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

Committee on TOXICITY

Committee on MUTAGENCITY

Committee on CARCINOGENICITY

Annual Report 2002

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

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About the Committees

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

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

The aim of this report is to provide a brief toxicological background to the Committees' decisions. The report also includes the Committees' published statements in full in order to fulfil the obligation to publish statements both electronically and in hard copy. Further information may be found on the Committee websites, including agendas, minutes, statements, reports and, where possible, the background papers discussed by the Committees. The internet addresses are:

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

COM: http://www.doh.gov.uk/com.htm

COC: http://www.doh.gov.uk/coc.htm

The year 2002 has seen a number of changes in the membership of the Committees and these are shown in the membership lists at the end of each Committee's report.

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

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

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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

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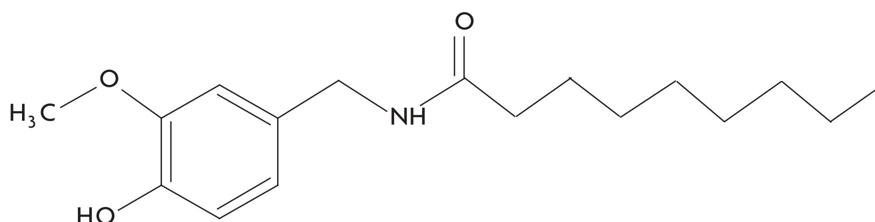
Statement on the use of PAVA (Nonivamide) as an incapacitant spray

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

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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

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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

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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.

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[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

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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

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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
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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

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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 (Chairman from 1 April 2002)	Pharmacia	Education Adviser	Academy of Med Sciences	IAH – Fellow
	BP Amoco	Shares	Soc for Endocrinology	IAH – Council Member
	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 to 31 March 2002)	HBOS (formerly Halifax Bank)	Shares	University of Sheffield, Faculty of Medicine	University of Sheffield, Faculty of Medicine
	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
Dr Sati Ariyanayagam	NONE	NONE	NONE	NONE
Dr P Carthew	Unilever	Employee	NONE	NONE
	Provalis	Share Holder		
Professor J K Chipman	Sequani	Training/	AstraZeneca	Research Support
		Consultancy	Glaxo Smith Kline	Research Support
	AstraZeneca	Consultancy	ICI	Research Support
	Inamed	Consultancy	HSE	Research Support
	Unilever	Consultancy	CEFIC-LRI	Consultancy
	Syngenta	Lecture fee	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

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

Preface

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

During 2002, the Committee finalised its review of dichlorvos and initiated a review of another insecticide, malathion. Reviews of the veterinary medicine dimetridazole, the incapacitant substance pelargonyl vanillylamide (PAVA), and the possible nitrosation of nicotine from nicotine patches were completed.

The Committee also reviewed its procedures in the light of new guidance from the Office of Science and Technology on a code of practice for Scientific Advisory Committees and the Government's response to the BSE enquiry report. The Committee adheres to most of the recommendations but agreed to publish more of the substantive background papers to discussions at the earliest opportunity. The COM devised a template showing its methods of working and expertise, which clarifies this for health professionals and members of the general public.

The Committee has an ongoing responsibility to provide Government Department's and Regulatory Authorities with advice on developments in procedures for the evaluation and risk assessment of mutagens. In this regard the Committee assessed new information from the ILSI/HESI (see below for definition) research initiative on the use of the Syrian Hamster Embryo (SHE) cell transformation assay as an *in-vitro* test for identifying potential mutagens. The COM confirmed its earlier views that this test is not suitable for screening or regulatory assessment of chemical mutagens. The Committee's views on the SHE assay have been published in the scientific literature (Toxicologic Pathology 2002 Jul-Aug;30(4):536-8).

Professor P B Farmer Chair
MA DPhil CChem FRSC

Dichlorvos

2.1 Dichlorvos (O-(2,2-dichlorovinyl)-O,O-dimethylphosphate, DDVP) is an organophosphorous insecticide. The COM provided advice to the Advisory Committee on Pesticides on the mutagenicity of dichlorvos during 2001. A full report was published in the 2001 Annual Report. The COM considered dichlorvos at three meetings in 2001 including an extraordinary meeting where industry made a presentation. A Judicial Review was sought by the data holder regarding the regulatory review of dichlorvos pesticides products. The Judicial Review took place during 5-9 November 2001. Mr Justice Crane handed down his judgement on the case held in the High Court between AMVAC Chemicals UK Ltd (Claimants) and Secretary of State for Environment, Food and Rural Affairs (DEFRA) and Secretary of State for Transport, Local Government and Regions (DTLR) (Defendants) on 3rd December 2001. Essentially the Claimants (AMVAC Chemical UK Ltd) won on one of the three issues they had raised, namely that the decision to suspend pesticide approvals was flawed in relation to the amount of notice given to the claimant and the decision was therefore quashed. Mr Justice Crane saw no reason why those advising ministers should not review their advice, taking into account all the Claimants submits, without significant delay.

2.2 This meant that the COM was asked to consider the relevant material submitted to the court by AMVAC to ascertain whether this warranted any revision of the COM statement on dichlorvos. This information essentially comprised exhibits from a number of independent scientific experts and a number of papers on the generic issues of evaluation of various types of mutagenicity assays. In addition other pesticide approval holders were asked to forward any further data by 4 January 2002.

2.3 A further extraordinary meeting of COM was arranged for the 9 January 2002 to consider these data.

2.4 The Committee considered all of the relevant information provided to the court and concluded that dichlorvos should be regarded as an *in vivo* mutagen at the site-of-contact (i.e at the initial sites of exposure, the COM felt there was no evidence for systemic mutagenic effects.) High doses of dichlorvos induced mutagenic effects in the skin following topical application and in the liver following intraperitoneal dosing. The Committee noted the limited evidence for a carcinogenic effect of dichlorvos. This related to tumours of the forestomach in mice after gavage dosing and also the oesophageal tumours seen after dietary administration. There was no satisfactory explanation proven for the mechanisms of these tumours and the Committee felt, given the available mutagenicity data on dichlorvos, that it would be prudent to assume a genotoxic mechanism. The Committee agreed that in the absence of appropriate mechanistic data a precautionary approach should be adopted and no threshold could be assumed for the mutagenic and carcinogenic effects of dichlorvos.

2.5 The Committee considered a request from the Advisory Committee on Pesticides on what investigations might assist in further considering the potential for dichlorvos to act as a site of contact mutagen. Members suggested that good quality studies in transgenic animals using repeat dosing to investigate mutagenic activity in the skin following dermal application and in the gastrointestinal tract following oral administration would be appropriate.

2.6 The COM statement was published in January 2002 and is included at the end of this report.

Dimetridazole

2.7 Dimetridazole (DMZ) is an antiprotozoal substance that is used to maintain the health of certain farm animals. In the UK it has been permitted for use as a feed additive for turkeys and guinea-fowl and as a veterinary medicine for game birds (pheasants and partridges only). The European Commission was considering whether to suspend use as a feed additive, but this would not directly affect veterinary medicinal use. In the last few years, DMZ has been assessed by the EU's Committee on Veterinary Medicinal Products (CVMP)and by the EU's Scientific Committee on Animal Nutrition (SCAN). These two committees disagreed with one another over the interpretation of the mutagenicity data. The CVMP considered that more information was needed before it could conclude on mutagenicity, whereas the SCAN considered that there was sufficient information to allow it to conclude that there was no genotoxic hazard to consumers of foods derived from animals given DMZ. The specialist expert advice of COM was sought by the Food Standards Agency (FSA).

2.8 The COM reached the following conclusion regarding the mutagenicity of DMZ.

2.9 Dimetridazole (DMZ) has mutagenic activity *in vitro*. The Committee was provided with full reports of *in vivo* studies to investigate the ability of DMZ to induce micronuclei in bone marrow of mice, UDS in primary hepatocytes of male rats, and dominant lethal mutations in the germ cells of male mice. In all cases the oral route was used, with dose levels up to about 1 gram/kg per day. Negative results were consistently obtained. However the Committee concluded that the rat liver unscheduled DNA synthesis (UDS) assay had not been adequately conducted and recommended that a further rat liver UDS assay conducted in accordance with OECD guidelines (but with their recommendation to use 4 animals per dose/sampling time point) was required to provide full reassurance that DMZ (or its metabolites) does not have significant mutagenic activity in mammals in vivo.

2.10 The COM statement is included at the end of this report.

Malathion

2.11 Malathion is an organophosphorus insecticide. It has been marketed in the UK for use in agriculture and horticulture since 1956. There were three products with approvals for use in agriculture and horticulture, home garden and use in pigeon lofts at the time when this review was initiated in January 2002. A number of products containing malathion are also licensed as human medicines for use in the control of head lice.

2.12 The Advisory Committee on Pesticides was reviewing the available toxicological information on malathion as part of its ongoing review of organophosphorus compounds. The ACP asked for advice from COM and COC on mutagenicity and carcinogenicity at its 289th meeting on 17 January 2002. The Chairs of COM and COC agreed that a joint statement was required in view of the need for a full review of all mutagenicity and carcinogenicity data.

2.13 The COM undertook an initial consideration of the in confidence mutagenicity data provided by the pesticide data holder and the available published information provided by the data holder at its 25 April 2002 meeting. A number of additional published papers on malathion and impurities present in technical grade malathion were also considered at this meeting. At its meeting of 10 October 2002 the COM considered some additional information provided in confidence by the pesticide data holder (a report of one additional *in vivo* study and information on the potential for variation in impurities between different sources of malathion) together with a number of published studies not previously reviewed.

2.14 The Committee agreed a number of conclusions, which were forwarded to the pesticide data holder. Additional data on the conduct and results of the *in vivo* oral rat liver UDS assay were submitted by the pesticide data holder and were to be considered at February 2003 meeting of COM.

2.15 Malathion was also considered at the COC meeting of 27 June 2002. A full statement from COC and COM is in preparation.

PAVA: use as an incapacitant spray

2.16 Sussex Police Force have started to use an incapacitant spray based on pelargonyl vanillylamide (PAVA) as an alternative to CS spray. PAVA is the synthetic equivalent to capsaicin the active ingredient of natural peppers. It is permitted as a food flavour (at up to 10 ppm). It is also used in human medicine topically as a rubifacient. The Home Office had asked the COT for advice on the health effects of PAVA spray. The COT gave initial consideration to this at its meeting on 4th December 2001, and requested that the COM provide advice on the mutagenicity data. This comprised a package of 3 *in vitro* studies and one *in vivo* study that had been commissioned by Sussex Police.

2.17 The Committee agreed the following conclusions for inclusion in the COT statement:

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/weakly positive 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 (http://www.doh.gov.uk/com/guidance.pdf) , 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.

2.18 A full statement is included in the COT section of this report.

Possible nitrosation of nicotine from nicotine patches

2.19 A member of COM requested the views of the Medicines Control Agency (MCA) on the possible nitrosation of nicotine derived from nicotine patches applied to the skin, and any mutagenic and subsequent carcinogenic risk. MCA presented a paper to the Committee in response to this request.

2.20 Members heard that nicotine from patches is absorbed through the skin and passes into the systemic blood system, and is distributed throughout the body. Blood plasma concentrations rise more slowly from the use of these products than from cigarettes. Nicotine is continually released throughout the time of exposure. Nicotine has not been shown to be carcinogenic to animals. There was the possibility that nicotine could be demethylated and subsequently (or concomitantly) nitrosated to form N-nitroso-nornicotine, which is a genotoxic carcinogen. Although the amount of nitrosamine generated in this way was unknown, the MCA considered that it was likely to be minimal and very much less than the levels of other nitrosamines and other carcinogens present in tobacco smoke. In the licensing of a medicinal product one aspect to be considered was the benefit to health which had to be compared with any risk attributed to the use of the medicinal product. The MCA view was that the benefits clearly outweighed the risks in this case.

2.21 Members agreed that any concern over the risk of nicotine related mutagenicity and cancer from skin patches due to nitrosation is likely to be very small or insignificant in tobacco users who are or have been exposed to high concentrations of many carcinogens. It was noted that similar arguments also applied to chewing gum containing nicotine.

Review of Committee Procedures

OST Code of Practice for Scientific Advisory Committees and Committee procedures in the light of the Government's response to the BSE enquiry report

2.22 The Committee agreed to consider these two reports together as many of the topics were common in both reports. Members agreed that in general the COM procedures complied with most of the guidance given by OST.

2.23 The Committee agreed that it was difficult to publish a detailed forward plan, as much of the work of the Committee was reactive in response to requests for advice from other Government Department/Agencies, often at short notice. It was noted that the agenda was normally set by the secretariat in response to such requests but that members could make suggestions for consideration at anytime. Regarding 'horizon scanning' members believed that generic issues were often identified by the secretariat and members, but acknowledged that systematic searching of the literature to identify all relevant research was not possible.

2.24 The Committee considered that improving communications of its advice to the public was difficult because of its specialist and very technical nature. The Chairs of the COC/COM had both agreed with the proposal in the OST report to publish more background papers. A 'what's new' section had also been placed on the COM website, which would help improve communication. Members agreed that lay summaries of completed statements should be drafted as appropriate when particularly complex subjects were under discussion. It was agreed that a glossary of technical terms could help with public understanding and that COM would contribute to a joint COT/COC/COM glossary. With respect to dealing with dissenting views members agreed that it should be made clear in the minutes and statements when consensus had not been reached, and the dissenting view published alongside the statement agreed by the majority.

COM Template

2.25 The COM agreed a template diagram which provided an overview of how COM undertakes risk assessment of carcinogens and the interaction of COM with its sister Committees (COT and COC) and with Government Departments, Regulatory Agencies and the Chief Medical Officer Professor Sir Liam Donaldson. For ease of reference this template is reproduced at the end of this section of the Annual Report.

Test Strategies and Evaluation

ILSI/HESI research programme on alternative cancer models: results of Syrian hamster embryo cell transformation assay

2.26 The International Life Sciences Institute (ILSI) and the Health and Environmental Science Institute (HESI) have co-ordinated a multinational research programme from 1996 – 2001 to more fully characterise the responsiveness of several alternative cancer models. The research has focused predominantly on a number of proposed short-term *in vivo* test models for assessment of potential carcinogenic activity carcinogenicity in mice (in particular ras H2, Tg.AC, p53 +/-,Xpa-/-, Xpa-/-/p53+/- double knockout, neonatal mouse) but also included the *in vitro* cell transformation assay using Syrian hamster embryo cells (SHE cell transformation assay) The overall objective of the work was to evaluate the ability of these models to provide useful information for human cancer risk assessment. The research involved input from over 50 industrial, governmental (USA, Denmark, Netherlands and Japan) and academic laboratories and cost around $35M. The COC has considered all of the data submitted by ILSI/HESI

except for the results of the investigations using the SHE cell transformation assay. These data have been considered by Committee on Mutagenicity on Chemicals in Food, Consumer Products and the Environment (COM) whose conclusions are given below.

2.27 In 1994 the COM considered a number of cell transformation assays including the modified SHE method, and had agreed that transformation assays using Syrian Hamster cells were not yet ready for routine use, but warranted further work on both their validation and providing an understanding of the underlying mechanisms. The Committee considered a draft proposal for an OECD guideline for cell transformation using the modified SHE method (low pH culture). A number of supporting publications and papers claiming a predictive correlation between cell transformation in this test system and carcinogenicity were also reviewed. In 1996, the Committee agreed that there were no mechanistic data available which gave an insight into the relationship between cell transformation in the SHE assay and the carcinogenic process. Although apparently high correlations had been reported in trials using a range of animal carcinogens including non-genotoxic carcinogens, only a very limited number of laboratories were involved and little value could be attributed to these results in the absence of appropriate supporting mechanistic data. The COM concluded at the end of its 1996 review that it was not possible to support the proposed OECD guideline.

2.28 The Committee reached the following conclusions:

i) The SHE cell transformation assay should not be used for regulatory screening of chemicals for potential carcinogenicity.

ii) There are insufficient data on validation of the SHE cell transformation assay to justify the development of an OECD test guideline for this assay.

iii) The Committee has considerable reservations regarding the mechanistic basis underpinning the rationale for using the SHE cell transformation assay to screen for chemical carcinogenesis.

iv) The Committee has considerable reservations regarding the validity of using morphological assessment alone to define transformed foci in the SHE cell transformation assay. The development of an objective molecular marker is essential before validation work can proceed.

2.29 The COM statement is included at the end of this report.

In vitro micronucleus test

2.30 The 3rd International Workshop on Genotoxicity Testing (IWGT) was held in Plymouth in June 2002 and included a Working Group on the *In vitro* Micronucleus test. There was international consensus that this method was adequately validated, and also on the methodology for a guideline. The COM has a remit to advise on the development of test methods and strategies. Members welcomed the development of the *in vitro* micronucleus test. Members queried the consensus point that the experimental unit for statistical analysis was the cell and not the culture and noted that this did not

conform to the approach taken in other previously validated *in vitro* tests. The secretariat would raise this aspect with the IWGT on behalf of the committee.

Ongoing Reviews

Flunixin, Flunixin-meglumine, meglumine

2.31 Flunixin in the form of the meglumine salt is a non-steroidal anti-inflammatory (NSAID) drug and a non-narcotic analgesic drug with antipyretic activities. It dissociates to Flunixin and meglumine in the body. It is used in veterinary medicine (including food-producing animals). The FSA have asked for advice on the mutagenicity of Flunixin, Flunixin-meglumine, meglumine. The COM has evaluated the available mutagenicity data and is to consider a presentation from the data holder regarding a mutagenicity testing strategy at the February 2003 meeting.

Significance of in vivo mutagenicity at high doses

2.32 The COM has previously agreed that it is prudent to assume that there is no threshold for mutagenicity unless appropriate mechanistic data can be provided to identify a threshold related mechanism. *In vivo* studies in the bone marrow provide key data in identifying compounds as *in-vivo* mutagens. In some cases the only data available to indicate such *in vivo* activity is from mutagenicity studies using excessively high doses (by current guidelines) associated with severe toxicity/lethality, and there is no evidence from carcinogenicity bioassays to suggest that a compound is a genotoxic carcinogen. Due to the importance of these data in risk assessment, where positive results normally lead to a 'non-threshold' approach being adopted by regulatory agencies, it is important that the observed effects are not secondary to toxicity. The Committee considered a paper drafted by the Secretariat at its October 2002 meeting which presented "in-confidence" data from submissions received by regulatory authorities. These were to be used as examples in order to draft generic guidance which could be published. A further paper is to be considered at the February 2003 meeting.

