaluation Decision Document, RRD 2002-01. Personal insect repellents containing DEET (N,N-diethyl-m-toluamide) and related compounds, April 15-2002.

5. Schoenig GP, Hartnegal RA Jr, Osmitz TG, and Llonas S (1996). Absorption, distribution, metabolism and excretion of N,N-diethyl-m-toluamide in the rat. Drug Metabolism and Distribution, **24**, (2), 156-163.

6. Selim S, Hartnagel RA Jr, Osmitz TG, Gabriel KL, and Schoeng GP (1995). Absorption, metabolism and excretion of N,N-diethyl-m-toluamide following dermal application to human volunteers. Fundamental and Applied Toxicology, **225**, 95-100.

7. Ohayan A *et al* (1997). A blood level study in humans following topical application of N,N-diethyl-m-toluamide (DEET). LAB Pharmacological Research International, Canada. (In-confidence data from report submitted).

8. Badalone V (1997). Blood level study in dogs following oral administration via gelatin capsules, of N,N-diethyl-m-toluamide (DEET). BioResearch laboratories Quebec, Canada. (In confidence data from report submitted).

9. Laveglia J (1998). Blood level study in rats following a single oral bolus administration of N,N-diethyl-m-toluamide (DEET). MPI Research, Michigan, U.S.A.

10. Goldenthal EI (1999). 48-hour blood level study in rats following a single oral bolus administration of N,N-diethyl-m-toluamide (DEET) in the rat. MPI Research Inc, Michigan, U.S.A.

11. Laveglia J (1998). Blood level study in rats following single and repeated dermal applications of N,N-diethyl-m-toluamide (DEET). MPI Research Inc, Michigan, U.S.A.

12. Schoenig GP and Osimitz TG (2001). DEET. Chapter 63 in Handbook of Pesticide Toxicology, volume 2; Agents. pp 1439-1459, Academic Press.

13. Usmani K, Rose RL, Goldstein JA, Taylor WG, Brimfield AA and Hodgson E (2002). In-vitro human metabolism and interactions of repellent N,N-diethyl-m-toluamide. Drug Metabolism and Disposition, **30** (3), 289-294.

14. Verschoyle RD, Brown AW, Nolan C, Ray DE and Lister T (1992). A comparison of the acute toxicity, neuropathology and electrophysiology of N,N-diethyl-m-toluamide and N,N-dimethyl-2,2-diphenylacetamide in rats. Fundamental Applied Toxicology, **18**, 79-88.

15. Schoenig GP, Hartnegal RA Jr, Schardein JL and Vorhees CV (1993). Neurotoxicity evaluation of N,N-diethyl-m-toluamide (DEET) in rats. Fundamental and Applied Toxicology, **21**, 355-365.

16. Hoy JB, Cornell JA, Karlix JL, Schmidt CJ, Tebbett IR and van Haaren F (2002), Interactions of pyrodostigmine bromide, DEET, and permethrin on locomotor behaviour in rats. Veterinary and Human Toxicology, **42** (2), 65-71.

17. Van Haaren F, Haworth SC, Bennett SM, Cody BA, Hoy JB, Karlix JL and Tebbett JR. (2001). The effects of pyridostigmine bromide, permethrin and DEET alone, or in combination, on fixed ratio and fixed interval behaviour in male and female rats. Pharmacology, Biochemistry and Behaviour, **69,** 23-33.

18. Mac Rae SS, Brown BA, Ubels JL, Edelhouse HF and Dickerson CL, (1984). Ocular toxicity of diethyltoluamide (DEET). Journal of Toxicology, Cutaneous and Ocular Toxicology, **3**, (1), 17-30.

19. Abdel Rahman A, Shetty AK, Abou Donia MB. (2001). Subchronic dermal application of N,N-diethyl-m-toluamide (DEET) and permethrin to adult rats alone or in combination causes diffuse neuronal cell death and cytoskeletal abnormalities in the cerebral cortex and hippocampus and Purkinjie neuron loss in the cerebellum. Experimental Neurology, **172**, 153-171.

20. Abou-Donia MB, Goldstein LB, Dechovskaia A, Bullman S, Jones KH, Herrik E, Abdel-Rahman AA and Khan WA (2001). Effects of daily dermal application of DEET and permethrin, alone and in combination, on sensorimotor performance, blood-brain barrier, and blood-testis barrier in rats. Journal of Toxicology and Environmental Health, par A, 62, 523-541.

21. Abou-Doia MB, Goldstein, Jones KH, Abdel Rahman A, Tirupapuliyar V Damodaran, Dechkovskaia AM, Bullman SL, Amir BE and Khand WA. Locomotor and sensorimotor performance deficit in rats following exposure to pyrodostigmine bromide, DEET, and permethrin, alone and in combination. Toxicological Sciences, **60**, 305-314.

22. Schoenig GP, Neeper-Bradley TL, Fisher LC and Hartnegel RA Jr. Teratologic evaluations of N,N-diethyl-m-toluamide (DEET) in rats and rabbits. Fundamental and Applied Toxicology, **23**, 63-69.

23. Schoenig GP. Osmitz TG, Gabriel KL, Hartnagel R, Gill MW and Goldenthal EI (1999). Evaluation of chronic toxicity and oncogenicity on N,N-diethyl-m-toluamide (DEET). Toxicology Sciences, **47**, 99-109.

24. von Mayenburg, J. & Rakoski, J. (1983). Contact urticaria to diethyltoluamide. Contact Dermatitis *9*, 171.

25. Wantke, F., Focke, M., Hemmer, W., Gotz, M., & Jarisch, R. (1996). Generalized urticaria induced by a diethyltoluamide-containing insect repellent in a child. Contact Dermatitis *35*, 186-187.

26. Lamberg, S.I. & Mulrennan, J.A., Jr. (1969). Bullous reaction to diethyl toluamide (DEET) resembling a blistering insect eruption. Archives of Dermatology *100(5)*.

27. Reuveni, H. & Yagupsky, P. (1982). Diethyltoluamide-containing insect repellent: adverse effects in worldwide use. Archives of Dermatology *118*, REF.

28. Miller, J.D. (1982). Anaphylaxis associated with insect repellent. New England Journal of Medicine *307*, 1341-1342.

29. Amichai, B., Lazarov, A., & Halevy, S. (1994) Contact dermatitis from diethyltoluamide. Contact Dermatitis *30*, 188.

30. Maibach, H.I. & Johnson, H.L. (1975). Contact urticaria syndrome. Contact urticaria to diethyltoluamide (immediate-type hypersensitivity). Archives of Dermatology *111*, 726-730.

31. Vozmediano JM, Armario J and Gonzalez-Cabrerizo A. (2000). Immunologic contact uticaria from diethyltoluamide. International Journal of Dermatology, 39, 876-877.

32. Zadikoff MB (1979). Toxic encephalopathy associated with the use of insect repellent. J Pediatr., 95:140-142.

33. Heick, H.M., Shipman, R.T., Norman, M.G., & James, W. (1980). Reye-like syndrome associated with use of insect repellent in a presumed heterozygote for ornithine carbamoyl transferase deficiency. Journal of Pediatrics *97*, 471-473.

34. Pronczuk, d.G., Laborde, A., & Fogel, d.K. (1983). Toxicity of an insect repellent: N,N-diethyltoluamide. Vet.Hum.Toxicol. *25*, 422-423.

35. Gryboski J, Weinstein D, Ordway NK (1961). Toxic encephalopathy apparently related to the use of an insect repellent. N. Engl. J. Med., 264:289-291.

36. Roland, E.H., Jan, J.E., & Rigg, J.M. (1985). Toxic encephalopathy in a child after brief exposure to insect repellents. Canadian Medical Association Journal *132*, 155-156.

37. Lipscomb, J.W., Kramer, J.E., & Leikin, J.B. (1992). Seizure following brief exposure to the insect repellent N,N-diethyl-m-toluamide. Ann. Emerg. Med. *21*, 315-317.

38. Oransky S (1989). Seizures temporally associated with use of DEET insect repellent – New York and Connecticut. *MMWR*, 38: 678-680.

39. Tenenbein, M. (1987). Severe toxic reactions and death following the ingestion of diethyltoluamide-containing insect repellents. JAMA *258*, 1509-1511.

40. Edwards, D.L. & Johnson CE. (1987). Insect-repellent-induced toxic encephalopathy in a child. Clinical Pharmacy *6*, 496-498.

41. Briassoulis, G., Narlioglou, M., & Hatzis, T. (2001). Toxic encephalopathy associated with use of DEET insect repellents: a case analysis of its toxicity in children. Human & Experimental Toxicology *20*, 8-14.

42. Petrucci, N. & Sardini, S. (2000). Severe neurotoxic reaction associated with oral ingestion of low-dose diethyltoluamide-containing insect repellent in a child. Pediatr.Emerg.Care *16*, 341-342.

43. Snyder, J.W., Poe, R.O., Stubbins, J.F., & Garrettson,L.K. (1986). Acute manic psychosis following the dermal application of N,N-diethyl-m-toluamide (DEET) in an adult. Journal of Toxicology. Clinical Toxicology *24*, 429-439.

44. Hampers, L.C., Oker, E., & Leikin, J.B. (1999). Topical use of DEET insect repellent as a cause of severe encephalopathy in a healthy adult male. Academic Emergency Medicine *6*, 1295-1297.

45. Leo, R.J., Del Regno, P.A., Gregory, C., & Clark, K.L. (2001). Insect repellant toxicity associated with psychosis. Psychosomatics *42*, 78-80.

46. Schaefer, C. & Peters,P.W. (1992). Intrauterine diethyltoluamide exposure and fetal outcome. Reprod.Toxicol. *6*, 175-176.

47. Clem, J.R., Havemann,D.F., & Raebel,M.A. (1993). Insect repellent (N,N-diethyl-m-toluamide) cardiovascular toxicity in an adult. Ann.Pharmacother. *27*, REF.

48. Fraser,A.D., Macneil,A., Theriault,M., & Morzycki,W. (1995). Analysis of diethyltoluamide (DEET) following intentional oral ingestion of Muscol. J.Anal.Toxicol. *19*, 197-199.

49. Hall JG, McLaughlin JF and Stamm S (1975). Coarctation of the aorta in male cousin with similar maternal environmental exposure to insect repellent and insecticides. Paediatrics, *55*, 425-427.

50. Bell JW, Veltri JC and page BC (2002). Human exposures to N,N-diethyl-m-toluamide insect repellents reported to the American Association of Poison Control Centers 1993-1997. International Journal of Toxicology, 21, 341-352.

Statement on a research project investigating the effect of food additives on behaviour

1. We were asked by the Food Standards Agency to review the results of a study investigating the effects of particular food additives on the behaviour of pre-school children and to advise on the significance of the study for public health.