Glossary of Terms

2.33 A document is in preparation.

Statements of the COM 2002

Mutagenicity of dichlorvos

Dimetridazole

Malathion

PAVA: use as an incapacitant

Possible nitrosation of nicotine from nicotine patches

Mutagenicity of dichlorvos

Introduction

Background to COM review

1. Dichlorvos (O-(2,2-dichlorovinyl)-O,O-dimethylphosphate, DDVP) was first introduced into the UK as an agricultural pesticide in 1962. Non-agricultural uses were first assessed under the voluntary Pesticides Safety Precaution Scheme in 1975-78. A review by the ACP of approvals issued under the Control of Pesticides Regulatory (COPR 1986) was undertaken in 1994. Currently dichlorvos is widely used by amateur and professional users as a public hygiene insecticide (e.g. use of hand held aerosols for surface/space spray and slow release products e.g. strips, cassettes). A relatively small number of products are approved for use in animal husbandry and in agriculture and horticulture on edible crops (e.g. cucumbers) and on non-edible crops (e.g chrysanthemums). Dichlorvos is currently used in a veterinary medicinal product for control of fleas in cats and dogs. The use of dichlorvos in pesticide products and in veterinary medicines is currently being reviewed.

2. The COM considered generic aspects arising from the paper by Sasaki YF et al[(1)] on the performance of the *in vivo* COMET assay with respect to the newly published strategy in the COM guidelance at its 8 February 2001 meeting. The Committee agreed that the positive results reported in the COMET assay using dichlorvos suggested that a full review of all the mutagenicity data was required.

COM reviews

3. The toxicokinetics, mutagenicity and carcinogenicity data sections from the draft evaluation prepared by HSE for the Advisory Committee on Pesticides meeting on 5 April 2001 were made available to the COM. The Committee evaluated the data from all of the mutagenicity studies cited in the HSE review.[(1-102)] The COM also considered additional information and mutagenicity data submitted by industry to HSE prior to the COM meeting on 26 April 2001.[(103-107)] A further meeting was held on 23 July 2001 to consider additional submitted information and to hear a presentation from industry. The COM advice was forwarded to the regulatory authorities (the Biocides and Pesticides Authorisation Unit (BPAU) at HSE and the Pesticides Safety Directorate (PSD)) at the end of July 2001 and was subsequently published in December 2001 after a judicial review of the regulatory decisions regarding dichlorvos. An extraordinary meeting of COM was held on 9 January 2002 to consider the new information submitted to the High Court during a judicial review of the regulatory decision on the pesticide products containing dichlorvos and data provided to regulatory authorities up to 4 January 2002 and to decide whether this warranted any revision of the COM statement on dichlorvos.

Overall Assessment of In vitro mutagenicity studies

4. Members agreed that dichlorvos is a weak methylating agent (compared to methyl methanesulphonate; MMS). The Committee concurred with the following assessment of the *in vitro* mutagenicity studies.

i) Dichlorvos is mutagenic, both in the presence and absence of exogenous metabolism, to bacteria, yeast cells and in mammalian cell gene mutation assays, chromosome aberrations assay, the *in vitro* micronucleus test and sister chromatid exchange assays.

ii) Positive results have been reported in *in vitro* UDS assays using human lymphocytes and human epithelial-like cells.

iii). Dichlorvos has been shown to methylate nucleophiles and to induce strand breaks in isolated DNA.

5. Members agreed that DNA methylation induced by dichlorvos contributed towards the mutagenicity reported in *in vitro* test systems but noted that other mechanisms might also be involved. Members considered that the positive results obtained in *in vitro* mutagenicity tests with dichlorvos in the presence of an exogenous metabolising fraction and in the assay for single strand breakage of DNA also suggested that dichlorvos and/or its metabolites were genotoxic. This might include dichloroacetaldehyde although the available evidence was insufficient to identify all potential mutagenic metabolites of dichlorvos.

6. The Committee concluded that dichlorvos is an in vitro mutagen

Assessment of *in vivo* mutagenicity studies

7. The Committee noted that there were a large number of *in vivo* studies available. Dichlorvos was negative in most published *in vivo* mutagenicity assays where it was administered as a single dose. These included mouse bone-marrow micronucleus (using i.p route)[(70,90,94)] and bone-marrow chromosome aberration studies in mice[(23,60)] and hamsters[(22)] using oral and, in two studies (mice/hamster) inhalation exposure. Negative results were also reported in SCE in mice[(44,53,95)] and UDS assays [liver (rats)/forestomach (mice)][(9,55,99,101)]. A negative result was also reported in an adequately conducted bone-marrow chromosome aberration study where mice were given daily oral doses of dichlorvos by gavage for five days.[(100)]

8. Members also noted that there were a number of positive studies and these are discussed below.

9. The Committee agreed that dichlorvos has been reported to induce micronuclei in keratinocytes in mice following the topical application to skin.[(81)] Members agreed that the approach used in this study had not been fully validated but agreed the authors had used an appropriate positive control chemical and that the results with dichlorvos were indicative of an in-vivo site-of-contact mutagenic effect. Members also noted a positive response in a nuclear anomaly assay in hair follicles of mice following topical application.[75] Although the latter is not considered to be a definitive genotoxicity assay, the results might be indicative of a biological effect in the skin.

10. Members agreed that the positive results reported in an abstract by Majeeth et al in a mouse bone-marrow micronucleus assay could not be interpreted, as insufficient information on the methods and results were available.[52] The Committee considered that equivocal evidence of chromosomal aberrations in bone-marrow smears had been reported in a study where hamsters were given a single oral dose of up to half the LD50 of the formulation.[30]

11. The Committee agreed that evidence for the induction of changes in chromosome number had been documented in the bone-marrow of rats following repeated oral dosing with dichlorvos for 6 weeks (5 days/week).[63] Members considered that the methods used were satisfactory and noted that, although the adequacy of reporting was limited, the results indicated a positive effect for the induction of numerical chromosome aberrations. It was noted that a clear dose-response would not be expected in this study as the dose range selected was relatively narrow.

12. Regarding the recently published COMET assay[1], Members considered that the approach adopted by Sasaki and colleagues to the mutagenicity testing of several hundreds of chemicals had a number of drawbacks, for example, limited reporting of signs of toxicity seen in animals. Members considered that the appropriateness of the isolated nuclei method used by Sasaki and colleagues had not been established and noted that there was no cellular measure of cytotoxicity or apoptosis in this study. In respect of the study on dichlorvos, members agreed that the dose level chosen (ca 80% of the LD50) was too high. Members agreed that in view of these limitations, little weight could be placed on this study. The positive data in all tissues examined was unexpected given all the available mutagenicity data on dichlorvos. Members considered that it was not possible to conclude that dichlorvos had mutagenic effects in a wide range of tissues on the basis of these data. Thus, although the authors suggested that dichlorvos had an *in vivo* genotoxic effect, the data were uninterpretable.

13. Members considered the in-vivo mutagenicity study in l lacZ transgenic (Muta™ Mouse) undertaken by Plesta and colleagues.[102] The authors had reported a statistically significant (3-fold) increase in mutant frequency in the liver and a slight non-statistically significant increase in mutant frequency in the bone-marrow following repeated dosing with dichlorvos (5 x 11 mg/kg) given intraperitoneally. Members noted that the dose levels used in this study were high and did induce severe toxicity in the animals. They agreed that although the methods used and standards of reporting used in this study had limitations, the data were indicative of a mutagenic effect of dichlorvos *in vivo* at the site-of-contact i.e. the liver. The Committee noted that the authors had failed to identify any O(6) and N-7 methylguanine adducts in tissue DNA from transgenic mice given a single intraperitoneal dose of either 4.4 mg/kg bw or 11 mg/kg dichlorvos bw but agreed that the methods used by the authors were of inadequate sensitivity and it was unlikely that any alkyl adducts could have been detected. In support of this conclusion Members commented that the levels of DNA adducts (O(6) and N-7 methylguanine) in transgenic mice (Muta™ Mouse) following repeated dosing with dimethyl sulphate (10 x 6 mg/kg bw i.p) were only approximately 4-fold higher than the limit of detection. Members considered that evaluation of DNA adducts in dichlorvos treated animals after the repeat dosing regime might have provided valuable information but these analyses had not been undertaken.

14. The Committee concluded that a consistent pattern of mutagenic effects had been documented in the in-vivo studies in which dichlorvos induced mutagenic effects at high doses in the skin following topical application[81], and in the liver following repeated intraperitoneal dosing[102], suggesting a potential site-of-contact effects (i.e. at initial sites of exposure).

Additional data submitted by industry: AMVAC (for 26 April 2001)

15. A number of papers had been submitted just prior to the COM meeting. Members agreed that no substantive new mutagenicity data had been submitted.[103-105] The additional data from the mouse lymphoma test undertaken as part of the US NTP assessment of dichlorvos was consistent with other assays and indicated a positive result in this assay. Regarding the specific comments on the most recent in-vivo mutagenicity assays, members agreed with the reservations proposed by industry regarding the interpretation of COMET assay[106] but did not agree with the views expressed regarding the conduct of the mutagenicity study in transgenic animals[107]. Members considered that the mutagenicity study in transgenic mice indicated a potential mutagenic hazard at the site-of-contact.

16. The Committee commented on the "Blue-Ribbon" evaluation of dichlorvos completed in July 1998[105] and noted that differences in the rate of methylation compared to the rate of phosphorylation could not be used to discount a potential in-vivo mutagenic hazard of dichlorvos. Additionally the role of phosphorylation in the induction of genotoxic effects could not be discounted.

COM review of submission from industry (23 July 2001)

17. A further meeting of the Committee was held on the 23 July 2001 to consider a presentation from industry on the mutagenicity of dichlorvos. This consisted mainly of a critique of the four positive in-vivo studies underpinning the COM statement (referred to in paragraphs 9-13 above), and of the Committee's approach to weight-of-evidence considerations. No additional information was provided to support the reference submitted by industry to the Committee on the possibility of oxidative damage (108) as a mechanism for the induction of mutations seen in the Muta™ mouse study.

COM consideration of additional information (9 January 2002)

18. The COM considered the new information submitted to the High Court during a judicial review of the regulatory decision on the pesticide products containing dichlorvos (held between 5-9 November 2001) and additional data provided to regulatory authorities up to 4 January 2002 to see if any revision of the COM statement on dichlorvos was warranted.

19. The COM considered the documents listed under reference 111 of this statement. Members only considered the information relating to the scientific assessment of dichlorvos and did not consider information of a legal nature. There were a number of topics raised in the documents.

20. Members agreed that mutagenicity studies that had not been subject to full international validation could be used to inform hazard assessment and regulatory decision making, This was particularly relevant when considering in-vivo activity at sites of initial contact for compounds shown to be direct acting mutagens in-vitro. The COM guidance (published in December 2000; See http://www.doh.gov.uk/com/guidance.pdf) recognised that such situations needed to be approached on a case-by-case basis. The current COM guidance listed a number of non-standard test methods which could be used including the use of transgenic animal models. Members concluded that the information available to the Committee to assess the potential for site-of-contact mutagenicity for dichlorvos was very limited, namely in-vivo skin micronucleus test, the intraperitoneal Muta™ Mouse study and forestomach UDS assay. The design of all these studies had limitations which had been noted by the COM. However in the absence of more definitive data, the results of these tests can be used to provide a provisional hazard assessment and could not be dismissed. Members considered the comments put forward on the conduct of the Muta™ Mouse study undertaken by Plesta and colleagues and reaffirmed that this study was acceptable for hazard identification and had given a positive result.

21. A number of the documents commented on the possibility that a threshold existed for the mutagenic efects of dichlorvos. Members reaffirmed their view (see COM statement COM/01/S3 published June 2001 http://www.doh.gov.uk/comivm.htm) that in the absence of specific investigations concerning mechanisms and possible thresholds, the prudent assumption was that there was no threshold for *in-vivo* mutagens. Members recalled that sufficient data had been provided to determine the existence of a threshold for aneugens that acted by inhibition of the mitotic spindle and also in the case of rapid detoxification of hydroquinone after oral administration; however this was not the case with dichlorvos.

22. COC Members attending the COM meeting of 9 January 2002 reviewed the available information from the carcinogenicity bioassays reviewed in the HSE review (submitted to ACP in April 2001) and in the documents submitted to the court and most recently to the regulatory authorities as part of the data call in up to 4 January 2002. COC members considered that it was extremely difficult to assess the extent of exposure from the available information. However the evidence suggested that some exposure of the skin would have occurred during the inhalation study undertaken by Blair et al in 1974. Regarding the other studies in rats, COC members considered there were limitations in all of the studies (e.g. age of study, numbers of animals used, extent of pathology investigations) and that, apart from evidence of mononuclear cell leukaemia in F344 male rats in two studies, there was no evidence for a carcinogenic effect in rats. It was noted that a Pathology Working Group (PWG) had subsequently discounted the finding of mononuclear cell leukaemia in the NTP bioassay in rats, but the report presenting the basis for this decision was not available to COC members. Regarding other studies in mice, COC members considered that there were limitations in the conduct of these studies similar to those undertaken in the rat. Members considered that it was not possible to undertake a comparison of the studies in mice where dichlorvos had been administered in corn oil and those where dichlorvos had been administered in the drinking water or as an aqueous solution by gavage. COC members reaffirmed, however that when the NCI and NTP bioassays in mice were considered together there was limited evidence for an effect on squamous epithelium of the forestomach and oesophagus in mice.

However the latter study should be viewed in terms of its age and small number of oesophageal tumours. On considering the overall package of carcinogenicity bioassays COC members felt that there was no consistent evidence for a genotoxic carcinogenic effect. COC members noted that there was no agreed mechanism for the forestomach tumours.

COM Discussion

23. The Committee agreed that there is clear unequivocal evidence that dichlorvos can induce DNA damage, chromosomal breakage and mutations in mammalian cells from *in vitro* studies. The compound has been shown to interact with DNA via methylation, however several other mechanisms are theoretically possible. *In vivo* dichlorvos can be rapidly detoxified by hydrolysis before it reaches the systemic circulation. Members noted from the HSE review that retention of 14C-vinyl-labelled dichlorvos in skin was recorded in a study where radiolabelled dichlorvos was applied to the skin on the backs of male rats. Several non-standard *in vivo* mutagenicity assays have indicated that dichlorvos can induce genetic damage when systemic detoxification mechanisms are bypassed, e.g. following exposure to the skin and exposure to the liver following intraperitoneal dosing. The COM agreed that there was a potential risk of mutagenicity at site of contact tissues, i.e. at the initial sites of exposure. The COM felt there was no evidence for systemic mutagenic effects. The Committee agreed that until evidence was provided to the contrary and in the absence of appropriate mechanistic data, a precautionary approach should be adopted and no threshold could be assumed for the mutagenic activity of dichlorvos.

24. Members were aware that there was some limited evidence for a carcinogenic effect in mice from standard bioassays.[109,110] This related to an increase in squamous cell papillomas of the forestomach in mice and carcinomas of the forestomach in female mice given gavage doses of dichlorvos[110] together with the finding of squamous cell papilloma and carcinoma of the oesophagus in a small number of mice[109] Members noted there was no evidence for carcinogenicity from a number of other carcinogenicity bioassays including an inhalation bioassay in the rat, although there were limitations with all of these studies.

25. Members noted that negative results had been obtained with dichlorvos in a single dose UDS assay in the forestomach of mice using gavage dosing.[9,99,101] An increase in replicative DNA synthesis had been reported in this study. Members noted that there were a number of proposals regarding the mechanism of dichlorvos tumourigenicity in the mouse forestomach including localised irritancy of dichlorvos in corn oil. The Committee agreed that this proposal had not been proven and considered that it was not possible to exclude a genotoxic effect from these data given the relative insensitivity of the method used as indicated by the response with the positive control chemical; they felt that repeat dosing would most likely be required to identify any mutagenic effect of dichlorvos in this assay.

Conclusion

26. The Committee concluded that dichlorvos should be regarded as an *in-vivo* mutagen at the site-of-contact (i.e. at the initial sites of exposure. The COM felt there was no evidence for systemic mutagenic effects.) High doses of dichlorvos induced mutagenic effects in the skin following topical application and in the liver following intraperitoneal dosing. The Committee noted the limited evidence for a carcinogenic effect of dichlorvos. This related to tumours of the forestomach in mice after gavage dosing and also the oesophageal tumours seen after dietary administration. There was no satisfactory explanation proven for the mechanisms of these tumours and the Committee felt, given the available mutagenicity data on dichlorvos, that it would be prudent to assume a genotoxic mechanism. The Committee agreed that in the absence of appropriate mechanistic data a precautionary approach should be adopted and no threshold could be assumed for the mutagenic and carcinogenic effects of dichlorvos.

January 2002

COM/02/S2

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111. Additional information submitted to high court during judicial review (5-9 November) and to BPAU/PSD up to 4 January 2002.

New Documents (AMVAC)

1. Cancer summary – 'Dichlorvos: An Assessment of Carcinogenic Potential'.

2. Letter to Ian Chart from J A MacGregor dated 7 December 2001.

3. Letter from Dr. Ward Richter, Director of Pathology Southern Research Laboratory, dated 4 December 2001.

New Documents (Other data holders)

1. Submission from Denka.

2. Submission from Product Safety Assessment Ltd.

Documents previously submitted during the Judicial Review proceedings

1. Written Comments submitted on 11 May 2001 to COM following the first draft COM Statement (F3 920-942).

2. Written summary submitted on 28 June 2001 prior to presentation to the 23 July 2001 COM Meeting (D1 86-93).

3. Written comments submitted on 26 July 2001 following the second draft COM statement (D1 98-104).

4. Expert opinion of David Brusick August 2001 (D1 117-122).

5. Expert opinion of John Ishmael August 2001 (D1 147-157).

6. Expert opinion of Karel de Raat August 2001 (D1 167-180).

7. Expert opinion of John Mennear August 2001 (D1 184-197).

8. First witness statement of J. A. MacGregor August 2001 (D1 217-243).

9. Second witness statement of J. A. MacGregor August 2001 (D1 312-326).

10. Third witness statement of J. A. MacGregor (D2 674-677).

11. Affidavit of Anju Sanehi (D1 1-7 paragraphs 13-18).

Dimetridazole (DMZ)

Background to COM review

1. Dimetridazole (DMZ) is an antiprotozoal substance that is used to maintain the health of certain farm animals. In the UK it is permitted for use as a feed additive for turkeys and guinea-fowl and as a veterinary medicine for game birds (pheasants and partridges only). The European Commission is currently considering whether to suspend use as a feed additive, but this would not directly affect veterinary medicinal use.

2. In the last few years, DMZ has been assessed by the EU's Committee on Veterinary Medicinal Products (CVMP)[1,2] and by the EU's Scientific Committee on Animal Nutrition (SCAN)[2]. These two committees disagreed with one another over the interpretation of the mutagenicity data. The CVMP considered that more information was needed before it could conclude on mutagenicity, whereas the SCAN considered that there was sufficient information to allow it to conclude that there was no genotoxic hazard to consumers. The specialist expert advice of COM was sought by the Food Standards Agency (FSA). The FSA is particularly concerned about the safety of consumers of foods derived from animals given DMZ.

Assessment of mutagenicity data

In vitro data

3. DMZ was mutagenic in bacteria.[4-6] Studies using nitroreductase proficient and deficient strains of *Salmonella typhimurium* TA100 showed that this pathway accounted for a proportion but not necessarily all of the mutagenicity seen in bacteria.[7] Positive results were reported in *Saccharomyces cerevisiae* D4 demonstrating that DMZ was mutagenic in eukaryotic cells.[8] Members noted that there was inconsistency in the interpretation of the data for the *in vitro* mutation assays in the mouse lymphoma assay. For the cytogenetics assay in CHO-K1 cells the data were reported as negative but were not traceable. In addition there were very limited details available regarding the *in vitro* UDS assay in V79 Chinese hamster lung fibroblasts and no conclusions could be reached on the results of this test.

4. However sufficient details were available to assess the genotoxicity of DMZ in human lymphocytes using the comet assay.[9] It was established that DMZ was genotoxic under aerobic conditions in the absence of exogenous metabolic activation. The addition of Aroclor 1254 induced rat liver S-9 fraction abolished the genotoxicity of DMZ in this test system. Using the same treatment concentration of DMZ, the magnitude of the response (mean tail moment derived from 200 cells) was reduced under anaerobic conditions and abolished by the addition of anti-oxidants (8-hydroxyquinoline, vitamin C, catalase or superoxide dismutase). Thus, DMZ was genotoxic in the *in vitro* comet assay in human lymphocytes and that the activity observed in this assay was consistent with oxidative DNA damage.

5. The Committee agreed that DMZ was mutagenic *in vitro*.

In vivo data

Insects

6. Members agreed that no weight could be given to the negative results reported in the sex-linked recessive lethal mutation assay in *Drosophila melanogaster*.[10]

Mammals

7. Negative results were reported in published *in vivo* micronucleus assays in the mouse using intraperitoneal and oral routes of administration.[11] However no conclusions could be reached by the Committee in view of the limited details of these tests that were available.

8. The full reports made available to the Committee of the three *in vivo* studies form the basis for the Committee conclusions regarding the ability of DMZ to express the mutagenic potential shown in vitro in mammals.

9. Negative results were obtained when DMZ was investigated in an adequately conducted bone marrow micronucleus test in the mouse, DMZ being given orally at 2 daily doses up to 915 mg/kg.[12] Negative results were also obtained in an *in vivo* liver UDS assay when DMZ was given orally to male rats at single doses up to 1000 mg/kg but the Committee noted that this study had not been performed to current standards since the analyses were limited to examination of primary hepatocytes at a single harvest time (15 hours).[13] [The OECD guideline (486) adopted in 1997, recommends an early sampling time (at 2-4 hours) as well as a later sampling time (12-16 hours). Compounds such as dimethylnitrosamine would not be detected as positive if only the later sampling time point was used.] Negative results were also obtained when DMZ was examined for its ability to induce mutations in germ cells in a dominant lethal assay in male mice.[14] Animals were given 5 daily doses of up to 1000 mg/kg and then subjected to sequential mating over 8 weekly periods.

10. The Committee agreed that the further data were needed to provide adequate reassurance that the mutagenic activity seen *in-vitro* was not expressed *in vivo*. This would comprise a liver UDS assay in rats conducted in accordance with the OECD guideline; it was however recommended that 4 animals should be analysed at each dose level and time point.

Carcinogenicity data

11. The Committee agreed that the available long-term carcinogenicity bioassays in rats had not, from the limited data available, been conducted to contemporary standards.[15] Benign mammary tumours had been documented in two of these studies and some limited evidence was available to support a hormonal mechanism for the induction of these tumours. However, the blood concentration of progesterone was raised in female rats but not in males, whereas benign mammary tumours had been produced in both sexes. Overall, no reassurance regarding the absence of genotoxicity could be derived from the available carcinogenicity bioassays.

Conclusion

12. Dimetridazole (DMZ) has mutagenic activity *in vitro*. The Committee was provided with full reports of *in vivo* studies to investigate the ability of DMZ to induce micronuclei in bone marrow of mice, UDS in primary hepatocytes of male rats, and dominant lethal mutations in the germ cells of male mice. In all cases the oral route was used, with dose levels up to about 1 gram/kg per day. Negative results were consistently obtained. However the Committee concluded that the rat liver UDS assay had not been adequately conducted and recommended that a further rat liver UDS assay conducted in accordance with OECD guidelines (but using 4 animals per dose/sampling time point) was required to provide full reassurance that DMZ (or its metabolites) does not have significant mutagenic activity in mammals in vivo.

June 2002

COM/02/S4

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ILSI/HESI research programme on alternative cancer models: results of Syrian hamster embryo cell transformation assay

Background

1. The International Life Sciences Institute (ILSI) and the Health and Environmental Science Institute (HESI) have co-ordinated a multinational research programme from 1996 – 2001 to more fully characterise the responsiveness of several alternative cancer models. The research has focused predominantly on a number of proposed short-term *in-vivo* test models for assessment of potential carcinogenic activity carcinogenicity in mice (in particular ras H2, Tg.AC, p53 +/-,Xpa-/-, Xpa-/-/p53+/- double knockout, neonatal mouse) but also included the *in-vitro* cell transformation assay using Syrian hamster embryo cells (SHE cell transformation assay). The overall objective of the work was to evaluate the ability of these models to provide useful information for human cancer risk assessment. The research involved input from over 50 industrial, governmental (USA, Denmark, Netherlands and Japan) and academic laboratories and cost around $35m.[1]

2. The Committee on Carcinogenicity of Chemicals on Food, Consumer Products and the Environment (COC) has considered all of the data submitted by ILSI/HESI except for the results of the investigations using the SHE cell transformation assay. These data have been considered by Committee on Mutagenicity on Chemicals in Food, Consumer Products and the Environment (COM) whose conclusions are given below.

Previous considerations of the SHE cell transformation assay by COM

3. The assessment of the SHE *in vitro* test system was considered by the COM in 1994 and in 1996. Full details of these considerations can be found in the relevant Annual reports.[2,3]

4. In 1994 the COM considered a number of cell transformation assays including the modified SHE method, and had agreed that transformation assays using Syrian Hamster cells were not yet ready for routine use, but warranted further work on both their validation and providing an understanding of the underlying mechanisms. The Committee considered a draft proposal for an OECD guideline for cell transformation using the modified SHE method (low pH culture). A number of supporting publications and papers claiming a predictive correlation between cell transformation in this test system and carcinogenicity were also reviewed.

5. In 1996, the Committee agreed that there were no mechanistic data available which gave an insight into the relationship between cell transformation in the SHE assay and the carcinogenic process. Although apparently high correlations had been reported in trials using a range of animal carcinogens including non-genotoxic carcinogens, only a very limited number of laboratories were involved and little value could be attributed to these results in the absence of appropriate supporting mechanistic data.

6. The COM concluded at the end of its 1996 review that it was not possible to support the proposed OECD guideline for the reasons given below:

   (i) The current draft of the guideline was poorly referenced and the text was vague and could apply to several different methods.

   (ii) The mechanisms underlying the changes observed in SHE cells were unknown.

   (iii) There were no objective criteria to define the end point (quantification of transformed foci) which is subjective.

   (iv) Data from a validation exercise involving a number laboratories testing compounds blind should be undertaken before consideration could be given to the proposed draft OECD guideline.

ILSI/HESI Alternative Cancer Test research

7. The Committee considered a draft paper by Mauthe RJ and colleagues which had been submitted to *Toxicologic Pathology*. The Committee had considerable reservations on several aspects of the ILSI study relating to the assay system, the study design, the data obtained and the data analyses. There is still no mechanistic basis to underpin the assay and the endpoint (transformed foci) remains subjective as there is a lack of a clear, objective definition of transformed foci. There were reservations about the selection and categorisation of test chemicals, concentration ranges were considered to be too narrow for reasonable analysis of dose-response and, concerns about the extent of repeat testing. Control values were unsatisfactorily low (0 to 6) and in some tests there was a lack of consistency of result between closely spaced doses. Finally, the definition of positive and negative results relied on statistical analyses that seemed inappropriate to the data. Most importantly, there was no correction for multiple comparisons. Also methods had been used (Fisher exact and binomial-based) that assume uniformity of sampling circumstance (i.e. that all cells have an equal chance of transformation under fixed experimental conditions). This is unlikely to be true for the mixture of cell types derived from embryos. No tables of historical negative and positive control ranges (and/or confidence limits) were given to enable general variability, test validity and biological importance to be assessed. Some members of the committee considered that two-sided (-tailed) statistical analyses would be more appropriate as it is probable that compounds may have negative as well as positive effects on transformation. Thus the Committee felt that the results of some tests reported as positive were most likely to be either equivocal or negative. Members also considered that the difficulties in objectively identifying transformed cells combined with the limited number of repeat trials suggested that interpretation of the ILSI/HESI data was particularly problematic. The results for a number of the chemicals tested are considered in detail below.

8. Phenacetin was included in the study as a definite human carcinogen, but was considered by the World Health Organisations International Agency for Research on Cancer (IARC) to be a probable human carcinogen. The IARC conclusions indicated that phenacetin was carcinogenic in rodents inducing malignant tumours of the urinary tract in rats and mice and nasal tumours in rats. IARC had noted that Phenacetin was mutagenic *in vitro* in Chinese hamster cells but gave equivocal results in *in-vivo* tests in rodents for chromosomal aberrations and micronuclei. Overall the data on phenacetin suggested that a positive response would have been expected in the SHE cell transformation assay whereas a negative response had been obtained with phenacetin. There was a need for further consideration of this result.

9. The COM's evaluation suggested that the positive responses obtained with melphalan (included as a human carcinogen) and sulphamethoxazole (rodent carcinogen) were equivocal and needed further trials to confirm the response obtained with this assay. Members noted that ampicillin had given a statistically significant increased response at the highest dose tested (13 transformed foci at 3000 mg/ml) whereas a dose level of 2450 mg/ml had not yielded any transformed cells. Overall ampicillin was classified by the authors as giving a positive response in the SHE assay. However, members considered that the outcome was equivocal or negative and that further testing was required to elucidate the response of ampicillin in this assay.

10. Members felt that the critical problem preventing the use of the SHE assay in a regulatory context was the difficulty in the morphological identification of transformed foci.

Use of SHE assay for regulatory screening of chemicals

11. The Committee considered on the basis of all the available information that some important generic comments could be made on the SHE cell transformation assay.

12. Members felt there was little prospect in obtaining reproducible results with the SHE cell transformation assay as a screen for the identification of chemical carcinogens in the absence of an objective molecular marker to identify cell transformation. Members stressed that there was a need to explain on a mechanistic basis why chemical carcinogens with disparate mechanisms of action should be expected to produce cell transformation in an embryonic cell line.

13. Members considered that whilst positive results could be obtained for benzo(a)pyrene indicating that SHE cells (and or its feeder layer) could activate carcinogens there was very little information available to define the metabolic competency of SHE cells and the feeder layer used.

14. Members noted that there was a tendency to report positive results for a diverse range of chemicals in the SHE cell transformation assay thereby demonstrating good sensitivity. However, there was a lack of chemicals reported to give negative data so that neither the specificity nor the accuracy of the test can be properly assessed. This problem had been illustrated by the data set from the ILSI/HESI trial. However, Members felt that no particular value could be currently ascribed to either positive or negative results obtained in the SHE cell transformation assay in the absence of an objective endpoint to measure, and an understanding of, the mechanism underlying the process of cell transformation. Many of the chemicals selected for this trial are rodent carcinogens that are not human carcinogens. There are concerns that this assay has poor specificity for the detection of human carcinogens.

Conclusion

15. The Committee agreed that the SHE cell transformation assay should not be used for regulatory screening of chemicals for potential carcinogenicity.

16. There are insufficient data on validation of the SHE cell transformation assay to justify the development of an OECD test guideline for this assay.

17. The Committee has considerable reservations regarding the mechanistic basis underpinning the rationale for using the SHE cell transformation assay to screen for chemical carcinogenesis.

18. The Committee has considerable reservations regarding the validity of using morphological assessment alone to define transformed foci in the SHE cell transformation assay. The development of an objective molecular marker is essential before validation work can proceed.

April 2002

COM/02/S3

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

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SECRETARIAT

R J Fielder BSc PhD Dip RCPath (Scientific Secretary)

Diane Benford BSc PhD (Scientific – Food Standards Agency)

K N Mistry (Administrative)

J M Battershill BSc MSc

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

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof P B Farmer (Chairman)	Celltech	Share Holder	NONE	NONE
	Abbey National	Share Holder		
	Bradford & Bingley	Share Holder		
	Friends Provident	Share Holder		
	Torotrak	Share Holder		
	SBS Group	Share Holder		
Prof J Ashby	Zeneca	Employee, Salary, Share option	NONE	NONE
	ML Labs	Share Holder		
	Phytopharm	Share Holder		
Dr G Clare	Huntingdon Life Sciences	Employee, Salary, Share option	NONE	NONE
	AstraZeneca	Share Holder		
	Syngenta	Share Holder		
	HBOS	Share Holder		
Dr J Clements	Covance	Employee Salary Share Option Share Holder	NONE	NONE
Prof C Cooper	HBOS	Share Holder	NONE	NONE
	Norwich Union	Share Holder		
Dr N Gooderham	Abbey National	Share Holder	GlaxoSmithKline	BBSRC Collaborative Studentship
	Friends Provident	Share Holder		
	Game	Share Holder		
	ML Laboratories	Share Holder		
	Northern Rock	Share Holder		
	Proctor & Gamble	Consultant		
	Protherics	Share Holder		
	Sunderland AFC	Share Holder		

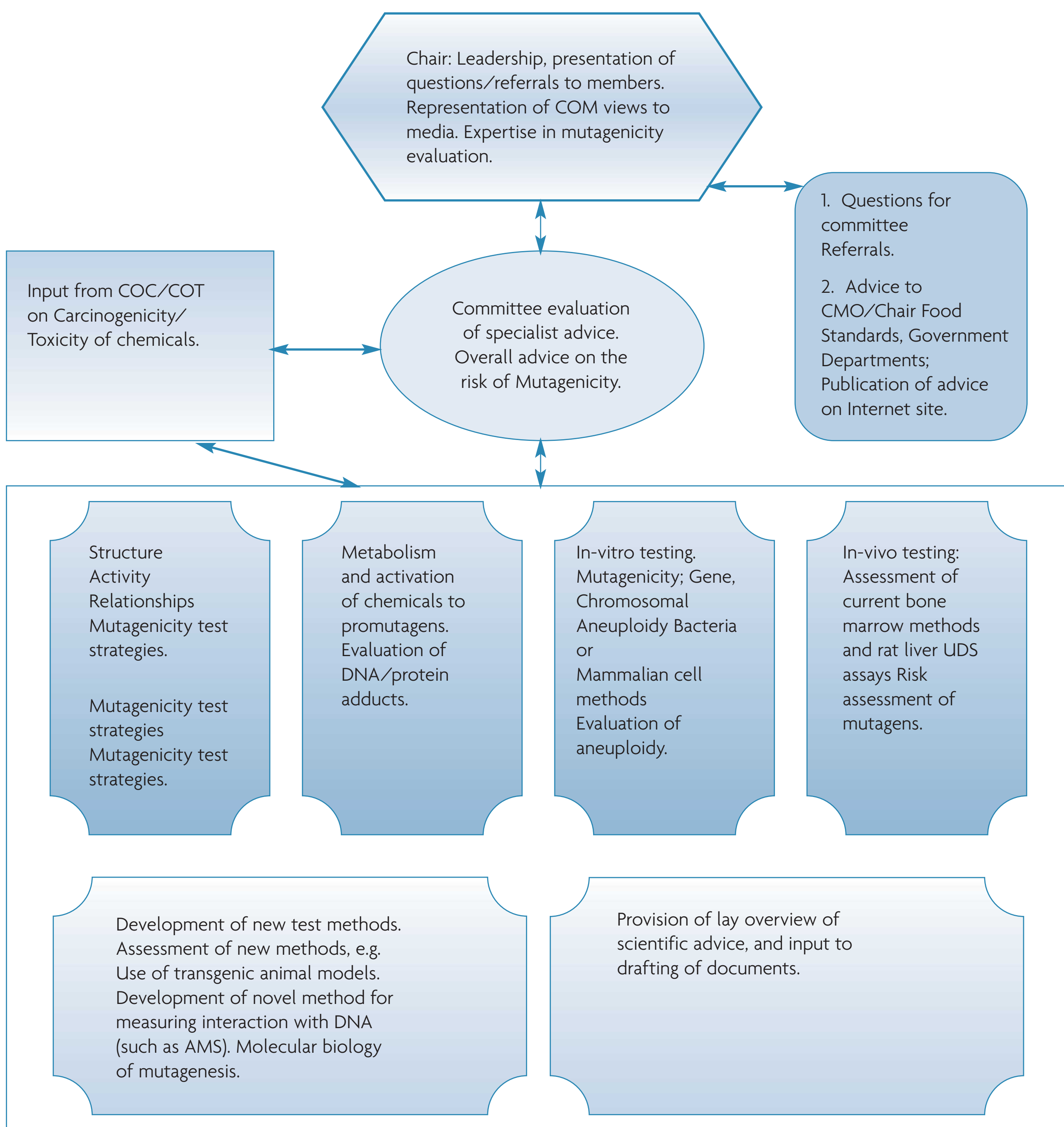
Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Ms M Langley	BT	Share Holder		
	Business Consolidating Services	Director		
	Ciebel	Share Holder	NONE	NONE
	CREE Research	Share Holder		
	Cyber Care	Share Holder		
	Eshelon	Share Holder		
	HBOS	Share Holder		
	MMO_2	Share Holder		
	Quelcom	Share Holder		
	Wibex	Share Holder		
Dr I Mitchell	Kelvin Associates	Associate Consultant		
	IM Enterprises	Director/Creditor		
	GlaxoSmithKline	Pension Option and Share Holder Consultant		
	Bass	Share Holder	NONE	NONE
	Cable & Wireless	Share Holder		
	Cadbury Schweppes	Share Holder		
	Marconi	Share Holder		
	Nokia	Share Holder		
	Pfizer	Share Holder		
	RTZ	Share Holder		
	Shell	Share Holder		
	Unilever	Share Holder		
	Vodafone	Share Holder		
	Whitbread	Share Holder		
	British Telecom	PEP Holder		
	Centrica	PEP Holder		
	Scottish Power	PEP Holder		
	Shire	PEP Holder		

Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Dr E M Parry	Invesco	PEP Holder		
	Fleming	PEP Holder	NONE	NONE
	Legal & General	PEP Holder		
Prof D Phillips	Abbey National	Share Holder		
	BG Group	Share Holder		
	Bradford & Bingley	Share Holder	NONE	NONE
	Centrica	Share Holder		
	CGNU	Share Holder		
	Lattice Group	Share Holder		
	National Grid	Share Holder		
	Servier	Consultant		
Prof D J Tweats	GlaxoSmithKline	Salary Employee Consultant Share Option Holder		
	Boots Healthcare	Consultant	NONE	NONE
	Charterhouse	Consultant		
	Therapeutic Ltd	Consultant		
	Gentronix Ltd	Consultant		
	Theravance Inc	Consultant		
	Watford FC	Share Holder		

Committee on Mutagenicity of Chemicals in Food Consumer Products and the Environment

DRAFT TEMPLATE FOR COM 2002

The template is designed to show the breadth of expertise available to the Committee and is intended to aide members in discussing future needs with regard to expertise necessary to fulfil the terms of reference of the COM. The compliment of COM is 10 members (9 specialists and one lay member) and one chair. A deputy chair has not been appointed at October 2002.