The study

2. A research team at the David Hyde Asthma and Allergy Research Centre, St Mary's Hospital, Isle of Wight carried out this study which was funded by the Food Standards Agency. The research has been submitted for publication and we were provided with a draft paper for comment. We initially reviewed this work in September 2000 when we discussed the results with members of the research team. At that time, we asked for additional data which was submitted for us to consider on 1st May 2001. The results were not available for consideration when our report on 'Adverse Reactions to Food and Food Ingredients' was published in June 2000.[2] Professor Eric Taylor of the Institute of Psychiatry, London, a member of the Working Group that drafted that report, assisted us in our deliberations.

3. The authors suggest that the study provides evidence that a mixture of five food additives, i.e. the preservative sodium benzoate and the food colours carmoisine, ponceau 4R, sunset yellow and tartrazine can affect the behaviour of three-year-old children.

The Committee's consideration of the study

4. The children were selected from the general population and the study involved putting the children on a diet which excluded the five additives for a period of one month. During this time the children were given, in a randomised double-blind protocol, coloured drinks with and without the five additives for separate periods of one week. We *note* that no statistically significant changes in the children's behaviour were apparent throughout the study when the children were assessed in a clinical setting. However, analysis of assessments made by the parents showed changes following challenge both with the additives and with the placebo. Compared with the effect of the placebo, challenge with the additives resulted in a small but significant increase in deterioration in the children's behaviour. This observation is consistent with results noted in our recent report,[2] i.e. that parents report behavioural changes in children that are not detected by observational assessment in a clinic.

5. We *note* that the reported effects on behaviour were small when compared to previous research. The researchers suggested that the behavioural effects occur irrespective of whether the children are atopic, hyperactive or neither. We have reservations about the generalisation and interpretation of these findings in view of some aspects of the study design. We therefore consider that the data, as reported, do not allow us to determine whether there was an adverse effect of the additives in all the children or a possible idiosyncratic effect in a susceptible sub-group.

Conclusions

6. Published data suggest exclusion of specific dietary components can affect some measures of behaviour in some children. The researchers suggest that this study provides evidence that food additives had statistically significant effects on some measures of behaviour, irrespective of whether the children were atopic, hyperactive or neither. We *acknowledge* that the study is consistent with published reports of behavioural changes occurring in some children following consumption of particular food additives. We also note that the authors suggest that this may apply to children who are not considered to be hyperactive. However, we *consider* that it is not possible to reach firm conclusions about the clinical significance of the observed effects.

June 2001

COT Statement 2001/03

References

1. Bateman B, Hutchinson E, Warner J, Dean T, Rowlandson P, Grant C, Grundy J, Fitzgerald C, Stevenson J (2000). The effects of a double blind placebo controlled artificial food colourings and benzoate challenge on hyperactivity in a general population sample of pre-school children. Unpublished manuscript.

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

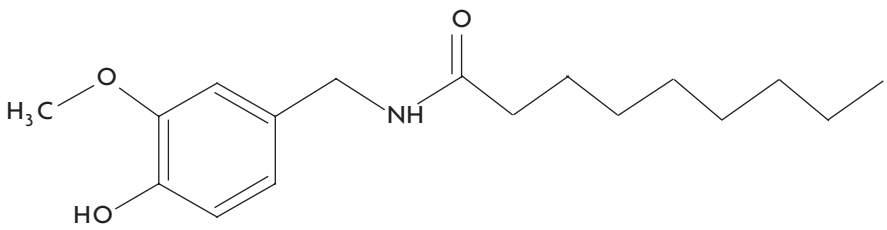
Statement on the use of PAVA (Nonivamide) as an incapacitant spray

Introduction

1. The Home Office has requested advice from the COT on the health effects arising from the use of a chemical incapacitant spray, containing pelargonyl vanillylamide (PAVA or Nonivamide) as the active ingredient. PAVA is the synthetic equivalent of capsaicin the active ingredient of natural pepper. It is a potent sensory stimulant. It is also used both as a food flavour (at up to 10 ppm in the diet) and in human medicine (topical application as a rubifaciant). In the USA it has been given GRAS (Generally Regarded as Safe) status by the Food and Drug Administration as a food flavour.

2. Following a pilot exercise last year the Sussex Police Force is now using PAVA spray as an alternative chemical incapacitant to CS spray. Recently Northamptonshire Police Force also started to use the spray. It is used by police forces in other European countries and in North America.

   PAVA has the following chemical structure:



Use of PAVA as incapacitant spray

3. The spray used by Sussex Police consists of a 0.3% solution of PAVA in 50% aqueous ethanol. It is dispensed from hand held canisters (containing nitrogen as propellant) as a coarse liquid stream; the spray pattern is stated to be directional and precise. The canisters contain 50 ml of solution. The instructions are to aim directly at the subject's face, especially the eyes, using a half second burst (still air) or one second burst (moving air), repeating if necessary. The maximum effective range is 8-15 feet and the instructions are not to use at a distance of under 3 feet because of the risk of pressure injury to the eye. The effectiveness of the spray depends on eye contact with small amounts reaching the eyes producing the desired effect. In some cases officers will miss and use more than one burst.

4. Studies on the particle size of the spray indicate that the bulk of the droplets are over 100 μm but a small proportion (1-2%) is in the range 2-10 μm, and there may be traces below 2 μm. Thus it is unlikely that large amounts of PAVA will reach the respiratory system, although the possibility of some reaching the lungs cannot be excluded. It is not possible to estimate the respirable dose.

Toxicity of PAVA

Absorption, Metabolism, Elimination and Excretion

5. Only limited data are available to assess oral absorption of PAVA; the compound shows higher acute toxicity by the parenteral rather than by the oral route suggesting relatively poor oral absorption. More extensive data are available regarding skin absorption, particularly from ointments designed for topical medical use. PAVA has been shown to be well absorbed through the rabbit skin (50-70% in 14 hours) when applied in such ointments (hydrophilic, oil-water emulsions) under an occlusive dressing.[1] More limited skin absorption (12% over 72 hours) was reported in the rat using an aqueous vehicle (phosphate buffered saline) and a non-occlusive dressing.[3] The only data available on skin absorption from aqueous ethanol are from *in vitro* studies using rat skin when the rate of absorption from 50% aqueous ethanol was shown to be considerably faster than that when phosphate buffered saline was used as vehicle.[3] It should therefore be assumed that there will be some absorption of PAVA following skin or eye contact with the spray.

6. Once absorbed PAVA is distributed throughout the body, extensively metabolised and rapidly excreted (most within 24 hours). The main route of metabolism is hydrolytic cleavage of the amide bond which occurs in liver and other tissue including the skin.[2] There is some evidence for aliphatic hydroxylation, as also occurs with capsaicin.[3]

Experimental studies in animals

7. Only very limited data are available on PAVA itself; it has a comparable toxicity, as measured by the LD_{50} value, to capsaicin when given by the intra-peritoneal route to mice (8 mg/kg).[4,5] Capsaicin has moderate toxicity by the oral route with LD_{50} values being reported as 119 and 97 mg/kg in male and female mice and 161 and 148 in male and female rats.[6] Data were provided on an acute inhalation study on PAVA in the rat using exposures of up to 3.6 mg/l for 4 hours, but the report is difficult to follow and no conclusions could be drawn.[7]

8. The skin irritancy of a 3.2% (v/v) solution of PAVA in polyethylene glycol has been investigated in the rabbit using an occlusive dressing and 4 hour exposure.[8] Animals were then observed for up to 3 days post exposure; no signs of irritancy were noted.

9. The ability of the in-use formulation (0.3% in 50% aqueous ethanol) to produce eye irritation has been investigated in the rabbit using the standard OECD test method.[9] Signs of significant irritation were seen from instillation until 3 days post-dose (including some evidence of opacity of the cornea and damage to the iris). However the eyes of all the animals had recovered by 7 days post exposure. These data indicate that the solution should be regarded as irritating to the eyes, but they do not suggest that any long term effects are likely.

10. No data are available on the potential of PAVA to cause skin sensitisation.

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

Reproductive toxicity

12. No data are available on reproductive toxicity studies on PAVA. Nor are any data available from fertility or developmental toxicity studies using standard methods for either PAVA or capsaicin. There is one report of the treatment of neonatal rats (2 day old) with a relatively high dose level of capsaicin (50 mg/kg using the subcutaneous route) resulting in retardation of sexual development and reduced fertility.[11] The dose level used resulted in growth retardation throughout adulthood. No conclusions can be drawn from this study regarding any effects of PAVA on the reproductive system. There are no reported investigations of developmental toxicity (teratogenicity), nor of any multigeneration studies to investigate effects on fertility.

Mutagenicity studies

13. The advice of our sister committee, the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) was sought on a package of mutagenicity data on PAVA that had been commissioned by the Sussex Police Force.[12,13,14,15] Its conclusions are given below:

    (i) The structure of PAVA suggests the possible formation of reactive oxygen species from the phenol moiety, and other possible active metabolites which may be mutagenic.

    (ii) Data are available from 3 *in vitro* studies done to current standards. The assay for gene mutation in bacteria gave negative results. Equivocal results were obtained in the mouse lymphoma assay. A clear positive result was however obtained in the assay for chromosome damage in CHO cells in the presence of the exogenous metabolic activation system which was not limited to concentrations producing excessive toxicity. These *in vitro* data indicate that PAVA has mutagenic potential.

    (iii) Negative results were obtained in a bone marrow micronucleus test, PAVA being given orally at up to dose levels that produced marked toxicity (some lethality).

    (iv) As noted in the COM guidelines, in the case of substances positive *in vitro* a negative result in a single tissue will not provide sufficient data to conclude that the chemical is inactive *in vivo*. Thus data from a second *in vivo* assay are necessary to provide adequate reassurance that the mutagenic potential identified in the *in vitro* studies cannot be expressed *in vivo*. In this regard members felt that data from an *in vivo* liver UDS assay would be appropriate in this case and that negative results in this assay would provide the necessary reassurance.

Effects in humans

Studies in volunteers

14. A series of studies have been carried out in volunteers to investigate the effect of inhalation of PAVA on the respiratory and cardiovascular system by measuring effects on heart rate, blood pressure, oxygen saturation and airways (FEV_1, Forced Expiratory Volume in 1 second).[16,17] These included studies in mild asthmatics. In order to maximise any response an aerosol of respiratory sized particles was produced using a nebulizer, rather than the coarse spray that is used by the police. Subjects (10 'normal' and 10 with mild asthma) were exposed to a range of concentrations, in the case of the normal subjects up to 0.3% PAVA in 50% aqueous alcohol to mirror the in-use condition. In the case of the asthmatics the maximum concentration used was 0.1%. Transient coughing was noted in normal subjects on exposure to the PAVA spray. Minimal effects were seen on FEV_1 heart rate and blood pressure (bp) in the normal subjects at the in-use concentration (1% reduction in mean FEV_1, 15% increase in mean heart rate and 8% increase in mean systolic bp compared to baseline). Similar effects were noted in the asthmatics exposed to 0.1% PAVA (mean reduction in FEV_1 3%, increase in heart rate 5% and increase in systolic bp 5%), although a transient but clinically significant reduction in FEV_1 ($>$0.5 l) was noted in 2 subjects; these were judged to have somewhat more severe asthma on the basis of their greater methacholine responsiveness. These data suggest that exposure to the coarse 'in-use' PAVA spray is unlikely to have any significant respiratory effects in normal subjects although some bronchospasm could be induced in asthmatics. It was noted that under operational use the subjects would be likely to be experiencing a high level of stress, and this could lead to clinically significant bronchospasm.

Experience in use

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

Quantification of exposure

16. It is extremely difficult to estimate accurately actual exposure levels in use due to the dynamics of the confrontation. The advice given to officers of the Sussex Police Force is to use a one second spray burst, and to repeat only if the first spray does not affect the eyes. However it is theoretically possible to discharge the whole of the container in a 6 second burst and this would be the most extreme (very unlikely) scenario. Assuming that half of this comes into contact with the skin, eye or mouth, this is equivalent to exposure to about 85 mg PAVA ie an external dose of the order of 1 mg/kg for an adult – some of which may be ingested. It was noted that the total exposure is about 150 times less than the estimated oral LD_{50} in the rat. A more realistic exposure scenario is to assume that all of a 1 second discharge comes into contact with the skin (ie about 28 mg PAVA). Assuming 10% absorption this will give a systemic dose of the order of 0.04 mg/kg (compared to a NOAEL of 10 mg/kg/day in the sub-

chronic rat study referred to in para 11). However it was noted that it was not possible to compare directly the dermal dose to that obtained orally, due to differences in systemic exposure to PAVA arising from the different routes. Any systemic exposure resulting from the use of PAVA spray was however likely to be low.

Conclusions

(i) We consider that it is not possible to make a complete assessment of the likely adverse health effects that could arise from the use of PAVA spray as a chemical incapacitant in view of the limited data available.

(ii) We recognise that exposures would be low and for a short period. It is impossible to calculate exposure with any accuracy but we note that dermal exposure would be of the order of 30 mg PAVA from a 1 second burst, with about 3 mg being absorbed. Any systemic exposure is likely to be low (of the order of 0.04 mg/kg bw).

(iii) The animal model data and experience in use do not give rise to any concerns regarding long term harm to the skin or eyes. However consideration needs to be given as to whether those wearing contact lenses might experience increased irritant effects. It is also noted that no data are available on the potential of PAVA to induce skin sensitisation.

(iv) The *in vitro* mutagenicity data, and consideration of metabolites, indicate that PAVA has some mutagenic potential; although negative results were obtained in an *in vivo* study to investigate mutagenic effects in the bone marrow, data from a further study are needed to provide adequate assurance that this activity cannot be expressed *in vivo*. An *in vivo* study to investigate the induction of unscheduled DNA synthesis (UDS) in the liver would be appropriate in this regard.

(v) No data are available to assess whether PAVA has any effects on the reproductive system. In particular the lack of any developmental toxicity studies is of concern as it is possible that pregnant women may be exposed to the spray.

(vi) The data from inhalation studies in volunteers, including those with mild asthma, indicate that there are unlikely to be any adverse respiratory reactions in normal individuals. Some respiratory effects may well occur in asthmatics, particularly since effects were observed in asthmatic volunteers at 0.1% PAVA, which is lower than the 0.3% used in the spray, and given the conditions of increased stress likely when the spray is used.

(vii) Further monitoring of experience in use, including the police officers using the spray, is recommended with particular consideration being given to eye irritancy in those wearing contact lenses and to effects in those with asthma or hay fever and in women who may be pregnant.

April 2002

COT Statement 2002/02

References

1. Fang JF, Wu PC, Huang YB, Tsai YH. In vivo percutaneous absorption of capsaicin, nonivamide and sodium nonivamide acetate from ointment bases: pharmacokinetic analysis in rabbits. Int. J. Pharmaceutics. **128** 169-77 (1996).

2. Kasting GB, Francis WR, Bowman LA, Kinnett GO. Percutaneous absorption of vanilloids: In vivo and in vitro studies. J. Pharm. Sci. **86** 142-6 (1997).

3. Surh YJ, Ahn SH, Kim KC, Park JB, Sohn YW, Lee S. Metabolism of capsaicinoids: evidence for aliphatic hydroxylation and its pharmacological implications. Life Sciences **56** PL305-311 (1995).

4. Janusz HM, Buckwalter BL, Young PA, LaHann TR, Farmer RW, Kasting GB, Loomans ME. Vanilloids I. Analogues of capsaicin with anti-nociceptive and anti-inflammatory activity. J. Med. Chem. **36** 1595-2004 (1993).

5. Glinsukon T, Stitmunnaithum V, Toskulkao C, Buranawisti T, Tangkrisanavinont V. Acute toxicity of capsaicin in several animal species. **18** 215-20 (1980) Toxicon.

6. Saito A and Yamamoto M. Acute oral toxicity of capsaicin in mice and rats. J. Toxicological Sciences, **21** 195-200 (1996).

7. Confarma AG. Report No 21101618C. Test of inhalation toxicity of Nonivamide/Nonyl acid capsaicin. Report for Swiss Police Technical Commission (1996).

8. Confarma AG. Report No: 21101618. Primary Dermal Tolerance of Intact and Scarified Skin Against Nonivamide. Report for Swiss Police Technical Commission (1995).

9. Chevarne FE. Technical/toxicological back up data to synthetic capsaicin solution (PAVA). Report by Analysis SA. Spain for IDC systems Switzerland.

10. Posternak JM, Linder A, Voduz CA. Toxicological tests on flavouring matter. Food Cosmetic Toxicol. **7** 405-7 (1969).

11. Traurig H, Saria A, Lembeck F. The effects of neonatal capsaicin treatment on growth and subsequent reproductive function in the rat. Naunyn-Schmiedeberg's Archives of Pharmacology **327** 254-9 (1984).

12. Nonivamide (PAVA). Testing for mutagenic activity with Salmonella typhimurium TA 1535, TA 1537, TA 98, TA 100 and Escherichia coli WP2 UvrA. Inveresk Report No 19490 (2001). Commissioned by Sussex Police.

13. Nonivamide (PAVA). Chromosomal aberration assay with CHO cells in vitro. Inveresk Report No 2039 (2001). Commissioned by Sussex Police.

14. Nonivamide (PAVA); mouse lymphoma cell mutation assay. Inveresk Report No 20250 (2001). Commissioned by Sussex Police.

15. Nonivamide (PAVA). Micronucleus test in bone marrow of CD-1 mice. Inveresk Report No 20903 (2002). Commissioned by Sussex Police.

16. Effects of high concentrations of inhaled nonivamide (PAVA) in normal subjects. Report by Dr P Ind *et al*. Imperial College School of Medicine, London for Sussex Police (2001).

17. Effect of inhaled Nonivamide (PAVA) in subjects with asthma. Report by Dr P Ind *et al*. Imperial College School of Medicine, London for Sussex Police.

Statement on phenol: Tolerable Daily Intake (oral)

Introduction

1. The United Kingdom Government is in the process of providing guidance on the health risks from contaminated land[1]. The guidance provides land-use-specific Soil Guideline Values (SGVs) for a range of chemical contaminants. Phenol has been identified as a soil contaminant of possible concern. To help derive a SGV for phenol, we were asked to recommend an appropriate No Observed Adverse Effect Level (NOAEL) and Tolerable Daily Intake (TDI) for oral exposure to phenol.

Toxicology

2. In 1994, the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM) agreed that phenol should be regarded as a somatic cell in-vivo mutagen. In 2000, the COM reviewed the available mutagenicity, toxicokinetic and metabolism data on phenol and agreed conclusions regarding risk of mutagenicity following ingestion, which are reproduced below:

   (i) Any risk to human health by ingestion would be likely to be greatly reduced by rapid conjugation and detoxification via the glutathione pathway. Furthermore mutagenicity also appeared to be positively related to peroxidase activity while catalase could also have a protective role. Actual systemic exposure levels in humans would be very much lower than levels at which positive results had been achieved in studies in animals

   (ii) The Committee concluded that by the oral route there was potential for a threshold of activity based on the protective mechanism outlined at (i).

3. The most recent addition to the toxicological data on ingested phenol, suitable for consideration for the purposes of risk assessment, is the two-generation reproduction study (drinking-water) in Sprague-Dawley rats by Ryan *et al.*, published in 2001[3]. The study was conducted as part of a negotiated consent agreement between the United States Environmental Protection Agency and the Phenol Panel of the Chemical Manufacturers Association. It was consistent with previous studies in finding evidence of parental and fetal toxicity at a dose of approximately 310 mg/kg bw/day. At the two lower doses (about 14 and 70 mg/kg bw/day), the only notable findings were reductions in absolute and relative prostate and uterine weights in the F1 generation. The authors argued that, given the absence of functional reproductive effects or adverse histological changes at these doses, the reductions in prostate and uterine weight did not represent an adverse effect of phenol. We *note* that this view is also consistent with the absence of effects on these organs in the NCI 1980 103-week drinking-water studies in F344 rats and B6C3F1 mice[4]. The authors therefore proposed 70 mg/kg bw/day as the NOAEL in this study. In the light of this proposed NOAEL, the following paragraphs discuss several earlier studies which reported findings at lower doses.

4. Hsieh *et al.* (1992) reported a reduced red cell count and haematocrit in male CD-1 mice at 1.8, 6.2 and 34 mg/kg bw/day for 28 days (*via* drinking-water), and suppressed antibody production response at their two highest doses[5], but Ryan *et al.* (2001) found no such changes at comparable and higher doses, and longer duration of dosing, in male Sprague-Dawley rats [3]. We *note* that the paper by Hsieh *et al.* provided no information on impurities in the reagent grade phenol used in the study, and did not indicate that any precautions had been taken to minimise oxidation and degradation of the test substance.

5. Hsieh *et al.* (1992) also reported reductions in neurotransmitter levels in several brain regions, in their study on male CD-1 mice[5]. No other study has examined these endpoints, and the functional significance of the reported reductions is unknown. Moser *et al.* (1995) reported a change in behaviour (increased "rearing") in a battery of neurobehavioural tests in female F344 rats given phenol by gavage at 40 mg/kg bw/day for 14 days; again, the functional significance is unknown[6]. In contrast, no relevant dose-related clinical or neuropathological findings were reported in the NCI 1980 103-week drinking-water studies in F344 rats and B6C3F1 mice, at much higher doses[4].

6. According to the USEPA online chemical toxicity information service[7], an unpublished 1945 Dow Chemical Company study revealed liver changes in rats (strain unknown) given phenol by gavage at 72 mg/kg bw/day for 6 months, and kidney damage at this dose and at 36 mg/kg bw/day, but no further details are available to us, and no useful conclusions can be drawn. The study by Berman *et al.* (1995) reported renal and hepatic pathology in female F344 rats given phenol for 14 days by gavage at 40 mg/kg bw/day, and thymic necrosis was found at 12 and 40 mg/kg bw/day[8]. In contrast, no gross pathology in these three organs was reported by Hsieh *et al.* (1992) in male CD-1 mice at 34 mg/kg bw/day (via drinking-water) for 28 days[5]. No histopathological changes in these organs were reported in the NCI 1980 (F344 rats, B6C3F1 mice)[4] and Ryan *et al.* (2001) (Sprague-Dawley rats)[3] drinking-water studies for longer periods at much higher doses. We note, however, that the thymus was not one of the tissues routinely examined microscopically in the NCI 1980 study.