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

Preface

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

During the year 2002 the Committee has provided advice on a wide range of chemicals including malathion (a pesticide) and polycyclic aromatic hydrocarbons (contaminants which may be present in air and food). The COC also discussed an approach to using estimates of the upper bound risk estimate for carcinogenic air pollutants at environmental levels of exposure. The COC agreed the approach provided it was based on good quality epidemiological data and that risk estimates were not quoted as if they were real estimates of risk but were used in the consideration of risk management options.

The Committee discussed its procedures in the light of the new code of practice for Scientific Advisory Committees published by the Office of Science and Technology (OST). The Committee adheres to most of the recommendations and agreed to publish more of the substantive background papers to discussions at the earliest opportunity. The COC devised a template showing its methods of working and expertise which is easy to follow.

The Committee also discussed the use of uncertainty factors in its evaluations, the minimum duration of carcinogenicity tests in animals and the use of short term tests in the future. The Committee finished a major piece of work on the investigation of interaction between environment and genotype in the induction of cancer by chemicals. A detailed statement and a lay statement have been published.

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

Malathion

3.1 Malathion is an organophosphorous insecticide. It has been marketed in the UK for use in agriculture and horticulture since 1956. There were three products with approvals for use in agriculture and horticulture, home garden and use in pigeon lofts at the time when this review was initiated in January 2002. A number of products containing malathion are also licensed as human medicines for use in the control of head lice.

3.2 The Advisory Committee on Pesticides is reviewing the available toxicological information on malathion as part of its ongoing review of organophosphorous compounds. The ACP asked for advice from COM and COC on mutagenicity and carcinogenicity at its 289th meeting on 17 January 2002. The Chairs of COM and COC agreed that a joint statement was required in view of the need for a full review of all mutagenicity and carcinogenicity data. There were inconsistent results in mutagenicity studies (both *in-vitro* and *in-vivo*) and there was evidence for the mutagenic activity of some impurities which might be present in some batches of technical malathion.

3.3 There was some limited evidence for tumourigenicity in rats at high oral doses given via the diet which adversely affected growth and survival of the animals. This included the occurrence of benign nasal tumours in a few animals given high oral doses of technical grade malathion in the diet and benign liver tumours in female rats at the highest dose level. An increased incidence of benign liver tumours was reported in male and female B6C3F1 mice at high dietary levels which were associated with reduced weight gain.

3.4 The COC reviewed the available carcinogenicity data on malathion which included in confidence reports (provided by the Pesticide Data Holder regarding two studies in rats one in mice) and published reports of long-term bioassays in rats and mice at its 27 June 2002 meeting. Three long-term bioassays using malaoxon (the principle metabolite of malathion and also present in technical grade malathion as an impurity) were available which included two in rats and one in mice. In addition the Committee also considered in confidence reports of Peer Reviews of the histology slides from the 1993-96 malathion bioassay in F344 rats, the 1992-94 bioassay in B6C3F1 mice and some additional supplemental information for the 1992-1994 bioassay in B6C3F1 mice and the 1993-1996 bioassay of malaoxon in F344 rats provided by the Pesticide Data holder.

3.5 A number of additional follow up reports from the contract laboratory concerning the 1993-96 bioassay of malathion in F344 rats were also reviewed. In addition the Pesticide Data Holder submitted a response to questions from COC secretariat which provided an overall summary of the histology of the nasal tissue in animals with tumours and additional evaluation of the historical control data on nasal tumours in F344 rats and possible mechanisms for nasal tumours induced in F344 rats fed high doses of technical grade malathion. A published Peer Review of a number of the older carcinogenicity bioassays was also available. The COC also considered expert reports from the EPA and a Scientific Advisory Panel established by EPA to review malathion.

3.6 The Committee reached the following overall conclusion.

3.7 "The COC agreed that technical grade malathion had been tested in four long-term dietary bioassays in rats and two long-term dietary bioassays in mice. The most recent studies undertaken in F344 rats (1993-96) and in B6C3F1 mice (1992-94) were adequate for the evaluation of carcinogenicity. There is evidence for tumourigenicity in the nasal tissue and liver (females only) of F344 to rats fed malathion. The nasal tumours were associated with severe ongoing inflammation, which is most likely involved in the mechanism of tumourigenesis. There was evidence for liver tumours in female F344 rats and male and female B6C3F1 mice. The weight of evidence suggested that these liver tumours were induced through a non-genotoxic mechanism and were not relevant to human health."

3.8 Malathion was also considered at the COM meetings of 25 April and 10 October. A full statement from COC and COM is in preparation.

Polycyclic Aromatic Hydrocarbons: Advice on Dibenzo (a,l)pyrene

3.9 Polycyclic aromatic hydrocarbons (PAHs) are a large group of highly lipophilic chemicals that are present ubiquitously in the environment as pollutants. Many of them are generated as by-products of the combustion of organic material and they occur in particulate and/or vapour phases. Humans are widely exposed to low levels of mixed PAHs in air, food and drinking water. Higher levels of atmospheric exposure are encountered by workers employed in industries such as aluminium production, coal gasification, coke production and iron and steel founding. Cigarette smoke is also a major source of PAHs.

3.10 The COM and COC were asked by DoE and MAFF for a scheme to evaluate and rank 25 selected PAHs which could be used as a basis for further monitoring and/or surveillance. When COC started this work in 1994 it was originally intended to use a ranking system based on 'toxic equivalency factors' with benzo(a)pyrene as the comparator substance. The data were, however, inadequate for some of the listed PAHs and a simple 5 category system was devised:

(Group A) There is a high level of concern about a carcinogenic hazard for humans because the compound is an *in vivo* mutagen and/or a multi-site carcinogen in more than one species.

(Group B) There is concern about a carcinogenic hazard for humans, but the data are incomplete or the mechanism is unclear.

(Group C) The compound is a non-genotoxic carcinogen. (This category may contain compounds with an equal amount of evidence for carcinogenic hazard as compounds in categories A or B, but these are placed in a separate category because subsequent management may be different). In practice none of the 25 PAHs considered fell into this group.

(Group D) The data are inadequate for assessment.

(Group E) There is no concern about carcinogenic hazard, ie the compound is non-genotoxic and non-carcinogenic or the mechanism of carcinogenesis is not relevant to humans.

3.11 Specific information regarding the classification of the 25 PAHs considered in 1994/5 can be found in the 1995 Annual report.

3.12 The COC consideration of dibenzo(a,l)pyrene is given below.

3.13 The COC agreed that the *in-vitro* mutagenicity tests and information on *in-vivo* DNA adduct formation was consistent with dibenzo(a,l)pyrene being an *in-vivo* mutagen. Members also agreed that dibenzo(a,l)pyrene was carcinogenic in mice and rats. Dermal application to mice produced skin tumours (squamous cell carcinomas) and tumours at a number of sites (such as lungs, spleen and lymphomas) and intraperitoneal administration to rats produced lung tumours. Intramammary instillation in rats resulted in mammary tumours. Dibenzo(a,l)pyrene also acted as an initiator in mouse skin carcinogenicity promotion assays. The COC therefore considered that dibenzo(a,l)pyrene should be assigned to group A of its hazard ranking scheme for PAHs.

3.14 Regarding carcinogenic potency, the committee agreed that dibenzo(a,l)pyrene was a very potent genotoxic carcinogen and that potency varied depending on factors such as species, route of administration, dose and site of tumour produced. From the available data where a comparison could be made, members considered that the dibenzo(a,l)pyrene carcinogenic potency was likely to be in the range of 10-100 times more potent than benzo(a)pyrene depending on the test system used.

3.15 A full statement on dibenzo(a,l)pyrene is in preparation. Further consideration of the relative potency of dibenzo (a,l)pyrene compared to other PAHs is underway.

Quantification of risks associated with carcinogenic air pollutants

3.16 In the air pollution area, the non-cancer health effects of these pollutants are quantified for cost-benefit analysis using dose-response functions from epidemiological studies of environmental exposure to air pollutants. The Department of Health had been asked whether the benefits (ie reduction in cancer incidence), which could be attributed to lowering levels of carcinogenic air pollutants below current standards, could be quantified. Simple linear extrapolation using WHO unit risk factors were used to highlight relevant issues for discussion. The Committee was asked for its views on possible approaches (eg risk estimation and relative ranking) to quantification of effects of carcinogenic air pollutants at the March 2002 meeting.

3.17 At the November 2002 meeting, Members considered a proposal to use the upper bound estimate from the one-hit model to set upper bounds of risk at low levels of exposure on the basis of data from human epidemiology studies. Members were advised that the intention was to use this very conservative approach to advise on the practicality of risk management options for air pollutants and there was no intention to publish risk estimates based on this approach. The primary objective would

be to assess the cost of reducing levels of air pollution to the exposures associated with the upper bound estimate of risk based on the one hit model. The COC was content with the approach provided that it was limited to chemicals for which there was good cancer epidemiology data and that data were used only as a guide when considering risk management options. Members felt it important to restate that extrapolation of risk estimates below the observed range was very problematic, as no model was completely satisfactory.

Review of Committee Procedures

OST Code of Practise for Scientific Advisory Committees

3.18 A copy of the new "Code of Practice" for Scientific Advisory Committees published by the Office of Science and Technology (OST) on 19th December 2001 was provided to members for information and comment. Many of the issues were considered at the last (November 2001) COC meeting when the Committee reviewed the Government's response to the BSE enquiry. Members agreed that most of the COC procedures conform to the new code of practice and where this is not the case steps are being taken to comply. The Committee noted that substantantive background papers would be published (excluding those containing commercial in-confidence data). Due to the highly technical nature of the work it would be difficult for papers to be truly comprehensible to the non-specialist, but it was hoped that the 'what's new section' of the COC internet site and lay summaries would help in this regard. Additionally, overview lay summaries should accompany some statements and a glossary of technical terms could help with public understanding. The COC would also contribute to a joint COT/COC/COM glossary. With respect to dealing with dissenting views members agreed that it should be made clear in the minutes and statements when decisions were not unanimous.

COC Template

3.19 The COC agreed a template diagram which provided an overview of how COC undertakes risk assessment of carcinogens and the interaction of COC with its sister Committees (COT and COM) and with Government Departments, Regulatory Agencies and the Chief Medical Officer Professor Sir Liam Donaldson. For ease of reference this template is reproduced at the end of this section of the Annual Report.

Test Strategies and Evaluation

IGHRC paper on uncertainty factors

3.20 The Interdepartmental Group on Health Risks from Chemicals (IGHRC) is developing cross-Government guidance on the handling of uncertainty in the toxicological hazard aspects of human health risk assessment. IGHRC intends to produce a document setting out a harmonised framework for UK Government departments, agencies and their advisory committees on how to address the uncertainties in toxicological hazard aspects of risk assessment including the derivation and application of uncertainty factors. The Committee heard a short presentation from Professor Iain Purchase (Chair of Executive Committee of IGHRC).

3.21 The document provided a review of the approaches used in chemical risk assessment in the UK. The draft document reflected the current position in relation to the assumption of absence of a threshold for genotoxic carcinogens as laid out in the 1991 COC *Guidelines for the Evaluation of Chemicals for Carcinogenicty* (see section on ongoing work). It was noted that the COM had recently reaffirmed its position that no threshold could be assumed for *in-vivo* mutagens in the absence of compound specific mechanistic data to suggest otherwise. (http://www.doh.gov.uk/comivm.htm.) The draft IGHRC document indicated that for carcinogenicity believed to arise through a non-genotoxic mechanism of action, a conventional approach using estimation of a No Observed Adverse Effect Level (NOAEL) and application of uncertainty factors could be adopted. It was also recognised that in some cases an extra uncertainty factor has been applied to non-genotoxic carcinogens because of concern over the severity (and irreversible nature) of the effect or uncertainties in the mechanism of carcinogenicity.

3.22 The COC confirmed that the approach set out in the document was acceptable. It was pointed out that in some instances there would be a limited range of risk management options and this could influence the approach to risk assessment used, but not the outcome of any risk assessment. Members commented that the Bench Mark Dose (BMD) approach made use of the data from all dose levels and avoided uncertainties in trying to set a NOAEL. However the BMD required more doses at levels expected to result in some toxic effects and might result in greater animal usage. In respect of risk assessment of chemical carcinogens, members cautioned against a “numerical” approach to the use of data from long term bioassays in animals in risk assessment. It was noted that the interpretation of long-term cancer bioassays was influenced by, often subtle, interpretation of histology.

Minimum duration of carcinogenicity studies in rats

3.23 The proper conduct of carcinogenicity studies in rats is an important part of the evaluation and prediction of potential human carcinogens. Significant reductions in the number of control rats surviving to termination have been widely reported in the scientific literature. This is a matter of concern since inadequate carcinogenicity studies could be important in decisions regarding the identification of potential human carcinogens and in particular the failure to identify such compounds. In addition there is a possibility that inadequate studies could be rejected by regulatory agencies with the consequent need for use of further animals to obtain a valid result. For a negative result from a rat carcinogenicity bioassay to be considered acceptable, survival at 24 months should be 50% or greater in all groups.

3.24 The Committee had reviewed the evidence for application of dietary restriction techniques in 2000 and a statement was published (http://www.doh.gov.uk/longevity.htm). It was concluded that the available information supported the view reached by the COC in its guidelines published in 1991 that dietary restriction in carcinogenicity studies should be applied with caution and is the responsibility of the toxicologist undertaking the study. The COC had agreed that the subject of dietary restriction should be reviewed when more information is available. Some investigators have also proposed that reducing the duration of carcinogenicity bioassays undertaken in rats would have the desired effect of terminating studies before survival was reduced to below 50% in tests groups and that such bioassays

would also be adequate. The Committee reviewed two published papers which had evaluated published data on the duration of carcinogenicity studies. These two papers came to contrasting views (see statement published at end of this Annual Report for overview). The Committee was asked to provide generic advice on the desirability of reducing the minimum duration of carcinogenicity studies in rats from 2 years as currently stated in international guidelines for the conduct of such studies.

3.25 The COC concluded that there was insufficient evidence from the new publications to recommend a change to the international guidelines for the conduct of long term carcinogenicity bioassays. The current guideline is that for a negative result to be acceptable in a rat carcinogenicity bioassay, survival should be at least 50% in all groups at 24 months. The Committee reaffirmed that it was the responsibility of the study director to use rat strains that would ensure adequate survival at 24 months. The COC statement is included at the end of this report.

Short term tests for carcinogenicity (ILSI/HESI research programme on alternative cancer models)

3.26 The International Conference on the Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) has agreed that the required bioassay data may be derived from only one species, i.e. the rat. This would be supported by appropriate mutagenicity and pharmacokinetic data and also information that could come from newly proposed short-term *in-vivo* test models for assessment of potential carcinogenic activity in mice (in particular heterozygous p53+/- deficient Tg.AC model, and ras H2 models). Although these recommendations apply only to human medicines, any decision could have significant implications for other categories of chemical (e.g. food additives, pesticides, industrial chemicals etc). The key public health issue is whether the proposed short term tests in transgenic mice are appropriate adjuncts to the rat carcinogenicity bioassay in the identification of chemical carcinogens.

3.27 The COC has as part of its remit to advise on issues relating to chemical carcinogenesis and to present recommendations for testing strategy. The Committee was asked by the Department of Health in 1997 to consider the available literature on the proposed short-term *in-vivo* tests for assessment of carcinogenic activity in mice, and specifically, on the transgenic mice models (heterozygous p53 +/- deficient, Tg.AC model, and ras H2 model). The conclusions reached by the Committee can be found in full in the Annual report for 1997. Overall the COC agreed that much of the current research effort had been placed on the evaluation of the three short-term animal model tests systems reviewed but little interest had been devoted to the underlying mechanistic basis for these tests and also to the most appropriate transgenic animal model for screening for potential human carcinogens. The Committee agreed that many transgenic models were likely to be developed over the next few years which might be applicable to specific areas of interest, such as the identification of tumour promoters. This was an area to keep under review.

3.28 The International Life Sciences Institute (ILSI) and the Health and Environmental Science Institute (HESI) have co-ordinated a multinational research programme, from 1996 –2000, on the use of alternative cancer models. The research involved input from over 50 industrial, governmental (USA,

Denmark, Netherlands and Japan) and academic laboratories and cost around $35m. The data from the project along with a number of evaluation papers from independent experts and abstracts of additional work were published as a supplement to volume 29 of Toxicologic Pathology in November 2001 pp1-351.

3.29 The COC acknowledged the considerable administrative and practical problems that had confronted ILSI/HESI in co-ordinating this work. It was considered that the programme had provided a large amount of information on the evaluation of performance of these assays but the data were not sufficient to validate the use of any of the assays for regulatory testing.

3.30 The COC agreed an overall conclusion that none of the models used in the ILSI/HESI Alternative Cancer Test programme were suitable as a replacement for the mouse carcinogenicity bioassay (the primary purpose for the development of these models) and that further research should look to identify models with a greater relevance to mechanisms of carcinogenicity in humans. Of the animal models assessed there was evidence that p53+/- transgenic mouse model could identify some genotoxic carcinogens. There was insufficient data to suggest that the animal models under consideration (*RasH2, Tg.AC, Xpa, Xpa/P53+/- and p53+/-*) provide essentially similar results. The COC statement is included at the end of this report.

The investigation of interaction between genotype and chemicals in the environment on the induction of cancer

3.31 Many diseases (such as cancer) are thought to be due to a combination of heredity and other factors in the environment (such as lifestyle, diet and to a lesser extent exposure to chemicals in the environment). The DNA sequence of an individual (his or her genotype) may be one factor which contributes to whether a person who is exposed to chemical carcinogens (e.g. from tobacco smoke) may develop cancer. The Human Genome Project is showing that there are a great many small differences between individuals in their DNA sequences.

3.32 The Committee was asked by the Department of Health to review the available information on the interaction between genotype and exposure to chemicals in the environment and the induction of cancer. The Committee was asked to provide advice on the methods of epidemiological research used in this area and the approaches to identifying genes of interest for such studies. Of particular importance is the evaluation and significance of data from relevant studies in cancer risk assessment.

3.33 The Committee reviewed the methods used to investigate possible interactions between genotype, exposure to chemicals and occurrence of cancer. The types of study, which all involved investigating genotype and exposures to chemicals in humans, could be separated into two types, i. gene characterisation studies, which aim to investigate the nature and strength of interactions and ii. gene discovery studies, which are intended to screen for genes which might be of importance for future gene characterisations studies.