7. Narotsky and Kavlock (1995) reported "altered respiration (eg rales and dyspnoea)" and "severe respiratory signs" in F344 rat dams given phenol by gavage at 40 and 53.3 mg/kg bw/day on days 6-19 of pregnancy[9]. No similar finding was reported in other studies (eg NCI, 1980 and Ryan *et al.*, 2001)[4, 3], and it may be that problems with gavage, rather than intragastric phenol, were responsible. This may also explain the litter losses at these doses in this study, since all of the dams that fully resorbed their litters experienced severe respiratory effects. Comparable fetal and maternal toxicity were not seen at higher doses in the gavage studies by Jones-Price *et al.* (Sprague-Dawley rats; CD-1 mice)[10,11] or in the Ryan *et al.* drinking-water study (Sprague-Dawley rats)[3].

8. The reasons for the apparent discrepancies between studies are unclear, but may be related to factors such as manner of exposure (abnormal findings reported at lower doses when phenol is administered as bolus doses by gavage than in studies using administration *via* drinking-water) and impurities, including oxidation and degradation products, in the test agent.

Conclusions

9. We *note* the opinion of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (2000) that, by the oral route, there is potential for a threshold of activity for the mutagenicity of phenol.

10. The data which we have considered on the toxicity of ingested phenol are sufficient to identify a No Observed Adverse Effect Level (NOAEL) for other endpoints. The critical study is the enhanced two-generation reproductive and developmental toxicity study in rats, by Ryan *et al*. (2001), in which the overall NOAEL was 70 mg/kg bw/day[3]. Although several studies have reported abnormal findings in animals at lower doses, these results were not consistent with the absence of comparable or related findings in other well-conducted studies at higher doses and longer periods of exposure to ingested phenol.

11. Standard uncertainty factors of 10 for extrapolation from rodent data, and 10 for variability within the human population, are appropriate.

12. The Tolerable Daily Intake for ingested phenol is therefore 0.7 mg/kg bw/day.

October 2002

COT Statement 2002/03

References

1. DEFRA/EA (2002) Department of the Environment, Food and Rural Affairs and the Environment Agency. Contaminants in soil: collation of toxicological data and intake values for humans. R&D Publication CLR 9. Environment Agency, Bristol.

2. COM (2000) Department of Health. Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment. Statement on the mutagenicity of hydroquinone and phenol. COM/00/S1.

3. Ryan B M, Selby R, Gingell R, Waechter J M, Butala J H, Dimond S S, Dunn B J, House R, Morrissey R (2001). Two-generation reproduction study in immunotoxicity screen in rats dosed with phenol via the drinking water. *Int J Toxicol* **20**, 121-142.

4. NCI (1980) National Cancer Institute. Bioassay of phenol for possible carcinogenicity. Technical Report TR-203. Prepared by the NCI for the National Toxicology Program, Research Triangle Park, NC, USA.

5. Hsieh G C, Sharma R P, Parker R D R, Coulombe R A (1992). Immunological and neurobiochemical alterations induced by repeated oral exposure of phenol in mice. *European Journal of Pharmacology – (Environmental Toxicology and Pharmacology Section* **228**, 107-114.

6. Moser V C, Cheek B M, MacPhail R C (1995). A multidisciplinary approach to toxicological screening. III. Neurobehavioral toxicity. *J Toxicol Environ Health* **45**, 173-210.

7. USEPA (2001) United States Environmental Protection Agency. Phenol. Integrated Risk Information System (IRIS, the USEPA online chemical toxicity information service). Accessed August 2002.

8. Berman E, Schlicht M, Moser V C, MacPhail R C (1995). A multidisciplinary approach to toxicological screening: I. Systemic toxicity. *J Toxicol Environ Health* **45**, 127-143.

9. Narotsky M G, Kavlock R J (1995). A multidisciplinary approach to toxicological screening. II. Developmental toxicity. *J Toxicol Environ Health* **45**, 145-171.

10. Jones-Price C, Kimmel C A, Ledoux T A, Reel J R, Langhoff-Paschke L, Marr M C (1983a). Final study report – (teratologic evaluation of phenol (CAS No. 108-95-2) in CD-1 mice (NTP Study No. TER-80-129). Research Triangle Institute, Research Triangle Park, NC, USA (NTIS Report No. PB85-104461).

11. Jones-Price C, Kimmel C A, Ledoux T A, Reel J R, Fisher P W, Langhoff-Paschke L, Marr M C (1983b). Final study report (teratologic evaluation of phenol (CAS No. 108-95-2) in Sprague-Dawley rats (NTP Study No. TER-81-104). Research Triangle Institute, Research Triangle Park, NC, USA (NTIS Report No. PB83-247726).

Statement on a survey of mercury in fish and shellfish

Introduction

1. We have been informed of the results of a Food Standards Agency (FSA) survey of the mercury levels in imported fish and shellfish and UK farmed fish and their products[1]. We were also informed of provisional results of blood mercury levels in 1320 adults participating in a recent National Diet and Nutrition Survey (NDNS)[2].

2. In an initial response to the estimations of mercury intake derived from the fish survey results, the FSA released a precautionary interim statement following consultation with the COT Chairman. This statement advised the general population to restrict consumption of shark, swordfish and marlin to no more than one portion a week of any of these fish. It also gave precautionary advice to pregnant women, women intending to become pregnant and children to avoid consumption of these three species of fish.

3. We were asked to consider the most appropriate safety guidelines to use in assessing the health implications of mercury in fish, and to consider possible health concerns associated with the estimated mercury intakes and blood level data provided.

Background

4. The toxicity of mercury is dependent on whether it is inorganic, elemental or organic (e.g. methylmercury). Methylmercury affects the kidneys and also the central nervous system, particularly during development, as it crosses both the blood-brain barrier and the placenta[3]. Both neuro- and nephrotoxicity have been associated with acute methylmercury poisoning incidents in humans, and neurotoxicity, particularly in the developing fetus, has been associated with lower level chronic exposures.

5. Exposure of the general population to mercury can occur via inhalation of mercury vapour from dental amalgam fillings (elemental), or through the diet (methylmercury and inorganic mercury). Methylmercury in fish makes the most significant contribution to dietary exposure to mercury, although smaller amounts of inorganic mercury are present in other food sources. All forms of mercury entering the aquatic environment, as a result of man's activities or from geological sources, are converted into methylmercury by microorganisms and subsequently concentrated in fish and other aquatic species. Fish may concentrate the methylmercury either directly from the water or through consuming other components of the food chain. Methylmercury has a half-life of approximately 2 years in fish thus large older fish, particularly predatory species, will have accumulated considerably more mercury than small younger fish.

Previous COT evaluation

6. COT previously considered the results of a survey of metals and other elements in marine fish and shellfish[4] published by the Ministry of Agriculture, Fisheries and Food (MAFF) in 1998. The survey examined a number of fish and shellfish species landed in the UK or imported from overseas ports including cod, haddock, herring, mackerel, lobster, mussels, crab and shrimps and samples of cod fish fingers. The survey also produced estimates of the mean and 97.5th percentile dietary intakes of the elements surveyed.

7. The 1998 survey demonstrated that the levels of mercury in the fish and shellfish tested were low and that average and high level fish and shellfish consumers in the UK would not exceed the provisional tolerable weekly intake (PTWI) for mercury or methylmercury. The estimated mercury intake for the highest level consumer was 1.1 μg/kg bw/week including mercury intake from the rest of the diet. The main conclusion drawn from the survey was that "dietary intakes of the elements surveyed were below safe limits, where defined, and did not represent any known health risk even to consumers who eat large amounts of marine fish or shellfish".

International Safety Guidelines

Joint FAO/WHO Expert Committee on Food Additives (JECFA)

8. In 1972, JECFA established a PTWI of 5 μg/kg bw/week for total mercury, of which no more than two thirds (3.3 μg/kg bw/week) should be from methylmercury[5]. The PTWI of 3.3 μg/kg bw/week for methylmercury was subsequently confirmed in 1989 and 2000[6,7]. The PTWI was derived from toxicity data resulting from poisoning incidents at Minamata and Niigata in Japan. In these incidents the lowest mercury levels associated with the onset of clinical disease in adults were reported to be 50 μg/g in hair and 200 μg/L in whole blood. Individuals displaying clinical effects, such as peripheral neuropathy, at these mercury levels were considered to be more sensitive than the general population, because there were a number of persons in Japan and other countries with higher mercury levels in hair or blood who did not experience such effects. However the methods employed in determining the intake associated with toxicity, and the subsequent establishment of the PTWI are unclear.

9. In 1989, JECFA had noted that pregnant women and nursing mothers may be at greater risk than the general population to adverse effects from methylmercury. Therefore it subsequently paid particular attention to possible effects of prenatal and postnatal exposure. Large long-term prospective epidemiological studies have been conducted in the Seychelles Islands and the Faroe Islands which attempted to identify the lowest dietary mercury exposure associated with subtle effects on the developing nervous system[8,9,10,11]. The studies have followed the neurological development of the children by testing their learning and spatial abilities at a number of time-points during their childhood. A number of smaller studies were also available.

10. Comparing the two main studies, the Faroe Islands cohort was tested up to the age of 7 years, whereas the Seychelles cohort has so far been tested up to the age of 5.5 years. Exposure in the Seychelles is through consumption of a range of fish species mostly with mercury concentrations between 0.05 and 0.25 mg/kg. In the Faroe Islands, most of the population consume fish at least three times a week and there is also occasional (approximately once per month) consumption of pilot whale which contains up to 3 mg/kg mercury. Pilot whale also contains high concentrations of polychlorinated biphenyls (PCBs), but a recent reanalysis of the data indicate that the effects seen could not be attributed to confounding by the PCBs[12]. There are also differences in the methods used to assess exposure. The Seychelles study used maternal hair samples (approx. 9cm long), one taken shortly after birth to estimate methylmercury exposure during pregnancy and one taken 6 months later. The Faroe Islands study used cord blood and maternal hair (various lengths) taken at birth. The studies used different batches of tests to assess the effects of methylmercury on neurological development. The tests used in the Faroe Islands study each examined specific domains in the brain (visual, auditory, etc.) however the Seychelles study used tests of a more global nature, with each test examining a number of domains.

11. These studies are continuing, but the results at present are conflicting. The mean mercury exposures (assessed by maternal hair mercury) during pregnancy were similar (Seychelles: arithmetic mean 6.8 μg/g, range 0.5-26.7 μg/g; Faroes: geometric mean, 4.27 μg/g, the upper mercury level in maternal hair is not clear from the reported data but may be as high as 70 μg/g). In the Faroes study, regression analysis showed an association between methylmercury exposure and impaired performance in neuropsychological tests, an association that remained even after excluding the results of children with exposures associated with greater than 10 μg/g maternal hair mercury. However in the Seychelles study regression analysis has identified no adverse trends, but a small statistically significant increase in test scores on several of the developmental outcomes. The investigators noted that this was probably due to beneficial nutritional effects of fish. A secondary analysis was performed where the results were split into sub-groups based on the maternal hair mercury level. Test scores in children with the highest mercury exposures (12 – 27 μg/g maternal hair) were not significantly different from the test scores in children with lowest exposure ($<$ 3 μg/g maternal hair).

12. A smaller study carried out in New Zealand on 6 year-old children[13] used a similar batch of tests to the Seychelles study and had similar exposure to methylmercury, yet found methylmercury related effects on behavioural test scores. However there were possible confounding factors that may have influenced the results of the New Zealand study, such as the ethnic group and social class of the children studied.

13. Having considered all of the epidemiological evidence, JECFA concluded that the Faroe Islands and Seychelles Islands studies did not provide consistent evidence of neurodevelopmental effects in children whose mothers had hair mercury levels of 20 μg/g or less. Because there was no clear indication of a consistent risk in these epidemiological studies, JECFA did not revise the PTWI, but recommended that methylmercury should be re-evaluated when the 96-month evaluation of the Seychelles study and other relevant data become available[7].

Environmental Protection Agency (EPA)

14. In 1997 the US EPA established a reference dose of 0.1 μg/kg bw/day for methylmercury[14]. This was based on a peak maternal hair mercury level during pregnancy of 11 μg/g, which was associated with developmental effects (e.g. late walking, late talking, mental symptoms, seizures) in children exposed *in utero* during a poisoning incident in Iraq in 1971.

15. In 2000, the US National Research Council (NRC) published a review of this EPA reference dose[15]. Following analysis of the data resulting from the available epidemiological studies, the NRC identified a benchmark dose of 58 μg/L in cord blood (corresponding to 12 μg/g in maternal hair). This was the lowest dose considered to produce a sufficiently reliable neurological endpoint (a 5% increase in abnormal scores on the Boston Naming Test*) in the Faroe Islands study. The NRC made a number of assumptions in deriving an estimate of methylmercury intake and included a composite uncertainty factor of 10, resulting in the same reference dose of 0.1 μg/kg bw/day, as had previously been used by the EPA. This reference dose is approximately one fifth of the current JECFA PTWI of 3.3 μg/kg of body weight/week.

Survey of the mercury levels in fish

16. The new FSA survey complements the previous MAFF survey since it has examined a wider range of fish, including imported exotic species of fish that have become more widely available on the UK market. These included shark, swordfish, marlin, orange roughy, red snapper and monkfish, as well as UK farmed fish such as salmon and trout[1].

17. Of the fish species covered by the survey, all but 3 species had mean mercury levels falling within the range 0.008 – 0.88 mg/kg of fish. This range is in line with the levels defined by European Community Regulation 466/2001 as amended by European Community Regulation 221/2002 (0.5 mg of mercury/kg for fish in general and 1.0 mg mercury/kg for certain larger predatory species of fish including shark, swordfish, marlin, tuna and orange roughy).

18. The 3 species with the highest mercury content were shark, swordfish and marlin. These fish had mean mercury levels of 1.52, 1.36, and 1.09 mg/kg respectively and were therefore above the levels defined in European Community Regulation 221/2002. Fresh tuna contained mercury levels ranging from 0.141 to 1.50 mg/kg with a mean of 0.40 mg/kg (only one sample out of 20 exceeded 1 mg/kg, the maximum mercury concentration in the other 19 samples was 0.62 mg/kg), whereas canned tuna had a lower mean mercury level of 0.19 mg/kg. It is assumed that canned tuna have lower mercury levels than fresh tuna, because the fish used in the production of canned tuna are smaller and younger and subsequently have accumulated less methylmercury.

* The Boston Naming Test is a neuropsychological test that assesses an individual's ability to retrieve a word that appropriately expresses a particular concern, for example naming an object portrayed by a simple line drawing.

19. Dietary exposure to mercury has been estimated for those fish species for which reliable consumption data are available[16, 17, 18, 19] (salmon, prawns and canned tuna) together with exposure from the rest of the diet (Table 1). Of these fish, canned tuna provided the largest contribution to dietary mercury exposure for high level consumers. Total fish consumption by the high level consumer was equivalent to approximately five portions per week (688g).

20. Estimates were also made of the methylmercury intake resulting from consumption of one portion of shark, marlin, swordfish or fresh tuna, for which consumption data are not available (Table 2), using portion sizes as recorded in the NDNS for fish consumption[17, 18, 19]. For comparative purposes similar estimates were made for canned tuna. For children up to the age of 14 and adult consumers, the mercury intake resulting from one portion of shark, marlin or swordfish per week would be close to or above the PTWI for methylmercury.

Blood mercury levels in British adults

21. We also considered a report produced by the Medical Research Council Human Nutrition Research in March 2002 detailing the provisional blood total mercury data obtained from 1320 adults (aged 19-64 years) participating in the National Diet and Nutritional Survey (NDNS) [2].

22. The mean and 97.5th percentile blood mercury levels in the survey were 1.6 and 5.88 μg mercury/L respectively. The highest blood mercury level found in the study was approximately 26 μg/L in an individual with a high fish intake. If the blood mercury level was at steady state, and assuming a body weight of 70 kg, then this would correspond to a mercury intake of approximately 2.6 μg/kg bw/week, which is within the JECFA PTWI.

23. Of the population covered by the survey, 97.5% had blood mercury levels indicating that their mercury intakes were below the EPA reference dose.

COT evaluation

24. The Committee discussed the possible risks associated with dietary exposure to methylmercury, in the light of the new information on intakes from fish and on blood mercury levels in the UK population.

Toxicokinetic considerations

25. Following ingestion, approximately 95% of methylmercury is absorbed through the gastrointestinal tract, and is subsequently distributed to all tissues in about 30 hours with approximately 5% found in blood and 10% in the brain. The methylmercury concentration in red blood cells is approximately 20 times higher than that in the plasma. Methylmercury readily crosses the placental barrier. Fetal brain mercury levels are approximately 5-7 times higher than in maternal blood. Methylmercury readily accumulates in hair and the ratio of hair mercury level (μg/g) to blood mercury level (μg/L) is approximately 1:4. Based on comparisons to hair concentrations, cord blood concentrations are reported to be 25% higher than the concentrations in maternal blood.[8] Table 3 shows the blood and hair mercury concentrations associated with exposures resulting in adverse effects and with the JECFA PTWI and EPA reference dose.

26. The excretion process for methylmercury involves transfer of the glutathione-mercury complex into the bile, demethylation by gut microflora to the inorganic form, then elimination from the body in the faeces. The half-life of mercury in the body is approximately 70 days in adults, with steady state being reached in about one year. Significant amounts of methylmercury also pass into the breast milk of lactating women, resulting in a decreased mercury half-life of approximately 45 days[20].

27. Doherty and Gates (1973)[21] reported that the excretion rate of mercury in the suckling rodent is less than 1% of the adult excretion rate. Sundberg *et al.* (1998)[22] reported a low elimination of mercury in suckling mice until lactational day 17. This is probably because biliary secretion and demethylation by microflora (which lead to faecal excretion) do not occur in suckling animals. The role of these processes in suckling human infants is unknown[3].

28. The concentration of mercury in breast-milk is approximately 5% of the blood mercury concentration of the mother[20]. Amin-Zaki *et al.*[23] reported that in women exposed to high levels of methylmercury during the Iraqi poisoning incident, 60% of the mercury in breast-milk was in the form of methylmercury. Therefore it may be estimated that the concentration of methylmercury in the breast-milk is approximately 3% of the total mercury concentration in the blood. If a breastfeeding mother was exposed to methylmercury at the JECFA PTWI then the suckling infant would be exposed to the following approximate amount of methylmercury:

    Blood mercury level = 33 μg/L
    Amount of methylmercury in milk = 0.99 μg/L

    Assuming a daily milk intake of 150 mL/kg bw
    Methylmercury intake = 0.15 μg/kg bw/day

29. Therefore the infant is exposed to methylmercury at a level substantially below the JECFA PTWI but 50% above the EPA reference dose.

Susceptible populations

30. The Committee noted that the JECFA PTWI may not be sufficiently protective for high-risk groups and therefore gave particular consideration to determining which groups are at higher risk.

31. The critical effect of methylmercury is on the developing central nervous system and therefore pregnant women are considered to be the most susceptible population because of the risk to the fetus. There have been no studies of the effects of exposure prior to becoming pregnant. However, because the half-life of methylmercury in the human body is approximately 70 days, steady state concentration is attained in approximately one year and a woman's blood mercury level at the time of becoming pregnant is dependent on the exposure to methylmercury during the preceding year. The Committee therefore agreed that women who may become pregnant within the next year should also be considered as a susceptible population.

32. The evidence regarding consideration of other susceptible populations is not conclusive. Animal experiments indicate that exposure via breast-milk has less serious consequences to the central nervous system than prenatal exposure. Spyker and Spyker[24] reported that the effects of prenatal exposure to methylmercury dicyandiamide on the survival and weight gain of the offspring were more severe than those seen with postnatal exposure, and were greatest when the methylmercury was administered late in the period of organogenesis. However, these results are not necessarily relevant to the health effects of concern in human exposure.

33. Data from a 5-year longitudinal study following the Iraq poisoning incident have suggested that some children exposed to methylmercury via breast-milk demonstrated delayed motor development [23]. The maternal blood mercury levels immediately following the incident were estimated by extrapolation to be in the range of approximately 100 μg/L to 5000 μg/L. Mothers who showed signs and symptoms of poisoning (ataxia, dysarthria, visual disturbance etc.) had the higher blood levels (3000 to 5000 μg/L) although some women with levels in this range were asymptomatic.

34. The affected infants all had blood mercury levels above those associated with the JECFA PTWI, and most of them had blood mercury levels higher than the minimum toxic level of 200 μg/L, defined by JECFA. There was no paralysis, ataxia, blindness or apparent sensory change and there were no cases of the severe mental destruction and cerebral palsy that were seen in the prenatally exposed infants of Minamata. However, language and motor development of the children were delayed. The authors of the study concluded that breast-fed infants are not as much at risk as the fetus, since most of the brain development has already occurred and the effects seen in the breast-feeding infant are different from those seen in infants exposed prenatally and not as severe.

35. There is no evidence that chronic exposure to methylmercury via the breast milk at levels below those observed in the Iraqi incident has any adverse effect on the neurophysiological/psychological development of the child. Data from the Faroe Islands study suggests that the beneficial effects of nursing on early motor development are sufficient to compensate for any adverse impact that prenatal exposure to low concentrations of methylmercury might have on these endpoints[25, 26]. Grandjean *et al*[25] looked at the relationship between seafood consumption and concentrations of contaminants in breast-milk in the Faroes Island population. Of 88 samples of breast-milk, three had a mercury level that would cause the infant to exceed the PTWI for mercury.