3.34 The Committee agreed that the available data had so far failed to show any consistent and strong interaction between genotype and chemically-induced cancer.

3.35 Key conclusions reached are highlighted below. The COC statements (including a lay statement) are appended at the end of this report.

- The most appropriate study designs for gene characterisation investigations will vary according to study purpose. Many of the currently available studies are either too limited in size or relied on *post hoc* analyses to highlight selected results. It is essential that such studies should involve *a priori* hypotheses.

- The rapid development of DNA sequencing techniques means that many gene discovery studies will become available in the future.

- Before the results of genotype-environment interaction studies can be used in risk assessment it is necessary to establish whether there is a reasonable case to infer that the genotype-environment interaction is associated with a real and important increased frequency of cancer A tiered approach has been recommended.

- It was unlikely that the interactions studied to date (which mainly concerned genes responsible for the metabolism of chemicals) were of importance to public health.

- There is little value in using genetic screening to identify individuals with particular genotypes of interest for carcinogenesis induced by environmental chemicals.

- The possibility could not be excluded that important genotype-environment interactions involved in chemically induced cancers would be identified in the future.

Ongoing Reviews

Alcohol and Breast Cancer

3.36 The Committee heard a presentation by researchers from the Department of Epidemiology and Public Health, Imperial College of Science Technology and Medicine on the finalised results of a formal systematic review (meta-analysis) of the association between drinking alcohol and breast cancer. The COC also considered an important paper from the Collaborative Group on Hormonal Factors in Breast Cancer recently published in the *British Journal of Cancer* (2002, vol 87, 1234-1245) at its meeting of 22 November 2002. A number of questions have been forwarded to the authors. The Committee will further consider this topic at its March 2003 meeting.

Glossary of Terms for COT/COC/COM

3.37 A document is in preparation.

Prostate Cancer

3.38 There is evidence for an increase in the number of diagnosed cases of prostrate cancer. The COC are to consider a review of the literature on the aetiology of prostrate cancer. A review paper is in preparation for the March 2003 meeting.

Revision of COC guidelines

3.39 The COC guidelines are used by Government Department as the basis for risk assessment of chemical carcinogens. The current guidelines were published in 1991. The Committee agreed to update its guidance on approaches to risk assessment in the light of developments over the last decade.

Statements of the COC

Statement on ILSI/HESI research programme on alternative cancer models

The minimum duration of carcinogenicity studies in rats: review of two selected papers published in 2000

Statement on the investigation of interaction between genotype and chemicals in the environment on the induction of cancer

Statement on ILSI/HESI research programme on alternative cancer models

Introduction

1. The International Conference on the Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for human use (ICH) has agreed that the required bioassay data may be derived from only one species, i.e. the rat[1] This would be supported by appropriate mutagenicity and pharmacokinetic data and also information that could come from newly proposed short-term *in-vivo* test models for assessment of potential carcinogenic activity in mice (in particular heterozygous p53+/- deficient Tg.AC model, and ras H2 models). Although these recommendations apply only to human medicines, any decision could have significant implications for other categories of chemical (e.g. food additives, pesticides, industrial chemicals etc). The key public health issue is whether the proposed short term tests in transgenic mice are appropriate adjuncts to the rat carcinogenicity bioassay in the identification of chemical carcinogens.

2. The COC has as part of its remit to advise on issues relating to chemical carcinogenesis and to present recommendations for testing strategy. The Committee was asked by the Department of Health in 1998 to consider the available literature from three research groups (namely NTP/NIEHS, ILSI/HESI and CIEA*) on the proposed short-term *in-vivo* tests for assessment of carcinogenic activity in mice, and specifically, on the transgenic mice models (heterozygous p53 +/- deficient, Tg.AC model, and ras H2 model). The Committee reached the following conclusions in 1998, which were published.[2]

   i) The Committee recognises that the three transgenic models considered in this paper (p53+/- , Tg.AC, ras H2) appear to be highly sensitive to carcinogens but questions whether data from such tests would add much to the information which can be derived from a well conducted *in-vivo* evaluation of mutagenic potential.

   ii) Dose-response data from tests in transgenic animals might be useful but at present there is no way of interpreting these data and extrapolating them to humans.

   iii) The Committee considers that the further development and validation of short-term *in-vivo* models to evaluate non-genotoxic carcinogenesis and tumour promoters may be valuable. However, there is likely to be less scope for the use of the proposed short-term animal models for other categories of chemicals, such as pesticides and industrial chemicals, where the available supporting information, such as the results of metabolism studies, is likely to be more limited. Hence the development of short-term carcinogenicity tests for these chemicals needs to be considered carefully.

   iv) The Committee concludes that, in view of the lack of appropriate validation data, it would not be appropriate to use the data from short-term transgenic bioassays considered in this statement to support regulatory decisions at the present time.

* NTP National Toxicology Program U.S.A. NIEHS National Institute for Environmental Health Sciences U.S.A. ILSI International Life Sciences Institute. HESI Health and Environmental Sciences Institute U.S.A. CIEA Central Institute for Experimental Animals, Japan.

Introduction to ILSI/HESI Programme in Alternative Cancer Models

3. The International Life Sciences Institute (ILSI) and the Health and Environmental Science Institute (HESI) have co-ordinated a multinational research programme, from 1996 –2000, on the use of alternative cancer models. The research involved input from over 50 industrial, governmental (USA, Denmark, Netherlands and Japan) and academic laboratories and cost around $35m.[3]

4. The data from the project along with a number of evaluation papers from independent experts and abstracts of additional work were published as a supplement to volume 29 of Toxicologic Pathology in November 2001 pp1-351. These papers were distributed first in draft form then as a final version to Regulatory Authorities and Advisory Committees, world wide, for comment. ILSI/HESI have co-ordinated a collaborative research project using 21 chemicals, including six known human carcinogens (three genotoxins, one immunosuppressant and two hormonal carcinogens), 12 rodent specific carcinogens (presumed on the basis of epidemiology and /or mechanism of action data) and three non-carcinogens. Chemical selection was targeted predominantly at non-genotoxic carcinogens in view of the need to examine specific mechanisms of chemical carcinogenicity in the animal models under consideration. In addition appropriate data were already available on a number of genotoxic carcinogens in some of these animal models. All chemicals used were readily accessible to test laboratories and certain core data were available; i.e. 2 year bioassay data in 2 species, established toxicology database, data on human exposure and effects.

5. The research programme was overseen by a Steering Committee of scientists drawn from academia and from pharmaceutical companies. The models under consideration were: p53+/-, ras H2+/- , Tg.AC, Xpa-/- , Xpa-/-/p53+/- double knockout, neonatal mouse, and Syrian Hamster Embryo (SHE) assay.

6. The protocols used were based on existing knowledge for each model. Positive control chemicals were used to demonstrate that each testing laboratory could undertake and report a positive assay for the model under test. Participating laboratories volunteered to act as compound co-ordinators, identifying sources of supply, co-ordinating the characterisation of chemicals and analytical methods for toxicokinetic studies. They also provided advice on the evaluation of 4-week dose range funding studies. It is noted that in practice the high dose level used equated to the Maximum Tolerated Dose (MTD). Assay Working Groups (AWGs) were formed for each assay, initially to refine protocols and to make recommendations on dose levels but eventually provided considerable assistance in resolving practical issues which arose during the research programme. AWGs also acted to collate data and to as a focal point for review of data and the application of the evaluation criteria. A Pathology Subcommittee and Statistics Subcommittee of the Alternative Cancer Test Committee were established to help set consistent criteria for evaluating studies.[4] The AWG acted as peer-review for data assessment before the results of studies were entered into the ILSI Alternatives to Carcinogenicity Testing Database. The database will eventually be made publicly available. A workshop was held 1-3 November 2000 in Leesburg, Virginia, USA to review the data from the research programmes.

7. The Committee's assessment was based predominantly on pre-publication reports submitted to the June 2001 COC and a brief consideration of the published results. The Committee's comments focused on the proposal that the alternative cancer tests models under consideration could be used as replacements to a long-term carcinogenicity bioassay in the mouse. The Committee made a number of general comments on the strategy used by ILSI/HESI before considering the results of each model. The Committee agreed to consult the COM for additional advice on the conduct of the Syrian Hamster Embryo cell transformation assay.

General Comments on ILSI/HESI strategy

8. Members welcomed the opportunity to comment on the pre-publication papers and raw data from the AWGs. Members acknowledged the considerable administrative and practical problems that had confronted ILSI/HESI in co-ordinating this work. It was considered that the programme had provided a large amount of information on the evaluation of performance of these assays but the data were not sufficient to validate the use of any of the assays for regulatory testing. Members asked for a number of comments to be forwarded to the ILSI/HESI Alternative Cancer Test Committee for inclusion in the peer review process.

9. The Committee noted that one of the aims in the selection of test chemicals had been to expand the available data set to included non-genotoxic carcinogens as data were already available on a range of genotoxic carcinogens. A key aim was to examine the ability of the individual alternative cancer models to detect human carcinogens. The carcinogens selected by ILSI/HESI were considered to act by a range of mechanisms including immunosuppression, enzyme induction, cell proliferation, and receptor mediated. Members agreed the rationale proposed by the investigators but commented that the categorisation of some of the carcinogens based on mechanisms in rodents and epidemiology data was debatable. However, it was agreed that the categorisation as suggested by ILSI/HESI would be used in this statement.

10. The Committee agreed that it was important to have the results of tests for all of the 21 chemicals selected using all of the assays. Thus it was agreed that a good level of testing had been achieved with perhaps the exceptions being for some rodent carcinogens in the Tg.AC, Xpa, Xpa/P53 and neonatal mouse models. Members also considered that the inconsistent response of some positive control chemicals in some of the assays confounded the evaluation of the data. With regard to the test methods, Members agreed the rationale of using 3 dose levels and a transgenic control, but noted that there would be only a minimal reduction in animal usage if it proved necessary to also undertake additional concurrent studies with non-transgenic animals in order to provide adequate results for regulatory assessments of chemicals. Members also commented that the duration of testing required in the *Xpa* assay (39 weeks) and the duration of observation required in the neonatal mouse tests (1 year) were such that these two assays could not be called "short-term" assays.

Comments on Alternative Cancer Tests

11. The Committee then discussed the results from each of the assays included in the ILSI/HESI programme.

12. **With regard to the p53+/-** transgenic mouse model, Members confirmed their previous conclusion that there was a rationale for assuming that this model could identify genotoxic carcinogens. All 21 chemicals selected by ILSI/HESI had been tested.[5,6] The Committee agreed that there were a number of queries regarding the results of some of the tests undertaken to be resolved before definite conclusions on assay performance could be reached. Members noted that a negative result had been obtained with phenacetin whereas a positive result should have been obtained. Members considered the positive result reported for cyclosporin but noted that there was little difference between the tumourigenicity observed in P53 +/- transgenic mice compared to wild type mice. Inconsistent results had been obtained with diethylstilbestrol and oestradiol whereas positive results should have been obtained. Members commented that the inclusion of hyperplasia as a positive result was not justified and overall diethylstilbestrol had, in their view, given a negative response in this assay. Equivocal responses had been found with chloroform and DEHP whereas negative responses should have been obtained. Members noted that there were inconsistencies between laboratories with regard to the performance of *p*-cresidine as a positive control in one study (negative result obtained) and the inadequate results obtained with benzene in one study. These data suggested a possible lack of reproducibility of the assay. Members confirmed their previous conclusion that the p53+/- mouse model could identify some genotoxic carcinogens.

13. **With regard to the Tg.AC** transgenic mouse model, Members confirmed their previous conclusion that there is a mechanistic rationale which could potentially support the use of this model to identify chemical carcinogens and potentially tumour promoters. It was noted that 14 out of the 21 chemicals selected by ILSI/HESI had been tested, and that data for only 6 out of the 13 rodent specific carcinogens had been presented.[7,8] The incomplete testing with this model therefore limited the conclusions which could be reached from the ILSI/HESI project. The Tg.AC transgenic mouse model identified positive results for 5 out of the 6 human carcinogens tested (including those acting by genotoxic, immunosuppressant and hormonal mechanisms) when data for dermal and oral tests were considered together. However there were inconsistencies in the current trial such that the genotoxic carcinogens cyclophosphamide and mephalan gave equivocal results when tested dermally but positive results when tested by oral administration. Cyclosporin, diethylstilboestrol and oestradiol gave positive results in dermal tests and equivocal (cyclosporin) or negative results in oral tests. A negative result was obtained for phenacetin in both oral and dermal tests. With regard to the rodent specific carcinogens tested, the positive response with topically applied clofibrate and equivocal response with Wy-14, 643 needed further explanation. Taking all of the available data on the Tg.AC transgenic mouse model, Members agreed that further explanation of the results for glycidol (false negative) and resorcinol (false positive) were required before the utility of his model could be further considered. It was noted that the problems with non-responder phenotype reported in earlier studies with the Tg.AC transgenic mouse model had been overcome. However, Members were concerned that the rate of

spontaneous tumours was significantly higher in the ILSI/HESI sponsored studies than in previous investigations using Tg.AC mice. Members were also concerned, for animal welfare reasons, at the sensitivity of these mice to audio induced seizures but were reassured to note in practice that such reactions were very rare. Members agreed that the available data on the Tg.AC transgenic mouse model showed that there were problems in consistently identifying human carcinogens which needed to be resolved. This suggested a need for further optimisation of methods, an understanding of the mechanisms underpinning differences between dermal and oral tests with the same chemical and a greater database before the performance of the model could be evaluated.

14. **With regard to the Xpa-/- and Xpa-/- p53+/-** transgenic mice models, Members observed that selection of the Xpa gene was only of relevance to the identification of bulky genotoxic carcinogens and possibly cross linking agents. Members agreed that there was no mechanistic rationale for producing a transgenic animal model with which was deficient for Xpa and heterozygous for p53 gene other than maximising the predisposition to detection of specific categories of genotoxic carcinogen such as cross linking agents. It was noted that 13 out of the 21 chemicals selected by ILSI/HESI had been tested in the Xpa transgenic mouse model.[9,10] Negative results had been obtained with phenacetin and oestradiol in Xpa mice but this was not unexpected given the specificity of the transgenic modification used in this particular assay. The positive results obtained in the Xpa mouse for Wy-14,643 needed further explanation. An inconclusive result had been obtained for clofibrate. Members also noted that there was significant interlaboratory variation in results for the positive control chemical p-cresidine with a negative result reported for one laboratory. Fewer results were available for the Xpa/p53+/- transgenic mouse model, with results for only 10 out of the 21 ILSI/HESI selected chemicals available. Members noted that oestradiol had given a positive result in Xpa/p53+/- transgenic mice in contrast to the negative result with Xpa-/- and agreed that an explanation for the difference in results would be valuable. It was also noted that the peroxisome proliferators Clofibrate and Wy-14,643 had not been tested in Xpa-/- p53+/- which might have given some insight into the unexpected results with these two chemicals reported for Xpa-/- transgenic mouse model. Overall few conclusions could be drawn from such limited data with these two models. The Committee felt that a valid rationale for developing these two particular transgenic animal models for short-term testing of potential carcinogenicity had not been proposed.

15. **With regard to the *rasH2+/-*** transgenic mouse model, members reiterated their previous conclusion that there was uncertainty about the relevance of this model, which entailed the integration of multiple copies of the c-Ha-ras gene into the CB6F1mouse in respect of the relevance of the model to the carcinogenic process in humans. Data were available for 20 out of 21 test chemicals selected by ILSI/HESI.[11,12] The study with Wy-14,643 was ongoing at the time of publication. Members noted that the immunosuppressant cyclosporin A and the hormonal human carcinogen oestradiol were negative in this model. Members considered that further explanation of the positive results with the peroxisome proliferators clofibrate and DEHP was required. It was noted that the papers supplied by ILSI contained the postulation that overexpression of the ras transgene followed by mutation of the transgene was the probable mechanism of carcinogenicity. Overall the Committee agreed that very little weight could be attached to results from this particular transgenic animal model given the proposed mechanism of carcinogenicity.

16. **With regard to the neonatal mouse model,** Members recalled the conclusion reached in 1998 that there was no evidence to support the use of either the neonatal rat or mouse bioassays as a part of regulatory testing strategies.[13] The new information from the ILSI programme, where 13 out of the 21 selected test chemicals had been tested in neonatal mice, supported this view.[14,15] Five out of 6 human carcinogens had been tested and a positive response had only been documented for cyclophosphamide and for oestradiol (in one out of three studies). Members reiterated their animal welfare concerns about the evidence of considerable animal mortality during these experiments. Overall there was no rationale for including the neonatal mouse model in carcinogenicity testing strategies.

17. With regard to the available data from the Syrian Hamster Embryo test[16], the COC noted that full consideration of these data would be given by the COM and a separate statement published in due course.

Conclusion

18. The COC agreed an overall conclusion that none of the models used in the ILSI/HESI Alternative Cancer Test programme were suitable as a replacement for the mouse carcinogenicity bioassay (the primary purpose for the development of these models) and that further research should look to identify models with a greater relevance to mechanisms of carcinogenicity in humans. Of the animal models assessed there was evidence that p53+/- transgenic mouse model could identify some genotoxic carcinogens. There was insufficient data to suggest that the animal models under consideration (RasH2, Tg.AC, Xpa, Xpa/P53+/- and p53+/-) provide essentially similar results. (A separate statement from the COM on the ILSI/HESI evaluation of the Syrian Hamster Embryo test would be published in due course).

April 2002

COC/02/S3

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The minimum duration of carcinogenicity studies in rats: review of two selected papers published in 2000

Introduction

1. The proper conduct of carcinogenicity studies in rats is an important part of the evaluation and prediction of potential human carcinogens. Significant reductions in the number of control rats surviving to termination have been widely reported in the scientific literature.[1-5] This is a matter of concern since inadequate carcinogenicity studies could be important in decisions regarding the identification of potential human carcinogens and in particular the failure to identify such compounds. In addition there is a possibility that inadequate studies could be rejected by regulatory agencies with the consequent need for use of further animals to obtain a valid result. For a negative result from a rat carcinogenicity bioassay to be considered acceptable, survival at 24 months should be 50% or greater in all groups.[6,7]

2. The Committee reviewed the evidence for application of dietary restriction techniques in 2000 and a statement was published (COC/00/S3). It was concluded that the available information supports the view reached by the COC in its guidelines published in 1991 that dietary restriction in carcinogenicity studies should be applied with caution and is the responsibility of the toxicologist undertaking the study. The COC agreed that the subject of dietary restriction should be reviewed when more information is available.

3. Some investigators have also proposed that reducing the duration of carcinogenicity bioassays undertaken in rats would have the desired effect of terminating studies before survival was reduced to below 50% in tests groups.[8] The Committee reviewed two published papers which had evaluated published data on the duration of carcinogenicity studies. These two papers (see paras 4 and 5 below) came to contrasting views. The Committee was asked to provide generic advice on the desirability of reducing he minimum duration of carcinogenicity studies in rats.