36. There have been few studies of the effects of methylmercury on young children. Most information has come from the poisoning incidents in Minamata, Niigata and Iraq. In all of these cases the exposures were very high, and in Iraq, the exposure was acute. Methylmercury is excreted by children as efficiently as by adults[2]. In the incidents where children were exposed directly to methylmercury and not prenatally, the damage seen in the brain was similar to that seen in adults: focal lesions of necrosis. The damage seen when the fetus is exposed is much more widespread[3].

37. The longitudinal study in the Seychelles has attempted to examine the effects of postnatal exposure to methylmercury[10]. This is complicated by the facts that in the Seychelles, the children exposed to methylmercury postnatally are also exposed prenatally, and the study has been unable to demonstrate any mercury-related deficits in the neurological development of children. However higher postnatal methylmercury exposure had a positive association with test scores. It was suggested that this may be because a higher mercury level indicates a high fish intake and therefore a diet rich in n-3-polyunsaturated fatty acids and vitamin E, which have beneficial effects and may mask any subtle neurological deficits due to chronic low level exposure to methylmercury. Fish consumed in the Seychelles Islands contain similar concentrations of methylmercury to fish commonly consumed in the UK[10], but methylmercury intakes appear to be higher in the Seychelles than in the UK. The FSA survey indicates that in the UK high level dietary exposure to methylmercury from commonly consumed fish ranges from about 0.8 μg/kg bw/week for adults to 2 μg/kg bw/week for toddlers. Based on the correlations in Table 3, it can be estimated that the most exposed individuals in the Seychelles studies had intakes in the region of 4.8-10.8 μg/kg bw/week.

38. The risk is greater for women who are pregnant or likely to become pregnant within the following year because of the effects of methylmercury on the developing central nervous system of the fetus. There is uncertainty with respect to whether infants and young children are at greater risk of methylmercury toxicity whilst the central nervous system is still developing. The limited data available indicate that this is not the case for children but the possibility of increased sensitivity of infants cannot be discounted.

39. The EPA reference dose is derived from data on neurobehavioural development in the Faroe Islands and the Iraqi incident. The population studied in the Seychelles Islands had similar maternal hair mercury concentrations to those in the Faroes and Iraq (Table 3). So far the Seychelles study has been unable to demonstrate any subtle neurobehavioural effects associated with *in utero* exposure, although effects may become apparent when the Seychelles cohort of children is subjected to comparable tests to those used on the Faroe Island children at age 7. Thus there are currently inconsistencies in the evidence of whether adverse effects are expected to arise from maternal exposures resulting in hair mercury concentrations in the region of 10-20 μg/g (corresponding to exposure of 4 – 8 μg/kg bw/week). However in view of the Faroe and Iraqi data, we consider it appropriate to refer to the EPA reference dose (which is equivalent to 0.7 μg/kg bw/week) in assessing the methylmercury intakes of pregnant women, women who may become pregnant within the next year, and breast-feeding mothers. The intakes for other subgroups should be compared with the JECFA PTWI of 3.3 μg/kg bw/week.

Assessment of dietary exposure estimates

40. The estimates of average and high level total dietary exposure to mercury, including from fish for which consumption data are available, are within the JECFA PTWI for methylmercury for all age groups.

41. The estimated high level intake by adult women exceeds the EPA reference dose by 10%. Total dietary exposure to mercury includes inorganic mercury, which is less toxic than methylmercury, and therefore comparison with the reference dose for methylmercury represents an overestimation of the risk. It is therefore unlikely that this small exceedance of the reference dose could result in adverse effects on the developing nervous system of the fetus or of the breast-fed infant.

42. Because of their nutritional requirements, dietary mercury exposure is comparatively higher in children than in adults. However, taking into account the evidence for the beneficial effects of fish consumption, we consider that there is an adequate margin of safety between the estimated total dietary exposure for average and high level child consumers and methylmercury exposures that could result in neurotoxicity.

43. For adults, consumption of one portion of shark, swordfish or marlin per week could result in a mercury intake close to the PTWI for methylmercury, before considering intake from the rest of the diet. To exceed the PTWI would require consumption of four portions of fresh tuna, or 8 portions of canned tuna per week by an adult. Children under the age of 14 consuming one portion of shark, swordfish or marlin per week, when considered with the rest of the diet, would exceed the PTWI. To exceed the PTWI would require consumption of about 3 portions per week of fresh tuna, or 6 portions of canned tuna.

44. Consumption of one portion of shark, swordfish or marlin by pregnant or breastfeeding women together with the rest of the diet would exceed the EPA reference dose for methylmercury by about 4-fold. One portion of fresh tuna or 2 portions of canned tuna would slightly exceed (about 10%) the EPA reference dose, which is not expected to result in adverse effects.

Conclusions

45. We *consider* that the JECFA PTWI of 3.3 μg/kg bw/week is sufficiently protective for the general population. We *recognise* that the PTWI may not be sufficiently protective for women who are pregnant, or who may become pregnant within the following year, or for breast-feeding mothers. This is due to the potential risk to the developing fetus or neonate. We therefore consider that the EPA reference dose of 0.1 μg/kg bw/day (0.7 μg/kg bw/week) is more appropriate for these groups.

46. We *consider* the NDNS data are reassuring with respect to average and high level consumption of fish. The adults surveyed had blood mercury levels that indicate that the JECFA PTWI for methylmercury was not being exceeded.

47. We *note* that estimates of average and high level dietary mercury exposure, resulting from fish for which consumption data are available, are within the JECFA PTWI for methylmercury for all age groups. Adult women who are high level consumers of these commonly eaten fish may marginally exceed the EPA reference dose. However we *consider* that this dietary exposure is not likely to be associated with adverse effects to the developing fetus.

48. We *note* that consuming one weekly portion of either shark, swordfish or marlin would result in a dietary exposure close to, or exceeding, the PTWI and therefore exceeding the EPA reference dose for methylmercury in all age groups. We *consider* that this consumption would not be expected to result in adverse effects in the general adult population, but could be harmful to the fetus and to the breast-fed infant. The exceedance of the PTWI is relatively greater for children under 14 years, because their food intake is greater, on a bodyweight basis, than that of adults. However, taking into account the evidence for the beneficial effects of eating fish, consumption of one portion per week of these fish is not expected to result in adverse health effects.

49. We *note* that the mercury content of tuna is lower than that of shark, swordfish or marlin, but higher than that of other commonly consumed fish. We *consider* that consumption of one portion of fresh tuna, or two portions of canned tuna, per week, by pregnant or breast-feeding women is not expected to result in adverse effects on the developing fetus or infant.

50. We *recommend* that further research is required to provide evidence on potential inter-individual differences in the toxicity of methylmercury, including susceptibility of children at different ages particularly infancy, and of factors that may influence its toxicity.

51. We *recommend* that these conclusions should be reviewed following the JECFA evaluation of methylmercury in 2003.

December 2002

COT Statement 2002/04

References

1. Food Surveillance Information Sheet XX/02 (2002): Mercury in imported fish and shellfish and UK farmed fish and their products. *to be published*

2. National Diet and Nutrition Survey (2002), Adults aged 19 – 64. to be published.

3. Clarkson, T. W. (2002). The three modern faces of mercury. *Environ. Health Perspect.* **110 (1):** 11-23.

4. Food Surveillance Information Sheet 151 (1998): Survey of the concentrations of metals and other elements in marine fish and shellfish. http://archive.food.gov.uk/maff/archive/food/infsheet/1998/no151/151fish.htm

5. WHO (1972). Evaluation of Mercury, Lead, Cadmium and the food additives Amaranth, Diethylpyrocarbonate and Octyl Gallate. FAO Nutrition Meetings Report Series, No. 51A: WHO Food Additives Series No. 4.

6. WHO (1989). Toxicological evaluation of certain food additives and contaminants. Cambridge, Cambridge University Press. (WHO Food Additives Series, No. 24).

7. WHO (2000). Safety Evaluation of Certain Food Additives and Contaminants. WHO Food Additives Series 44.

8. Grandjean, P., Weihe, P., White, R. F., Debes, F., Araki, S, Yokoyama, K., Murata, K., Sorensen, N., Dahl, R., Jorgensen, P. J. (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol. Teratol.* **19:** 417-428.

9. Crump, K.S., Van Landingham, C., Shamlaye, C., Cox, C., Davidson, P.W., Myers, G.J., Clarkson, T.W. (2000). Benchmark concentrations for methylmercury obtained form the Seychelles Child Development Study. *Environ. Health Perspect.* **108:** 257-263.

10. Davidson, P.W., Myers, G., Cox, C., Axtell, C., Shamlaye, C., Sloane-Reeves, J., Cernichiari, E., Needham, L., Choi, A., Wang, Y., Berlin, M., Clarkson, T. W. (1998). Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment. Outcomes at 66 months of age in the Seychelles Child Development Study. *JAMA* **280:** 701-707.

11. Myers, G. J., Davidson, P. W., Shamlaye, C. F. (1998). A review of methylmercury and child development. *Neurotoxicology* **19:** 313-328.

12. Budtz-Jorgensen, E., Keiding, N., *et al.* (1999). Methylmercury neurotoxicity independent of PCB exposure. *Environmental Health Perspectives*, **107 (5):** 236-237.

13. Kjellstrom, T., Kennedy, P., Wallis, S., Stewart, A., Friberg, L., Lind, B., Witherspoon, P., Mantell, C. (1989). Physical and mental development of children with prenatal exposure to mercury from fish. Stage 2: Interviews and psychological tests at age 6. National Swedish Environmental Protection Board, Report 3642, Solna, Sweden.

14. EPA (U.S. Environmental Protection Agency). (1997). Mercury Study Report to Congress. Vol. IV: An assessment of exposure to mercury in the United States. EPA-452/R-97-006. U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards and Office of Research and Development.

15. National Research Council (2000), Toxicological effects of Methylmercury. National Academy Press, Washington, DC.

16. Mills, A. & Tyler, H. (1992). Food and Nutrient Intakes of British Infants Aged 6-12 Months, HMSO.

17. Gregory, J., Lowe, S., Bates, C.J., Prentice, A., Jackson, L.V., Smithers, G., Wenlock, R., and Farron, M. (2000). National Diet and Nutrition Survey: young people aged 4 to 18 years. Volume 1: Report of the diet and nutrition survey. London: TSO.

18. Gregory, J., Collins, D.L., Davies, P.S.W., Hughes, J.M. and Clarke, P.C. (1995). National Diet and Nutrition Survey: Children aged one-and-a-half to four-and-a-half years. Volume 1: Report of the diet and nutrition survey. London, HMSO.

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

20. WHO (1976). Environmental Health Criteria 1. Mercury. United Nations Environment Programme and the World Health Organisation. Geneva.

21. Doherty, R.A. and Gates, A.H. (1973). Epidemic human methylmercury poisoning; application of a mouse model system. *Paediatric Research,* 7(4): 319/91.

22. Sundberg, J. *et al*. (1999). Lactational exposure an neonatal kinetics of methylmercury and inorganic mercury in mice. *Toxicology and Applied Pharmacology,* **154:** 160-169.

23. Amin-Zaki, L. and Majeed, M.A. *et al.* (1981). Methylmercury poisoning in the Iraqi suckling infant: a longitudinal study over five years. *Journal of Applied Toxicology*, **1 (4):** 210-214.

24. Spyker, D.A, and Spyker J.M. (1977). Response model analysis of cross-fostering studies: Prenatal versus postnatal effects on offspring exposed to methylmercury dicyandiamide. Toxicol. Appl. Pharmacol, 40 (3): 1977 511-527.

25. Grandjean, P., Weihe, P., Needham, L.L., Burse, V.W., Patterson, D.G., Sampson, E.J., Jorgensen, P.J., Vahter, M. (1995). Relation of a seafood diet to mercury, selenium, arsenic, and polychlorinated biphenyl and other organochlorine concentrations in human milk. *Environ. Res.* **71 (1):** 29-38.

26. Grandjean, P., Weihe, P., White, R.F. (1995a). Milestone development in infants exposed to methylmercury from human milk. *Neurotoxicol.* **16 (1):** 27-33.

27. Food Surveillance information Sheet 191, (1999). Total Dietary Study 1997 – Aluminium, Arsenic, Cadmium, Chromium, Copper, Lead, Mercury, Nickel, Selenium, Tin and Zinc. http://archive.food.gov.uk/maff/archive/food/infsheet/1999/no191/191tds.htm

Table 1: Estimated mean and high level dietary intakes of mercury from salmon, prawns, canned tuna and the whole diet.

Consumer group	Mercury Intake – µg/kg bw/week[1]							
	Salmon[2]		Prawns[2]		Canned Tuna[2]		Whole Diet [3,4]	
	Mean	97.5%	Mean	97.5%	Mean	97.5%	Mean	97.5%
Infants	0.01	0.01	0.00	0.00	0.04	0.13	0.06	0.14
Toddlers	0.18	0.53	0.13	0.45	0.81	2.45	0.84	2.03
Young People aged 4 – 6	0.18	0.39	0.09	0.34	0.53	1.61	0.77	1.82
Young People aged 7 – 10	0.11	0.36	0.06	0.15	0.39	1.26	0.62	1.40
Young People aged 11 – 14	0.09	0.23	0.04	0.13	0.32	0.98	0.43	1.19
Young People aged 15 – 18	0.08	0.15	0.04	0.11	0.27	0.68	0.36	0.84
Adults	0.06	0.24	0.04	0.14	0.25	0.62	0.35	0.84
Adults – Women only	0.06	0.18	0.04	0.12	0.27	0.62	0.34	0.77

1. Consumption data for salmon, prawns and tuna are taken from the following sources:
   - Dietary and Nutritional Surveys of British Adults.[19]
   - Food and Nutrient Intakes of British Infants Aged 6-12 Months[16]
   - National Diet and Nutrition Surveys Children Aged 1.5 – 4.5 years.[18]
   - National Diet and Nutrition Survey: young people aged 4-18 years. Volume1 report of the diet and nutrition survey.[17]
2. Mercury intake from eating the named fish only, for the mean and 97.5th percentile consumers.
3. Mercury intake from consumption of fresh salmon, prawns, canned tuna and the rest of the normal UK diet (based on the 1997 Total Diet Study) for consumers of fish[27]. The total mercury intakes do not equal the sum of the mercury intakes from the named fish because the populations of consumers differ (for example not all fish consumers eat prawns).
4. The measurement of mercury does not distinguish between inorganic and organic mercury. Therefore although methylmercury is the major contributor to mercury intake from fish, the estimate of intake from the whole diet also includes inorganic mercury.

Table 2: Mercury intake from one portion of shark, swordfish, marlin, fresh tuna or canned tuna.

Age group (years)	Body Weight (kg)	Av. Portion Size[a] (g)	Weekly methylmercury intake assuming one portion of fish per week[b] (µg/kg bw/week)				
			Shark	Swordfish	Marlin	Fresh Tuna	Canned Tuna
1.5 – 4.5	14.5	50	5.24	4.62	3.79	1.38	0.66
4 – 6	20.5	60	4.44	3.90	3.22	1.17	0.56
7 –10	30.9	85	4.17	3.69	3.04	1.10	0.52
11 – 14	48.0	140	4.44	3.92	3.21	1.17	0.55
15 – 18	63.8	105	2.51	2.21	1.82	0.66	0.31
Adults	70.1	140	3.04	2.68	2.20	0.80	0.38

a. The average portion size that each age group of the population would consume at a single meal event for fish consumption, as recorded in the following National Diet and Nutrition Surveys (NDNS):

- 1995 National Diet and Nutrition Survey: Children aged one-and-a-half to four-and-a-half years[18].
- 2000 National Diet and Nutrition Survey: young people aged 4 to 18 years[17].
- 1990 The Dietary and Nutritional Survey of British Adults[19].

b. This intake estimate does not include the intake from the rest of the diet, which is estimated to be 0.052 μg/kg bw/day for a 60kg average consumer (0.36 μg/kg bw/week)[27].

Table 3: Summaries of biomarkers and methylmercury intakes

(a) Epidemiological studies

Population studied	Biomarker		NOAEL or LOAEL	Associated weekly dietary intake of methylmercury
	Mercury in Blood	Mercury in Hair		
Adults in Minamata/Niigata	200 μg/L	50 μg/g	LOAEL	[20 μg/kg bw/week][1]
Children in Iraq[3]	[40 – 80 μg/L in maternal blood][1]	10 – 20 μg/g in maternal hair	LOAEL	[4 – 8 μg/kg bw/week]
7-year-old children in Faroes Islands[2 3]	58 μg/L (cord blood) [~48 mg/L in maternal blood]	12 μg/g in maternal hair	LOAEL	[4.8 μg/kg bw/week]
5.5 year-old children in Seychelles3	[48 – 108 μg/L in maternal blood]	12 – 27 μg/g in maternal hair	NOAEL	[4.8 – 10.8 μg/kg bw/week]

1. Values in square brackets [ ] and *Italics* have been calculated using the following assumptions:
   i. The hair mercury level (μg/g) to blood mercury level (μg/L) ratio is 1:4.
   ii. Daily intake at steady state (μg/day) for a 70 kg person equals the blood mercury level (μg/L), i.e. a blood level of 33 μg/L corresponds to an intake of 33 μg/**day** and therefore 3.3 μg/kg bw/**week** for a 70 kg person.
2. Levels in cord blood and maternal hair are associated with a 5% increase in abnormal scores in the Boston Naming Test. These levels were used in the calculation of the EPA Reference Dose.
3. Children exposed prenatally, biomarkers are therefore maternal or in the case of the Faroe Islands, fetal (cord blood).

(b) Safety guidelines

Safety Guideline	Mercury in blood	Mercury in hair	Associated weekly dietary intake of methylmercury
JECFA PTWI[1]	33 μg/L	8.25 μg/g	3.3 μg/kg bw/week
EPA RfD[2]	4.75 μg/L	1.2 μg/g	0.7 μg/kg bw/week

1. The blood and hair levels associated with the JECFA PTWI have been calculated using the following assumptions:
   i. The hair mercury level (μg/g) to blood mercury level (μg/L) ratio is 1:4.
   ii. Daily intake at steady state (μg/day) for a 70 kg person equals the blood mercury level (μg/L). A blood level of 33 μg/L corresponds to an intake of 33 μg/day and therefore 3.3 μg/kg bw/week for a 70 kg person.
2. The EPA reference dose calculates the intake using the following equation (NRC 2000):

$$\text{Daily intake } (\mu g/kg\ bw/day) = \frac{\text{concentration in blood x elimination constant x blood volume}}{\text{absorption factor x fraction of daily intake taken up by blood x body weight}}$$

The following values were used in calculating the blood mercury level associated with the reference dose in this table:

Daily intake	0.1 μg/kg bw/day
elimination constant	0.014 $days^{-1}$
blood volume	5 L
absorption factor	0.95
fraction of daily intake taken up by blood	0.05
body weight	70 kg

The body weight used by the EPA in calculating the reference dose is **60 kg**. However in order to make the values more comparable to the JECFA PTWI, the blood level associated with the reference dose in this table has been calculated using a bodyweight of **70 kg**.

The hair mercury level was calculated using the ratio of blood to hair mercury employed by JECFA.

Joint statement on a symposium held by the three committees on the use of genomics and proteomics in toxicology

Introduction

1. Sir Robert May's[1] "Review of Risk Procedures used by the Government's Advisory Committees dealing with Food Safety" [2] recommended greater collaboration between expert committees covering overlapping areas. A joint symposium to discuss the use of genomics and proteomics in toxicology was considered a suitable opportunity for collaboration between the three committees (COT, COM and COC). The meeting, held on 8 October 2001, included members of various other expert committees, delegates from government departments and invited speakers and was also open to interested parties.

2. The topic was chosen in response to an increasing need to consider the role that novel technologies such as proteomics (or protein profiling) and genomics (or more accurately in the context of this workshop, transcript profiling) may play in toxicological risk assessment. This area has been given added impetus since the publication of the human genome sequence[3] and it was considered important to consider if and how data obtained using such techniques may be incorporated into regulatory processes. The objectives of the meeting were thus defined as:

   - To provide advice to government departments and regulatory agencies on use of genomics and proteomics in toxicological risk assessment

   - To facilitate closer working and greater collaboration between the COT, COC and COM.

3. The symposium was divided into three sessions: an introduction, a detailed discussion of the subject areas and a final summary and conclusions session. Three working groups considered the issues of genomics, proteomics and the use of genomics and proteomics in risk assessment, respectively. Each group had a presentation from an expert in the field followed by a targeted discussion, facilitated by a member of one of the committees (COT, COC or COM).

Genomics

4. The working group concluded that data from toxicogenomic studies could not be used in isolation for risk assessment purposes but that such data could be considered as part of a 'weight of evidence' approach. Gene expression studies could not, at present, be used to define NOELs and NOAELs. It was considered that there was a need to correlate changes in gene expression to corresponding conventional toxicology data and histopathology. It was also agreed that it was essential to distinguish between changes in gene expression representing background variation, adaptive pharmacological effects and those that represent adverse effects.

5. It was agreed that genomics data might be useful for limited screening for toxicological mechanisms and endpoints such as hormonally mediated carcinogenesis, mutation in error-prone DNA synthesis caused by genotoxic carcinogens and adverse effects on reproduction other than teratogenicity. It may also be useful for investigating organ specific effects of chemicals earlier in their pathogenesis than the appearance of frank pathological lesions as detected by light microscopy. However, further research was required before the technology could be applied to other areas. For example, in neurotoxicology it is difficult to correlate structural targets in the nervous system to the neurotoxicant's site of action particularly as validation using conventional toxicological methods is limited. At present, genomics is also unlikely to be of use in teratology studies due to the rapid rate of change in the developing organism. It was proposed that transcript profiling might be valuable also for evaluation of adverse effects resulting from the interaction of chemicals with the immune system. Clearly there are important opportunities for the application of toxicogenomics, but also some limitations. Presently it is likely that the main benefits will be in characterisation of mechanisms of actions and the identification of new markers for hazard identification.