Review of two selected investigations

Davis et al, 2000[8]

4. Davis et al studied IARC chemical Monographs (Vols 1-70) to determine the time of onset to ‘treatment-related’ tumorigenicity in long-term rodent studies for chemicals classified by the IARC as showing evidence of carcinogenicity in animals. The chemicals were categorised as producing tumours at <12m, 12-18m, or >18m. The analysis excluded studies on metals and their salts, studies on particulates, studies by parental routes of administration that resulted in tumours only at the site of exposure, and studies that did not approximate to the current standard long term rodent carcinogenicity bioassay e.g. transplacental or multigeneration studies, initiator-promoter studies, lung tumour assays in ‘Strain A’ mice and studies in new born animals. Davis et al considered that from a total of 210 chemicals, overall, evidence of treatment related tumorigenicity was first apparent within 12 months for 66% of the chemicals and that studies longer than 18 months were necessary for 7%. All IARC Group 1 chemicals

were detected in animals within 18 months and most within 12 months. Most of the tumour types that required more than 18 months for detection were considered by Davis et al to be of "dubious" relevance to human risk assessment On this basis Davis et al concluded that termination of rodent carcinogenicity studies at 18 months or earlier was justified, and would greatly reduce the complications that arise in interpreting findings in aged animals.

Kodell et al, 2000[9]

5. Data from bioassay studies in rats using selected pharmaceuticals were used to formulate biologically based dose-response models of carcinogenesis based on the 2-stage clonal expansion model. These dose response models, which were chosen to represent 6 variations of the initiation-promotion-completion cancer model were employed to generate a large number of representative bioassay data sets using Monte Carlo simulations. The six variations of the model were based on data:

Model Variation	Data on which model variation was based
initiator only	anonymous drug 1 and pancreas adenoma in females
completer only	anonymous drug 1 and mammary adenocarcinoma in females
initator + completer	anonymous drug 1 and mammary adenocarcinoma in males
initator + promoter	anonymous drug 2 and pancreas acinar cell carcinoma in males
promoter + completer	anonymous drug 3 and thyroid follicular cell adenoma in males
promoter only	selenium sulphide and liver hepatocellular carcinoma in females

For a variety of tumour dose-response trends, tumour lethality and competing risk-survival rates, the power of age-adjusted statistical tests to assess the significance of carcinogenic potential was evaluated at 18 and 21 months and compared to the power at the normal 24 month termination time. Kodell et al results showed that termination at 18 months would reduce statistical power to an unacceptable level for all 6 variations of the 2-stage clonal expansion model, with the pure-completer models being most adversely affected.

COC Discussion

6. The committee agreed that some rat strains, namely, Sprague-Dawley (in certain labs) have inadequate survival at 24 months. Members noted the argument put forward by Davis et al that the pathology associated with old age might mask important cancer pathology in animals terminated at 24 months. Davis et al had also argued that it is possible that an earlier onset of the incidence of a common spontaneous tumour type could be detected at 18 months and missed at 24 months. However, members considered that in 24-month studies, autopsy of the dead animals and analysis of tumour incidence in deceedents would pick this up.

7. The committee considered that a single study would not be looked at in isolation and that consideration of the mechanism of an effect was crucial in the overall evaluation. Members were also concerned about modifying an already imperfect lifetime model, and agreed that possible dietary methods of extending life span, such as by caloric restriction, needed to be considered on a case-by-case basis with regard to laboratory historical control data on tumour incidence.

8. The committee did not agree with the conclusions drawn by Davis et al that carcinogens detected after 18 months were unlikely to be relevant to human health assessment. Members were concerned that such shortened studies might not be sufficiently sensitive to detect some human carcinogens. The Committee agreed that the approach taken by Kodell et al to the modelling of carcinogen dose-response was satisfactory.

COC Conclusion

9. The COC concluded that there was insufficient evidence to recommend a change to the international guidelines for the conduct of long term carcinogenicity bioassays, that for a negative result to be acceptable in a rat carcinogenicity bioassay, survival should be at least 50% in all groups at 24 months. The Committee reaffirmed that it was the responsibility of the study director to use rat strains that would ensure adequate survival at 24 months.

March 2002

COC/02/S2

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Statement on the investigation of interaction between genotype and chemicals in the environment on the induction of cancer

Background to review

1. The Committee was asked by the Department of Health to review the available information on the interaction between genotype and exposure to chemicals in the environment and the induction of cancer. The Committee was asked to provide advice on the methods of epidemiological research used in this area, the approaches to identifying genes of interest for such studies and the evaluation and significance of these data for cancer risk assessment.

2. The Committee was aware of the major technological advances in rapid DNA sequencing which had been published by the Human Genome Project (HGP) (http://www.ornl.gov/hgmis/project/.html) and the Environmental Genome Project (EGP) (http://www.niehs.nih.gov/envgenom/). [See Introduction to review and glossary for explanation of abbreviations and terms used in this statement] A draft scaffold sequence for the human genome was published in February 2001 and it has been proposed by HGP that a complete high quality DNA reference sequence will be available by 2003. These projects have as their major goal, the diagnosis, prediction and intervention in diseases where there is a genetic contribution to the cause of disease. However the EGP is focused on the role of genes implicated in cellular responses to environmental chemicals. Ambitious projects have been set up by EGP, for example, to identify Single Nucleotide Polymorphisms (SNPs) for up to 30,000 genes, new statistical methods to aid in the evaluation of the interaction effects of carcinogen metabolism and bioinformatics tools to assist in the evaluation of the large amounts of data generated from epidemiological studies. These, and other developments[1-3] are likely to lead to a rapid increase in the published information on the interaction between genotype and exposure to chemicals in the induction of specific cancers. It was considered timely to examine the questions raised by the Department of Health in order to draw conclusions on what advice could be given.

3. The Committee considered that it was necessary to set out a discussion of the key terms in the text of the statement. The Committee agreed that a concise "non-technical" summary was also required which should provide a glossary of key terms. Members considered it appropriate to discuss the critical areas of the review, particularly the design of epidemiology studies for genotype-environment interactions for specific cancers, the identification of genes of interest and risk assessment, before providing advice and suggestions for further research. The Committee was provided with a set of detailed papers drafted by the DH Toxicology Unit at Imperial College of Science, Technology and Medicine for use in their discussions.[4-7] The relevant papers will be published on the COC website (www.doh..gov.uk/coc.htm).

Introduction to review

Introduction to the review

*Background to terminology**

**(see HGP and EGP internet sites (para 2 for addresses) and refs 4-7)*

4. The human genome comprises all the genetic material (i.e sequence of DNA) in the 23 pairs of chromosomes present in all somatic nucleated cells in the body. Within the genome, the gene is the fundamental physical and functional unit of heredity. A gene is an ordered sequence of DNA located in a particular position on a particular chromosome that encodes for a specific functional product(s) (i.e. a sequence of RNA which may be translated to give a protein(s) which, with any subsequent necessary posttranslational modification, gives the functional protein). One key development arising from the expansion in DNA resequencing work described in paragraph 2 of this statement has been the recognition that the human genome does vary considerably between individuals (i.e it is subject to considerable interindividual variability). Thus it is estimated that approximately 1 in every 300-500 base pairs will differ between any two individuals. Variation in the DNA sequence of a particular gene between individuals comprising a single nucleotide difference is called a Single Nucleotide Polymorphism (SNP). The variations in the genome between individual ranging from SNPs, differences in small sequences of DNA, up to whole chromosomes are collectively referred to as "genotypic variation". During its discussions the Committee was principally concerned with SNPs. These may have no impact on the function of the encoded gene products and are called "non-functional but in some cases SNPs do result in variation between individuals in the function, e.g. qualitative and/or quantitative changes in protein function. This is referred to as phenotypic variation". The phenotype of an individual is defined as the observable physical biochemical or physiological characteristics of that individual.

5. Within the genome, SNPs can be found in the coding region of a gene; i.e. functional DNA, (cSNPs), in potential regulatory sequences, i.e. peri-genic regions (pSNPs) or in intervening stretches of DNA with no apparent function (intergenic DNA; iSNPs). The term genetic polymorphism is often used to indicate phenotypic variation and as such is frequently used in association with genetically-determined variations in the metabolising capacity for chemicals. The changes in DNA sequence responsible for metabolic polymorphisms are often SNPs. It is now easier and more pragmatic to identify the genotype in large numbers of individuals by DNA sequencing rather than to elucidate phenotype (i.e. measure the expression and function of genes) and thus it is possible that fewer studies of phenotype will be undertaken. However it is the phenotypic expression of genes that is most likely to be important with regard to the interaction between a gene and an environmental chemical in the induction of specific cancers.

6. The development in DNA sequencing techniques has allowed for the rapid and easy identification of SNPs, and hence closer examination of whether there is an interaction between the occurrence of a particular SNP in an individual and chemical exposure that is associated with adverse health effects such as cancer. Many epidemiological studies have investigated associations between cancer incidence and polymorphisms of the enzymes responsible for the metabolism of chemical carcinogens since many carcinogens require metabolic activation. It is therefore logical to suggest that variation in metabolism of these chemicals will accord with changes in risk of cancer development. The Committee reviewed

several examples, e.g. *N*-acetyltransferase 2 (NAT 2) and exposure to tobacco smoke associated with bladder cancer, and glutathione-S-tranferase M1 (GSTM1) and exposure to tobacco smoke associated with lung cancer.[7] However the Committee felt it was important also to consider target genes other than those associated with the metabolism of chemicals. A discussion paper was therefore drafted on this topic.[6]

7. The term "penetrance" is used in this statement to describe the frequency with which carriers (e.g. of a particular genotype) develop cancer, i.e. the ratio of carriers who develop cancer compared to all carriers. Inherited cancer genes are considered to be "high penetrance" if affected individuals have a high probability of getting cancer. An example is the breast cancer susceptibility gene BRCA1, where the lifetime cumulative risk of cancer in individuals carrying specific mutations within this gene has been estimated as approximately 90%. However "high penetrant" genes are usually rare , i.e their prevalence in the population is low. The genotypes under consideration in this statement are considered to be of low penetrance, i.e. the increase in risk of cancer is very low. However their prevalence in the population can be very high (e.g. 40-50% of the population as with N-acetyltransferase 2 slow acetylator (NAT2) allele polymorphism and GSTM1 null polymorphism). The Committee noted that the penetrance and prevalence of genotypes that were of importance to carcinogenesis induced by chemicals could vary and thus this should be considered in strategies to identify genes for research.

8. The use of the term "interaction" has been considered in detail.[8-10] There are two ways in which this term has been used in the scientific literature: either to describe a biological model of interaction between two or more factors in the aetiology of disease or to describe the statistical concept of interaction which describes the patterns of disease risks. Thus for genotype-environment interactions, a biologically significant effect infers that there is evidence for or there is a presumed (as yet unknown) biological consequence arising from the function of a particular gene variant and exposure to chemical(s) on the risk of cancer. The degree of statistical interaction can be measured in two ways, depending on whether it is the differences (i.e additive scale) or ratios of risks (i.e multiplicative scale) that are of interest. An illustrative numerical example is given in the Annex at Table 1 based on the lifetime risks of lung cancer. Further explanation of the example is given below in paragraphs 30-31 that concern risk assessment.

Discussion of critical areas to be considered

9. Members noted the rapid increase in the number of publications on genotype-environment interactions and in particular those concerning the potential impact of metabolic polymorphisms. They were also aware of the suggestion that genetic screening could be used to identify individuals carrying a particular genotype or to identify chemicals to which individuals should avoid exposure. The Committee therefore agreed there were two questions which needed to be addressed during the review namely;

   i) The extent to which subgroups of the population can be identified, who because they have a particular genotype, are at greater risk of developing cancer, when exposed to particular chemicals.

ii) Is it appropriate or desirable to use genetic screening to identify individuals with a particular genotype of importance to chemically induced cancers.

10. Members agreed that in order to consider these two questions, it would be important to review the epidemiological methods used and to comment on the significance and potential value of the results from these studies for risk assessment. An integral part of this consideration would be to provide advice on the numbers of individuals required in such studies. This review would also provide advice on gene selection, and the formulation of hypotheses for future epidemiological investigations.

11. Members agreed that a further critical area for review involved the discussion of the nature of the interactions between genotype and exposure to chemicals resulting in an increased risk of cancer. The objective was to define criteria which could help to assess whether an interaction existed between a particular genotype and exposure to chemicals leading to an increase in the frequency of cancer that was significant for public health. This will assist in differentiating between genotype-environment interaction associated with increased risk of cancer, and those which are chance findings, and therefore not relevant to risk assessment.

12. It would then be important to define the data necessary to assess the potential impact of interactions between genotype and exposure to chemicals and if possible to estimate potential numbers of cancer cases that might be involved. It would also be important to provide advice on the prospects and desirability in regard to the suggestion for genetic screening. In this regard, the Committee was of the view that a number of critical genotype-environment interactions have yet to be discovered.

13. The Committee agreed that any conclusions should be prefaced with a discussion of the uncertainties in the assessment.

The Assessment of Genotype-Environment interaction studies[4,5]

14. The recognition that many cancer susceptibility genes are likely to be of low penetrance has led to the evolution of two major study designs for the assessment of gene-environment interactions.

    i) epidemiological studies of candidate susceptibility genes (gene characterisation studies) and,

    ii genetic association studies (gene discovery studies).

15. In the first, the influence of known polymorphisms (or SNPs) on cancer risk is determined, usually in case-control or cohort studies, whilst in the second, cases and controls are genetically screened in an attempt to identify a clear difference in one or more gene loci. Most studies of the interaction between genotype and exposure to chemicals to date have involved the first design, but the increasing availability of dense SNP (single nucleotide polymorphism) maps and the technology to perform large numbers of genotyping tests is making the second design much more feasible.

Gene characterisation

16. Gene characterisation studies should involve the *a priori* selection of candidate genes to be included in the study protocol before the investigation is initiated. A number of different study designs had been used including case-control (with a variety of methods for choosing controls), cohort and case-only. Members noted that the use of case-only designs was relatively recent and could provide an estimate of the strength of interaction between genotype and exposure to chemicals but such studies assumed independence between the effects of genotype and exposure to chemicals. Members felt that overall many of the available genotype-environment interaction studies suffered from flaws in design and/or interpretation, reducing their potential value in cancer risk assessment. The Committee considered that apart from the limitations often found in epidemiological studies such as measurement error, bias and confounding, a key concern for many published studies was the absence of clearly stating the *a priori* hypotheses to be tested before undertaking the epidemiological investigation. The reliance of many research groups on *post hoc* analyses of sub-groups after data had been generated could yield biased statistical analysis of the multiple comparisons common in such studies. Members considered that the *a priori* hypotheses under investigation should be clearly stated in publications, perhaps even lodged with a third party before the analysis. The Committee reviewed study designs used for case-control gene characterisation studies and agreed that good study design would require careful selection of cases and controls from the same population, adequate exposure assessment, appropriate analysis strategy, and power calculations of necessary study size (given assumptions on penetrance, relative risk of disease and prevalence of susceptible genotype).

17. The Committee reviewed some model calculations for a case-control study design based on published approaches to the consideration of study size. The calculations assumed that genotype and chemical exposure had independent effects on cancer risk, there was no matching of cases and controls, and a mulitplicative interaction was of interest, a baseline cancer rate of 0.001 and the odds ratio (OR) for cancer in non-susceptible subjects from exposure was 1.5.

18. These calculations provide evidence to show that many of the currently published case-control studies are of insufficient size to identify moderate interactions between genotype and exposure to chemicals in the induction of cancer.

Table 1. Number of subjects required in case-control studies

Proportion of susceptible genotype in population	Strength of interaction to be detected	Number of subjects (equal number of cases and controls)
0.5 (50%)	2x	2,215
	5x	485
0.2	2x	3,891
	5x	1,017
0.05	2x	13,902
	5x	3,949

Two-tailed test of null hypothesis, $P < 0.05$; power, 0.8. (Calculations were performed using the “Power” program described by García-Closas and Lubin (1999), American Journal of Epidemiology, **131**, 552-566.

Gene Discovery

19. Until recently, gene discovery designs have not been used widely in genotype-environment studies of cancer. This is because of the impracticalities involved in screening the very large numbers of subjects and alleles that would be necessary for the detection of genes (i.e. sequence variants such as SNPs) of low penetrance. However, as indicated above, rapid advances in both knowledge and technology are making such study designs more feasible, and several groups have commenced or are about to commence such studies.

20. The Committee considered that there were at least four broad categories of gene for which it was reasonable to hypothesise that genotype-environment interactions might be of importance with regard to cancer.[6]

Table 2 Categories of gene associated with Genotype/Environment interactions

Category of gene	Examples
Increased metabolic activation and/or reduced detoxication, elimination.	Cytochrome P450 isozymes (e.g CYP1A1 and CYP2E1). Glutathione S-transferases (e.g GSTM1 and GSTT1). N-acetyltransferases (e.g NAT1 and NAT2). P-glycoprotein transporters.
Reduced capacity for DNA repair.	Very few studies to date have examined DNA repair capacity, suggestions include base and nucleotide excision repair genes.
Immune surveillance.	No data available in respect of immune surveillance, suggestions include human leukocyte antigen complex (HLA).
Increased potential for cell proliferation and survival resulting from alterations in control of cell cycle and apoptosis.	No data available. Suggestions for cell cycle control include cyclinD1 and HRAS1 and for apoptosis Bcl-2.

21. Members were aware that there was a large number of publications which had reported investigations of genetic polymorphisms of enzymes of metabolic activation and detoxication (e.g. cytochrome P450 dependent monooxygenases, glutathione-S-transferases and *N*-acetyltransferases) and some studies had included investigations of the combined effect of two or more metabolic polymorphisms for these enzymes. Comparatively few studies had investigated variants of the other categories of genes identified by the Committee, and the extent to which these might interact with environmental chemicals was unknown.

22. The Committee considered that it was difficult to know how to prioritise the search for gene variants with increased risks for environmentally induced cancers as this could plausibly involve many thousands of such variants. However, members believed that the benefits of improving and developing technology could result in this exercise being practical and useful in the future. Members noted that the Environmental Genome Project had identified similar categories of genes for inclusion in the first phase of its project on gene discovery.

23. Members agreed that as understanding of the pathways and genes involved in the biological processes critical to cancer development increases, the number of candidate genes within those pathways that may be relevant to study for interaction with environment would increase rapidly.

Criteria for assessing interactions between genotype and environment in the aetiology of cancer[7]

24. The Bradford-Hill criteria for causality[11] have been used in the past to investigate single risk factors (environmental or genetic) by both this Committee and the WHO International Agency for Research on Cancer (IARC) reviewed studies of selected metabolic polymorphisms and susceptibility to cancer. Whilst there was no formal attempt to establish causality, the conclusions reached were based on the Bradford-Hill criteria.[12]

25. In contrast to investigations of single factors, the Committee agreed that, consideration of genotype-environment interactions referred to the assessment of whether the occurrence of a particular genotype and exposure to chemicals was associated with an increased frequency of cancer that was of significance for public health . The Committee agreed that an assessment of genotype-environment interactions should ideally require information on the gene variants(s) under consideration, the mechanism of carcinogenicity of the chemical under consideration and evidence to link all of this information together to form a reasoned case.

26. Members acknowledged, however, that it was likely that future investigations would examine the potential role of several hundreds or thousands of genes simultaneously and felt that, for the assessment of genotype-environment interaction studies, initial emphasis would be placed on the strength and consistency of the association. This would require demonstration of consistency in both gene discovery and characterisation studies and preferably by several different methods in adequately conducted gene characterisation studies. There would also need to be a plausible rationale for the mechanism of carcinogenicity for the chemical under consideration. This assessment should ideally include information on phenotype, but it is recognised that such information may not always be available.

27. Members also highlighted the potential problem of random co-inheritance (i.e. linkage disequilibrium), where alleles of one gene (associated with increased risk) are inherited with specific alleles of adjacent genes (unrelated to risk) giving the false impression that these latter genes were also causally associated with increased risk. Therefore, in the absence of knowledge of which genes are co-inherited, it would be important to have some understanding of the mechanism of carcinogenesis of an environmental chemical before any final conclusions could be reached.

28. Thus the Committee agreed that a tiered approach to the assessment of genotype-environment interactions was required as outlined in paras 24-27 It would only be possible to undertake a quantitative risk assessment if there was compelling evidence that a true interaction existed.

Risk Assessment[7]

Significance of genotype-environment interactions for public health

29. The Committee agreed that a full assessment of the significance of genotype-environment interactions with regard to chemically induced cancer required considerable information to be available. Thus ideally data on the prevalence of chemical exposure, prevalence of susceptible genotype and the cancer incidence rate in those exposed with and without the genotype and in those non-exposed with and without the genotype of interest. However, some useful measures of the size of an interaction and its impact can be estimated if relative risks (or odds ratios) are available instead of incidence rates (See Annex). In many instances such data would not be available and thus any evaluation would be based on incomplete data.

30. The Committee reviewed a worked example where appropriate data were available, namely lung cancer, smoking and GSTM1 polymorphism. The rationale for choosing this example was that the particular cancer is common, there is good agreement regarding the exposed attributable fraction for lung cancer associated with smoking (cf 90%) and the polymorphism chosen was common (i.e. 50% of population).

31. The results of the model calculations are given in Tables 1 and 2 in the Annex at the end of this statement. Any measure of *population impact* needs to take into account the prevalence of both the high-risk genotype and the environmental exposure, as well as the risks of disease in each exposure combination. One approach would be to simply use this information to work out the numbers of cancer cases who would be predicted to occur in each exposure subgroup (as outline in para 29, exposed with and without the genotype and non-exposed with and without the genotype of interest) and hence the population impact. Another approach would be to use the *population attributable fraction* (PAF). For a single risk factor this is usually considered to be the fraction for exposure to a single factor of disease in a population that might be avoided if the exposure had not occurred (or by eliminating that exposure). The model calculation estimates the PAF for all potential exposure subgroups. It is also possible to calculate the *exposed attributable fraction* of disease which provides information on the fraction in the exposed subgroups which might be avoided by eliminating exposure. The results shown in the Annex Table 1 suggest that for the example of GSTM1 polymorphism and lung cancer there is a slight benefit to the population impact (in reduction of numbers of individuals with lung cancer) in targeting smokers with GSTM1 but only if effective intervention is feasible. This has important implications when reviewing the practicalities of screening (see para 34 below).

32. Members were aware that to date most studies had investigated the interaction between metabolic polymorphism (i.e variation in the metabolising capacity for chemicals) and cancer.[11] The majority of studies (using either case-control or cohort methods) report modest increases in relative risk in exposed individuals with the susceptibility genotype. Without information on the factors outlined in paragraph 29 above, it would be difficult to derive conclusions on the significance for public health of the genotype-exposure interaction. The Committee concluded that the available data on metabolic polymorphisms had failed to demonstrate any consistent strong association between any one

gene-environment interaction and cancer risk and therefore the interactions studied to date were likely to be of little importance for public health or risk assessment.[11] However, this did not exclude the possibility that genotype-environment interactions with a significant impact on cancer risk would be identified in the future.

33. The existence of an association between a genotype and chemical exposure in the induction of cancer (e.g. a phenotype that results in enhanced metabolic activation of the chemical) could provide supporting epidemiological evidence in the identification of human carcinogens. It would be important to demonstrate a plausible biological association between the mechanism of carcinogenesis and the genotype/phenotype measured.

Significance for genetic screening

34. A final measure of impact which can be derived is the Number Needed to Screen.[13] This combines together the prevalence of genotypes, the risks of cancer in each subgroup and the reduction in risks which could be achieved by screening identified individuals where effective intervention was possible. The Committee noted that the data for the example used in model calculations suggested that there was little value in screening for GSTM1 polymorphism. The Committee reaffirmed its view that when the environmental exposure is smoking, the only appropriate public health intervention was to aid all smokers in giving up smoking. The Committee reviewed some further published calculations which confirmed that it was impractical to screen for these low-penetrant genotypes in the general population.[13] The Committee noted that, at present, there is little value for risk assessment in screening for the genotypes identified to date in gene-environment interaction studies. This is because the number of individuals with the genotype of interest who would develop cancer would be small, whereas there would be large numbers of individuals with the genotype of interest who would not develop cancer. Members were also concerned that screening for such low penetrant genotypes was undesirable in that the information would not have any significant predictivity of individual risk of cancer. In addition, other risk factors for cancer such as diet and smoking were likely to be of much greater importance in determining individual risk. The Committee also noted that there were considerable ethical, legal and social issues to be considered with regard to any proposal for screening which were beyond the scope of this review.[14]

Discussion and conclusions

35. The Committee noted that there were considerable practical difficulties in assessing the significance for public health of the currently available genotype-environment interaction epidemiology studies of cancer. These related to the size and design of the investigations and the absence of clearly set out *a priori* hypotheses as an essential part of study design. Many of the studies published also had limited power to detect genotype-environment interactions. The Committee was aware of the rapid advances in DNA re-sequencing in the last few years which meant that many potential candidate genes and genotypes/SNPs for investigation in genotype-environment cancer studies were being identified and many more would be forthcoming. The Committee noted that several projects had been set up under the U.S Environmental Genome Project and other initiatives to address these issues.

36. The Committee agreed that there was a need to assess all the available information and to consider if there was compelling evidence that a true genotype-environment interaction existed before using the information in quantitative risk assessment. The Committee agreed that an assessment regarding a genotype-environment interaction should ideally require information on the gene variants(s) under consideration, the mechanism of carcinogenicity of the chemical under consideration and evidence to link all of this information together to form a reasoned case. It was necessary to consider the possibility of linkage disequilibrium. With regard to the assessment of genotype-environment interactions, initial weight should be placed on the strength and consistency of the association. There was also a need to provide a reasoned case linking the mechanism of carcinogenicity of the chemical with the genotype under consideration. This would involve some knowledge of the function of the gene in question. Members agreed it would be valuable to have full information on the phenotype including characterisation of the function of the gene product and information on chemical-phenotype interaction but acknowledged this might be a lengthy process. An interim assessment could be drawn on basic information on gene function.

37. The Committee concluded that the available data on metabolic polymorphisms had failed to demonstrate any consistent strong association between any one gene-environment interaction and cancer risk and therefore the interactions studied to date were likely to be of little importance for public health or risk assessment. However, this did not exclude the possibility that genotype-environment interactions with a significant impact on cancer risk would be identified in the future. The Committee discussed the likely scenarios under which genotype-environment interactions might be of significance for public health and also commented on the feasibility and desirability for genetic screening for low penetrance gene variants in genotype-environment interactions. It was acknowledged that future gene discovery studies might identify genotype-environment interactions involving gene variants of significant penetrance and prevalence for cancer and thus the literature on this subject should be kept under review.

38. The Committee agreed the following overall conclusions.

    i) The most appropriate study designs for gene characterisation investigations will vary according to study purpose. Many of the currently available studies are either too limited in size or relied on *post hoc* analyses to highlight selected results. Ideally, studies should include information on phenotypic variation, but it is unlikely that such data would be available for all candidate genes selected for investigation. It is essential that such studies should involve *a priori* hypotheses. There is an argument that such hypotheses should be lodged with a third party before epidemiological investigations are undertaken.

    ii) The rapid development of DNA sequencing techniques means that many gene discovery studies will become available in the future. There is currently no clear rationale for gene selection for gene discovery studies, other than to state broad categories of genes that could be prioritised for consideration (such as metabolic activation, DNA repair and immune surveillance, cell proliferation and cell cycle control).

iii) Before the results of genotype-environment interaction studies can be used in risk assessment (either for the identification of susceptible populations or identification of human carcinogens), it is necessary to establish whether there is a reasonable case to infer that the genotype-environment interaction is associated with a real and important increased frequency of cancer A tiered approach has been recommended. Initially the strength and consistency of evidence from the epidemiological studies should be considered. In addition information to establish if there is a credible link between the mechanism of carcinogenicity for the chemical and the function of the gene and genotype under investigation should be considered. This assessment should ideally include information on phenotype, but it is recognised that such information may not always be available.

iv) The Committee concluded that the available data on metabolic polymorphisms had failed to demonstrate any consistent strong association between any one gene-environment interaction and cancer risk and therefore the interactions studied to date were likely to be of little importance for public health or risk assessment.

v) There is little value in using genetic screening to identify individuals with particular genotypes of interest for carcinogenesis induced by environmental chemicals.

vi) The possibility cannot be excluded that genotype-environment interactions involving gene variants of significant penetrance and prevalence might be identified through gene discovery investigations in the future or that combinations of genotypes might result in significantly greater interaction with chemicals in the induction of cancer.

vii) The Committee recommended that it was important to keep this subject under review particularly in the light of expected developments arising from the Environmental Genome Project based in the U.S.A. and other initiatives in this area.

June 2002

COC/02/S4

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ANNEX

Calculating and interpreting genotype-environment interaction: an example using lung cancer, smoking and GSTM1 polymorphisms

The statistical definition of a genotype-environment interaction is that the effect of genotype on disease risk varies with the level of exposure to an environmental factor, or vice versa. The degree of statistical interaction can be measured in two ways, depending on whether it is the *differences* or *ratios* of risks that are of interest. For simplicity, it is assumed that the variables measuring disease, exposure and genotype are all dichotomous. An illustrative numerical example is given in Table 1 based on the lifetime risks of lung cancer. The environmental factor is cigarette smoking (+ = Yes — = No), and the genotype of interest is the GSTM1 polymorphism (+ = null — = wild). The lifetime risk of lung cancer in non-smokers with the low-risk genotype was assumed to be 1.2%, and the lung cancer risks in the other subgroups were plausible estimates from the literature.

Table 1 Lung cancer risks in each subgroup

Environmental factor (Smoking)	Genetic factor (GSTM1)	Lifetime lung cancer risk (%)	Relative risk
+	+	R++ 16.0	RR++ 16/1.2 = 13.33
+	-	R+- 12.0	RR+- 12/1.2 = 10
-	+	R-+ 1.6	RR-+ 1.6/1.2 = 1.33
-	-	R-- 1.2	Reference subgroup
Whole population		4.55	

Measures of genotype-environment interaction

If *differences* in lung cancer risk are of interest, the *Additive* measure of interaction contrasts the difference between the risks of those with the high and low risk genotype who are exposed to the environmental factor $(R_{++} - R_{+-})$, to the same difference for those unexposed to the environmental factor $(R_{-+} - R_{--})$.

i.e. There is no Additive interaction if $(R_{++} - R_{+-}) = (R_{-+} - R_{--})$
or equivalently, in terms of relative risks, if $(RR_{++} - RR_{+-}) = (RR_{-+} - 1)$

A measure of *Additive* interaction is therefore $(RR_{++} - RR_{+-} - RR_{-+-} + 1)$ and a value of 0 denotes no additive interaction[1].

In Table 1 $(RR_{++} - RR_{+-}) - (RR_{-+} - 1) = (13.33 - 10 - 1.33 + 1) = 3.0$, so there is some interaction on an additive scale, since there is a larger difference between the cancer risks for the null and wild genotypes for smokers than for non-smokers.

If the *ratios* of the risks are of interest, the *Multiplicative* measure of interaction contrasts the ratio of the risks between those with the high and low risk genotype who are exposed to the environmental factor $(R_{++} \div RR_{+-})$, to the same ratio for those unexposed to the environmental factor $(R_{-+} \div R_{--})$.

i.e. there is no Multiplicative interaction if $(R_{++} \div R_{+-}) = (R_{-+} \div R_{--})$
or equivalently in terms of relative risks $RR_{++} = (RR_{+-} \times RR_{-+})$

A measure of *Multiplicative* interaction is therefore $RR_{++} \div (RR_{+-} \times RR_{-+})$, and a value of 1 denotes no multiplicative interaction[1].

In Table 1 $RR_{++} \div (RR_{+-} \times RR_{-+}) = 13.33 \div (10 \times 1.33) = 1$, so there is no interaction on a multiplicative scale, since the cancer risk increases by the same ratio between the null and wild genotypes in smokers and non-smokers.

NB If the data come from case-control studies, then the absolute disease risks will not be available. However, relative risks (RR) can be estimated by odds ratios (OR) and the measures of genotype-environment interaction above can be estimated using the appropriate OR.

Population impact of genotype-environment interaction

These measures of genotype-environment interaction give an idea of the size and type of any interaction between two factors, but don't permit an assessment of the impact of the interaction on the whole population or selected subgroups. A variety of such measures are described below. Any measure of population impact needs to take into account the prevalence of both the high-risk genotype and the environmental exposure, as well as the risks of disease in each genotype-exposure combination. In this example, the prevalence of smoking was taken to be 25%, while that of the null genotype was 50%, and they were assumed to occur independently.

Table 2 Measures of population impact of genotype-environment interaction

Environmental factor (Smoking)	Genetic factor (GSTM1)	Exposure prevalence (%)	Cases in population of 50 million (000s)	Population attributable fraction (%)	Exposed attributable fraction (%)
+	+	P++ 12.5	1000	40.7	92.5
+	-	P+- 12.5	750	29.7	90.0
-	+	P-+ 37.5	300	3.3	25.0
-	-	P-- 37.5	225	–	–
Total population		100	2275		

One approach would be to assess the population impact simply by calculating the numbers of lung cancer cases that would be predicted to occur in each subgroup: multiplying together the absolute cancer risks in each genotype-exposure subgroup by the appropriate prevalence. Table 2 shows the predicted numbers of subjects in a population of 50 million, who would get lung cancer at some point in their lifetime, for each genotype-exposure subgroup. It can be seen that of the 2.275 million cases expected in the whole population, the highest numbers of cases occur in the GSTM1-null & smokers subgroup, followed by the GSTM1-wild &smokers subgroup. The lack of multiplicative interaction between GSTM1 and smoking has increased the risk of lung cancer for the GSTM1 -null genotype by 33.3% in both smokers and non-smokers. However, the addition interaction means that given the 10-fold extra risk for smokers, the GSTM1-null genotype has had a more noticeable impact on the expected number of lung cancer cases among smokers than non-smokers: an extra 75 thousand cases amongst non-smokers against an extra 250 thousand cases amongst smokers. Note that this approach requires knowledge of the absolute risks, rather than just relative risks.

Another approach would be to use the *population* attributable fraction. The population attributable fraction (PAF) for exposure to a single factor is often interpreted as the fraction of disease in a population that might have been avoided if the exposure had not taken place (or, making some strong assumptions, if the exposure could be eliminated). For a single factor it can be calculated as

(Risk in whole population – Risk in unexposed subgroup)(Risk in whole population

If smoking is the exposure of interest, the lung cancer risk in non-smokers is the average of that for non-smokers with both genotypes (since they have equal prevalence). So, using the information in Table 1, the population attributable fraction for smoking is (4.55 – 1.4) ÷4.55 = 0.692 (69.2%). So 69.2% of the cases of lung cancer could have been avoided if cigarette smoking had not occurred.

The PAF is often used in a public health context to help decide which exposures to target. If it can be assumed that a number of different exposures all cause lung cancer, and there is an intervention to prevent their effect (e.g. eliminating an exposure or prophylactic treatment) then efforts may be directed towards whichever of exposures have the largest PAF. However, this approach is less useful if the intervention is not fully effective.

The PAF can be extended when there is more than one exposure category. This could be ordered categories of the same exposure factor (e.g. None, Low, Medium & High) or, as in our example, a combination of two factors (e.g. Unexposed & Low-risk-genotype, Exposed only, High-Risk-Genotype only, and High-Risk-Genotype & Exposed). The aim is to measure the effect on the population if the exposure-genotype combination in one subgroup had not occurred, using the doubly unexposed group (i.e. unexposed and low-risk genotype) as a reference group.

Population attributable fraction (PAF) = $P_i \times (RR_i - 1) \div (1 + \Sigma P_i \times (RR_i - 1))$ [2]
– the subscript i refers to each of the three exposure combinations (++, +- & -+)

Using the information in Tables 1&2, for ++ subgroup
PAF = 0.125x (13.33 –1) ÷(1 + 0.125x(13.33 –1) + 0.125x(10-1)+0.375x(1.33-1))
= 0.407 (40.7%)

Values of PAF for the other subgroups are given in Table 2. This shows that the largest population impact comes from the Exposed & High-Risk-genotype subgroup. So, if intervention were feasible, there would be more benefit to the population as a whole in targeting the smokers & GSTM1-null subgroup.

Rather than look at the effect on the whole population, another approach is to use the *exposed* attributable fraction (EAF). The exposed attributable fraction for a single factor is the fraction of disease amongst the exposed subgroup that might have been avoided if that exposure had not occurred (or its effects could be eliminated). PAF is used more widely, but the EAF is included here to show the distinction between them. For a single factor it can be calculated as

(Risk in exposed subgroup – Risk in unexposed subgroup)÷Risk in exposed subgroup

So, using the example in Table 1, the exposed attributable fraction for the single factor smoking (irrespective of genotype) is (14 – 1.4) ÷14 = 0.9 (90%). This approach can be also be used when there is more than one exposure category: in our example the Non-smoker & GSTM1-wild subgroup is used as the 'unexposed' subgroup. The results are shown in Table 2. The subgroup with the largest population impact with this measure is still the Smoker & GSTM1-null genotype, but using this measure,the impact is only slightly greater than that of the Smoker & GSTM1-wild genotype subgroup.