Proteomics

6. It was concluded that proteomics could not be used for regulatory purposes at this stage. However, the technology might be useful for screening candidate compounds where there is some knowledge of the mechanisms of toxicity. Proteomics may also be useful in identifying novel mechanisms. Data from proteomic studies can only be used for refining NOAELs in defined circumstances where the pattern of protein changes under investigation can be causally related to the toxic mechanism and observed pathology, and the study includes dose-response data. The Committees noted that a collaborative initiative to provide validation data for proteomic studies was crucial as problems with reproducibility between studies has often compromised their potential for use in risk assessment. Similarly, the group agreed that there is an urgent need to develop databases to aid protein identification.

7. The potential for using proteomics in non-invasive human biomarker studies was acknowledged. However, the reproducibility and dose response of any potential proteomic biomarkers would need to be established and compared to existing methods with regard to specificity and sensitivity before they could used for risk assessment.

Risk Assessment

8. It was agreed that although genomics and proteomics show great potential for risk assessment, caution should be applied when drawing conclusions from data derived from such technologies. These may be difficult to interpret and input from bioinformatics specialists and statisticians with suitable expertise and experience is considered essential. Genomics and proteomics may be used to identify possible mechanisms of action, biomarkers of exposure and predictors of effect, but their use in these areas is dependent upon knowledge of gene function and the relationships between gene expression and biological effects. Further research into the natural background variation in gene expression is considered essential to aid in interpreting the data from such studies.

9. The use of proteomics and genomics in risk assessment was not considered likely to lead to a reduction in the use of animals in toxicology studies in the short-term. However, the use of genomics and proteomics should lead to more appropriate use of both *in vivo* and *in vitro* model systems where the extrapolation to man can be based on a fundamental understanding of differences in gene and protein expression between the systems. Future development of these technologies may provide sufficient data to enable toxicological studies to be more focused.

Overall conclusions

10. We *recognise* the future potential of proteomics and genomics in toxicological risk assessment.

11. We *note* that these techniques may serve as adjuncts to conventional toxicology studies, particularly where proteins under investigation are known to be causally related to the toxicity.

12. However, we *consider* that research and validation is required before these techniques can be considered for routine use in regulatory toxicological risk assessment. In particular, there is a need for more research leading to development of genomic/proteomic databases, methods of bioinformatic and statistical analysis of data and pattern recognition and for information on the normal range of gene expression.

---

[1] Sir Robert May was the Government's Chief Scientific Advisor from 1995 to 2000 when he was succeeded by Professor David King.
[2] Office of Science and Technology, July 2000; http://www2.ost.gov.uk/policy/issues/food_safety/index.htm
[3] International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome; *Nature* **409**, 860-921

2002 Membership of the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

CHAIRMAN

Professor H F Woods CBE BSc BM BCh DPhil FFPM FIFST HonFFOM FRCP (Lon & Edin) (to March 2002)
Sir George Franklin Professor of Medicine, Division of Molecular and Genetic Medicine, University of Sheffield

Professor I A Hughes MA MD FRCP FRCP(C) FRCPH F Med Sci. (from April 2002)
Professor and Head of Department of Paediatrics, University of Cambridge

MEMBERS

Professor P Aggett OBE MB ChB FRCP FRCPCH MSc DCH
Vice Chairman.
Head of Lancashire Postgraduate School of Medicine and Health

Dr Sati Ariyanayagam MBBS, MRCGP, MRCOG MFFP DCh Lond
Public Interest Representative

Dr P Carthew BSc MSc PhD FRCPath
SEAC Toxicology Unit, Unilever

Professor J K Chipman BSc PhD CBiol FIBiol FRCP
Professor of Cell Toxicology, University of Birmingham

Dr Peter R Jackson BA (Oxon), MA (Oxon), MB ChB, MRCP, PhD, FRCP
Reader in Clinical Pharmacology and Therapeutics, University of Sheffield

Dr M Joffe BA MB BChir MSc MD FRCP FFPHM MSc (Econ) PhD
Reader in Epidemiology, Imperial College Faculty of Medicine

Professor I Kimber BSc MSc PhD FIBMS CBiol MIBiol
Research Manager, Syngenta Central Toxicology Laboratory

Professor Joseph Lunec BSc (Hon) PhD FRC Path
Director of Clinical Pathology, University of Leicester

Dr Aldert Piersma MSc PhD
Senior Staff Scientist, National Institute of Public Health and the Environment, Holland

Dr L Rushton BA MSc PhD CStat
Head of Epidemiology, Institute for Environment and Health, University of Leicester

Professor I R Rowland BSc PhD
Northern Ireland Centre for Diet and Health (NICHE), University of Ulster

Ms J Salfield BSc MSc MIFST CERTED RPHN
Public Interest Representative

Dr A G Smith BSc PhD CChem FRSC
Molecular Toxicologist, Medical Research Council Toxicology Unit, Leicester

Dr Lesley Stanley BA PhD
Principal Lecturer in Biomedical Sciences, School of Pharmacy and Pharmaceutical Sciences, De Montfort University, Leicester

Professor S Strobel MD PhD FRCP FRCPCH
Institute of Child Health, London

Professor J A Timbrell BSc PhD DSc MRCPath FRFC FIBIOL (to July 2002)
King's College London

Dr M Tucker BSc PhD FRCPath
Independent pathologist and animal histopathologist

SECRETARIAT

D J Benford BSc PhD (*Scientific Secretary*)
J M Battershill BSc MSc (*Scientific, DH*)
K V Butler (*Administrative Secretary*)
N Ball BSc MSc (from April 2002)
D Gott BSc PhD
B Maycock BSc MSc
K Moizer BSc MSc (from September 2002)
C A Mulholland BSc
S Sivapathasundaram BSc PhD (from August 2002)
C S M Tahourdin BSc MSc PhD
N Thatcher BSc PhD
J Shavila BSc MSc PhD (to October 2002)

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

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Professor I Hughes	Pharmacia	Education Adviser	Academy of Med Sciences	IAH – Fellow
(Chairman from 1	BP Amoco	Shares	Soc for Endocrinology	IAH – Council Member
April 2002)	BP Amoco	Daughter is an employee of this company	Royal College of Paediatrics and Child Health	IAH – Fellow, Senior Examiner, Regional Academic Adviser
	Topical Endocrinology	Editorial Board Member	Medical Res Council	IAH – Member of Advisory Board
			Pharmacia Aventis NovoNordisk Diabetes UK Welcome Trust Juvenile Diabetes Fund	Funds received from all these sources for Departmental research and education in medicine and health related topics
Professor H F Woods (Chairman	HBOS (formerly Halifax Bank)	Shares	University of Sheffield, Faculty of Medicine	University of Sheffield, Faculty of Medicine.
to 31 March 2002)	HSBC	Shares	Wide range of national & international food & chemical companies.	Has extensive activity in teaching and research in nutrition and toxicology and in topics related to and supported by many companies in the food and chemical industry.
				Trustee of the Harry Bottom Charitable Trust.
Professor P Aggett Deputy Chairman	NONE	NONE	Astra Zeneca	Chairmanship (meetings) and lecture fees.
			Smith Nephew Nestec ILSI Abbott Food Standards Agency Welcome Yakult	Departmental research and education in medicine and health including food safety and metabolism.
			International Copper Association Many other food, pharmaceutical and chemical companies	
Dr Sati Ariyanaygam	NONE	NONE	NONE	NONE
Dr P Carthew	Unilever	Employee	NONE	NONE
	Provalis	Shareholder		
Professor J K Chipman	Sequani	Training/ Consultancy	Astra-Zeneca	Research Support
	AstraZeneca	Consultancy	Glaxo Smith Kline	Research Support
	Inamed	Consultancy	ICI	Research Support
	Unilever	Consultancy	HSE	Research Support
	Syngenta	Lecture fee	CEFIC-LRI	Consultancy
			Dept of Health	Consultancy
			Inamed	Consultancy

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Dr P Jackson	Bristol Myers Squibb	Lecture fees	Novartis	Research grant
	Merck Sharp Dohme	Lecture fees	Merck Sharp Dohme	Research grant
	British Heart Foundation	Lecture fees	Servier	Research grant
			Bristol Myers Squibb	Research grant
			Pfzier	Research grant
			AstraZeneca	Research grant
			Medtronic AVE	Research grant
			Department of Health	Research grant
			Boots Healthcare	Consultancy
Dr M Joffe	NONE	NONE	NONE	NONE
Dr I Kimber	British Airways	Share Holder	Unilever plc	Grant for Research
	BP-Amoco	Share Holder		
	ICI	Share Holder		
	Halifax/Royal Bank of Scotland	Share Holder		
	AstraZeneca	Share Holder		
	Syngenta	Share Holder		
	Syngenta	Employee		
	Lloyds TSB	Share Holder		
	Scottish Power	Share Holder		
Professor J Lunec	NONE	NONE	Scilucent LLC. USA	Funding research group to investigate toxicology of soya-bean oil implants
Dr A Piersma	NONE	NONE	NONE	NONE

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Professor I R Rowland	Colloids Naturels International (CNI)	Consultancy	Valio (Finland)	Departmental teaching & research funded by various food and pharmaceutical companies
	Cerestar	Consultancy	Alpro (Belgium)	
	Halifax	Share Holder	Alphro	
	Woolwich	Share Holder	VK Muelen (Germany)	
			Biohit (Finland)	
			Danone (France)	
			Orafti (Belgium)	
			Kelloggs UK	
			Unilever UK	
			Uniq plc	
			Scotia Pharmaceuticals UK	
			Robert Craig & Sons	
			Cultech UK	
			ILSI Europe	
Dr L Rushton	Institute of Petroleum	Consultancy, Contracts and Grants – completed	Concawe	Contracts to Institute of Environment and Health – completed
	Transport and General Workers Union	Consultancy – completed	European Silica Industry	Contract to IEH, ongoing cohort study
	Friends Provident	Shares	International Manganese Institute	Contract to IEH to prepare criteria document
	Northern Rock	Shares	American Chemistry Council	Contract to IEH for systematic review and meta-analysis
Ms J Salfield	NONE	NONE	NONE	NONE
Dr A Smith	Abbey National	Share Holder	Rhône Poulenc	Research Support
	British Telecom	Share Holder	Glaxo-Wellcome	Research Support
	MMO_2	Share Holder	CEFIC-LRI	Research Support
	HBOS	Share Holder		

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Dr L Stanley	CXR Biosciences	Employee	Cyclacel	Company contract
			Euro Chlor	Company contract
			Halogenated Solvents Industry Alliance	Company contract
			Association of Plastics Manufacturers, Europe	Company contract
			AstraZeneca	Company contract
Professor S Strobel	NONE	NONE	NONE	NONE
Professor J A Timbrell (resigned July 2002)	Shook, Hardy & Bacon (Law firm)	Occasional Fee	Glaxo Wellcome	Research Support
			Taisho Pharmaceutical Co	Research Support
	Sorex Ltd	Occasional Fee		
Dr M Tucker	Zeneca	Pension	NONE	NONE