All three measures of population impact indicate that the Unexposed & High-risk-genotype subgroup has the largest impact (to a greater or lesser extent) in the numerical example. However, there are very real practical difficulties in acting on this. Firstly, to identify this subgroup, the population members with the high-risk genotype would have to be identified by genetic screening and their environmental exposure determined. Secondly, having identified this subgroup, it may be difficult or impossible to reduce their cancer risks down to those experienced by the Unexposed & Low-risk genotype subgroup. It may not be possible to remove the environmental exposure from those so identified (e.g. smoking cessation programs are only partially successful, and then annual risks of lung cancer in ex-smokers take some years to be reduced to those of never-smokers). The example used also has the feature that the high-risk genotype increases lung cancer risk on its own. Even if the effects of the environmental exposure were eliminated, that would still only reduce the risk to that of the Unexposed &High-Risk-genotype subgroup: there may or may not be interventions that could reduce their risk further (e.g. prophylactic treatment). Unless interventions to reduce cancer risk in a genetic subgroup exist, there is no point in genetic screening.

If an effective intervention is possible, then a final, more recent, measure of population impact is the Number Needed to Screen[3]. This combines together the prevalence of the genotypes, the risks of cancer in each subgroup and the reduction in risks which could be achieved if screening identified individuals where intervention was needed. To provide comparability with the other measures discussed above, assume that lung cancer risks in smokers could be reduced to those of non-smokers by some treatment or intervention (e.g. via smoking cessation program and/or chemoprevention). If we consider smokers with the *high*-risk genotype, they have a lifetime lung cancer risk of 16% that could be reduced to 1.6% if they were identified. This gives a number needed to treat (NNT) of 1/(0.16 – 0.016) = 6.9. However only 50% have the high-risk genotype, so we have to screen 6.9/0.5 = 13.8 to prevent one case – so the Number Needed to Screen (NNS) is 13.8. A similar argument applies to the smokers with *low*-risk genotype giving a NNS= 18.5. With these assumptions there is little benefit to screening, since there is little difference between NNS for the two genotypes. However, a more reasonable assumption might be that lifetime lung cancer risk in smokers could only be reduced to a fraction of current levels, rather than down to the level experienced by non-smokers. A recent paper [3] used the NNS approach when assuming it was possible to reduce lifetime lung cancer risks in smokers by 50% (rather than to the level of non-smokers), and also concluded that there was little advantage to screening for GSTM1.

In the numerical example used here, there is limited benefit in screening for the high-risk genotype for any measure of population impact. However, since smoking is a lifestyle choice that considerably increases the risk of lung cancer (and many other diseases), it is extremely unlikely in practice that genetic screening would be considered: the obvious approach would be target all smokers to reduce their smoking. Screening is more likely to be considered if the exposure is involuntary (e.g. exposure to an industrial chemical or family history of cancer). Even then, there are ethical, legal and social issues to be considered.[4] It should be noted that all these calculations are sensitive to changes in any of the estimates of risks, prevalence and interaction. Given the sample size requirements in genotype-environment investigations, it is rare to have precise estimates of all of these.

None of these measures of interaction or population impact can be interpreted in isolation. A recent paper has suggested a tabular layout that includes many of the measures discussed previously[5]. It would be helpful if there were consistent reporting of all the measures needed to interpret genotype environment interaction in future studies: it remains to be seen if this will happen.

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Lay summary

Statement on the interaction between genotype and chemicals in the environment on the induction of cancer in risk assessment

Introduction and Background Information

1. The Committee was asked by the Department of Health to review the available information on the interaction between genotype and exposure to chemicals in the environment and the induction of cancer. The Committee was asked to provide advice on the methods of epidemiological research used in this area and the approaches to identifying genes of interest for such studies. Of particular importance is the evaluation and significance of data from relevant studies in cancer risk assessment.

2. A short summary of the conclusions is given on pages 2 and 3 of this statement. A brief overview of relevant information which will help in understanding the reasons for undertaking this review and conclusions reached is given below and some additional information on the term used is given in the glossary apended to this statement.

   - Many diseases (such as cancer) are thought to be due to a combination of heredity and other factors in the environment (such as lifestyle, diet and to a lesser extent exposure to chemicals in the environment). The DNA sequence of an individual (his or her genotype) may be one factor which contributes to whether a person who is exposed to chemical carcinogens (e.g. from tobacco smoke) may develop cancer. (Most chemical carcinogens exert their effects after prolonged exposure, e.g. over several decades)

   - The information coming from the Human Genome Project (http://www.ornl.gov/hgmis/project/.html) and the Environmental Genome Project http://www.niehs.nih.gov/envgenom/ is helping scientists to gain an understanding of the differences between people in their DNA sequences (genes) and thus more information about possible chances of getting diseases (such as cancer). These projects are showing that there are a great many small differences between individuals in their DNA sequences.

   - There is a lot of knowledge available on how carcinogens can cause cancer (for example how chemicals can be metabolised in the body to form carcinogenic chemicals, see glossary for more information) . It is therefore possible to identify differences in DNA sequences between individuals (for example in genes controlling the metabolism of chemicals) which might affect susceptibility to cancer.

   - It is already known that a few genes (such as the breast cancer susceptibility gene BraC1) have a very strong association (link) with the occurrence of cancer; in this case breast cancer. However such genes are very rare. It is much more likely that a gene will increase the tendency to develop cancer in a weaker fashion, with a low proportion of carriers actually getting cancer. This review is about whether we can identify any combinations of exposure to chemicals and occurrence of a particular DNA sequence (genotype) that is associated with a higher risk of cancer compared to individuals who may be exposed to the same chemical but do not have the same DNA sequence. [The types of gene concerned (such as those which metabolise chemicals) have, on the available evidence, little or no direct association with cancer.]

Conclusions of Review

3. The Committee reviewed the methods used to investigate possible interactions between genotype, exposure to chemicals and occurrence of cancer. The types of study, which all involved investigating genotype and exposures to chemicals in humans, could be separated into two types. Gene characterisation studies which aim to investigate the nature and strength of interactions. Gene Discovery studies which are intended to screen for genes which might be of importance for future gene characterisations studies.

   - There are problems in using the results of many of the available studies because these have investigated too few individuals to allow legitimate conclusions to be made. Scientists/ epidemiologists conducting such studies may not have formulated clear reasons for doing the research before conducting the work. These studies often produce a large amount of information and it is possible that some of the associations reported (between genotype and chemical induced cancer) arose by coincidence simply because of the large number of analyses undertaken and could be considered as "chance findings". The Committee felt that the possibility of chance findings was highly likely in the future because such studies would provide information on many hundreds of genes at a time. The Committee felt that investigators should be asked to lodge the reasons for undertaking the research with a third party before the investigations were undertaken so that the possibility of the information being used for purposes that it was never intended could be avoided. This might help to clarify which genes were of most importance in each study.

   - The Committee agreed that the most appropriate way to assess the results from many of studies investigating the possible interaction between a particular genotype and exposure to chemicals on the occurrence of cancer should involve the following information. In many cases a provisional assessment would have to be made without full information on number (iii)

     i) Clear information on the mechanisms of carcinogenicity of the chemical under consideration

     ii) An assessment of the strength of the interaction and consistency of the information from epidemiological studies of genotype-environment interaction.

     iii) An assessment of the information on the function of the particular gene under consideration.

   - The Committee concluded that an assessment of likely numbers of individuals with a particular genotype at risk of developing cancer following exposure to chemicals needed a lot of information before such calculations could be undertaken. This included information on the type and extent of chemical exposure, the numbers of people with genotype in the whole population and in exposed individuals. It would also be necessary to know the incidence of cancer in exposed and non exposed individuals either with or without the particular genotype. It would therefore only be possible to undertake such an assessment in a very few cases given the information currently available.

- The Committee agreed that the available data had so far failed to show any consistent and strong interaction between genotype and chemical induced cancer. It was unlikely that the interactions studied to date (which mainly concerned genes responsible for the metabolism of chemicals) were of importance to public health.

- There is little value in using genetic screening to identify individuals with particular genotypes of interest for carcinogenesis induced by environmental.

- However the possibility could not be excluded that important genotype-environment interactions involved in chemically induced cancers would be identified in the future.

- The Committee recommended that it was important to keep this subject under review particularly in the light of expected developments from the Environmental Genome Project based in the U.S.A and other initiatives in this area.

June 2002

Glossary of important terms

Association: The finding that the occurrence of disease and a factor (such as exposure to a chemical) is greater than expected by chance.

Consistency: The association has been consistently identified in studies using different approaches and by different research groups and in different populations.

Cancer: A malignant neoplasm (commonly called a tumour) that grows progressively, invades local tissues and spreads to distant sites.

DNA sequence: The carrier of genetic information for all living organisms except some viruses. Most cells in humans contain 46 chromosomes, each consisting of two strands of DNA which make up genes (see definition given below). Each DNA strand consists of two interwound chains of linked nucleotides. The nucleotides are the chemical building blocks of genes.

Environmental Genome Project: (EGP) was initiated by the US National Institute of Environmental Health Sciences (NIEHS) in 1998.The mission of the EGP is to improve understanding of human genetic susceptibility to environmental exposures. The EGP supports the mission of NIEHS, which includes the goal of understanding how individuals differ in their susceptibility to environmental agents and how these susceptibilities change over time. The EGP has a well developed internet site from where it is possible to obtain a lot of information on the subject of genotype-environment interactions (http://www.niehs.nih.gov/envgenom/home.htm)

Gene: The functional unit of inheritance: a specific sequence along the DNA, which codes for a product which a specific function in the cell.

Gene characterisation Studies: Epidemiology studies designed to give information on the nature and strength of interaction between genotype and exposure to chemicals in induction of cancer. There are many different designs which can basically involve either investigation of cases (i.e. individuals who have got cancer) to examine if exposure to chemicals and genotype were risk factors or investigation of large groups of individuals where disease status (i.e. cancer) is unknown. In this instance it may be possible to follow a group of individuals to see who gets cancer or to use records to retrospectively assess the occurrence of cancer within a group. All of these approaches need good information on genotype status of individuals and information on exposures to chemicals.

Gene Discovery Studies: Studies designed to screen many hundreds (possibly thousands) of genes and particular gene variants to see if there is a potential association with a disease such as cancer. Such studies are becoming feasible due to rapidly advancing methods for sequencing DNA.

Genotype: The particular DNA sequence seen in an individual.

Genotype Environment Interaction: A biologically relevant effect of two or more factors contributing to the risk (likelihood) of getting a disease (e.g the effect of a particular genotype and exposure to chemicals in the induction of cancer). The degree of interaction can be measured to examine whether the risk of disease is the sum of the risks associated with individual factors or whether the risk is greater than the sum (e.g a mulitplication of the risks of disease associated with two or more factors).

Human Genome Project (US): Begun in 1990, the U.S. Human Genome Project is a 13-year effort coordinated by the US Department of Energy and the US National Institutes of Health. The project originally was planned to last 15 years, but effective resource and technological advances have accelerated the expected completion date to 2003. Visit http://www.hgmp.mrc.ac.uk/About/ Project goals are to

- *identify* all the approximately 30,000 genes in human DNA,
- *determine* the sequences of the 3 billion chemical base pairs that make up human DNA,
- *store* this information in databases,
- *improve* tools for data analysis,
- *transfer* related technologies to the private sector, and
- *address* the ethical, legal, and social issues (ELSI) that may arise from the project.

Several types of genome maps have already been completed, and a working draft of the entire human genome sequence was announced in June 2000, with analyses published in February 2001. An important feature of this project is the federal government's long-standing dedication to the transfer of technology to the private sector. By licensing technologies to private companies and awarding grants for innovative research, the project is catalysing the multibillion-dollar U.S. biotechnology industry and fostering the development of new medical applications.

Human Genome Mapping Project (UK): The UK Human Genome Mapping Project Resource Centre (HGMP-RC) provides access to leading-edge tools for research in the fields of genomics, genetics and functional genomics. The Research Division and the Bioinformatics Division are located on the Hinxton Genome Campus along with the Sanger Centre and the European Bioinformatics Institute. The Biology Services Division of the HGMP-RC is located on the site of the Babraham Institute, Babraham. The Mission of the UK Human Genome Mapping Project are;

- To provide both biological and data resources and services to the medical research community, with a special emphasis on those relevant to the Human Genome Programme.
- To facilitate genomic research by the provision of cost effective centralised collaborative and training facilities.
- To encourage users to share their data, information and resources.
- To encourage the transfer of technology from the academic to commercial/industrial applications.

Mechanisms of chemical carcinogenicity: There are a wide diversity of mechanisms by which chemicals may cause cancer. However a basic distinction between two types can be made. Chemicals that are mutagenic (in-vivo, i.e in whole animals) are persumed to be potential carcinogens. Other chemicals act by various mechanisms but are not mutagenic (e.g effects on hormones or inducing high levels of irritation/cytotoxicity).

Metabolism of carcinogens: It has been established that a number of chemicals which are carcinogenic act only after they have been metabolised to chemical structures which are mutagenic. Thus a key step in the carcinogenic mechanism of these chemicals is the metabolism.

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

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Professor of Environmental Medicine, University of Newcastle,
Consultant Physician, Newcastle Hospitals NHS Trust and
Director, Chemical Hazards and Poisons Division (North), Health Protection Agency

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Diane Benford BSc PhD (*Scientific Secretary – Food Standards Agency*)

K N Mistry (*Administrative Secretary*)

R J Fielder BSc PhD Dip RCPath

Frances D Pollitt MA Dip RCPath

Declaration of COC Members' Interests during the period of this report

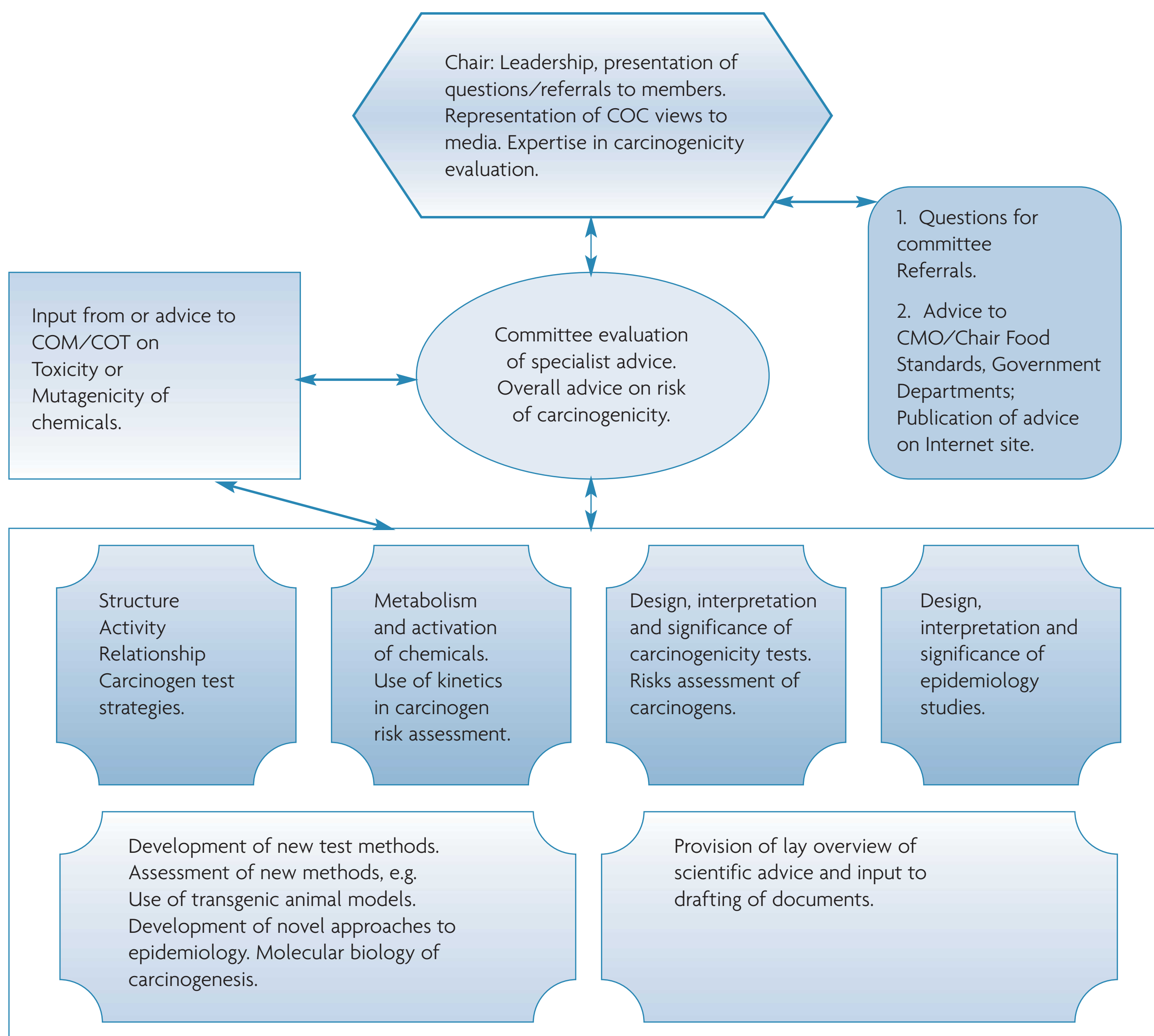
Member	Personal Interest		Non-Personal Interest	
	Company	Interest	Company	Interest
Prof P G Blain CBE (Chairman)	NONE	NONE	Unilever plc	Research Studentship
Prof C Cooper	HBOS	Share Holder	NONE	NONE
	Norwich Union	Share Holder		
Prof P B Farmer	Abbey National	Share Holder	NONE	NONE
	Bradford & Bingley	Share Holder		
	Celltech	Share Holder		
	Friends Provident	Share Holder		
	SBS Group	Share Holder		
	Torotrak	Share Holder		
Prof D Forman	Barclays	Share Holder	NONE	NONE
	Friends Provident	Share Holder		
	HBOS	Share Holder		
	Woolwich	Share Holder		
Prof D Harrison	Medical Solutions	Share Holder	NONE	NONE
	Quintiles	Consultant		
	Scottish Medicine	Consultant		
Ms D Howel	NONE	NONE	NONE	NONE
Dr S J Kennedy	Oxford GlycoSciences	Salary/ Share Holder	NONE	NONE
	Unilever	Share Holder		
Ms M Langley	BT Business Consolidating Services	Share Holder	NONE	NONE
	Ciebel	Director		
	CREE Research	Share Holder		
	Cyber Care	Share Holder		
	Eshelon	Share Holder		
	HBOS	Share Holder		
	MMO2	Share Holder		
	Quelcom	Share Holder		
	Wibex	Share Holder		

Member	Personal Interest Company	Personal Interest Interest	Non-Personal Interest Company	Non-Personal Interest Interest
Prof D Phillips	Abbey National	Share Holder	NONE	NONE
	BG Group	Share Holder		
	Bradford & Bingley	Share Holder		
	Centrica	Share Holder		
	CGNU	Share Holder		
	Lattice Group	Share Holder		
	National Grid	Share Holder		
	Servier	Consultant		
Prof A G Renwick OBE	International Sweeteners Association	Consultant	American Chemistry Council	Research Support
	Novartis	Share Holder	Pfizer	Research Support
	Targacept	Share Holder	GlaxoSmithKline	Research Support
Dr R Roberts	AstraZeneca	Share Holder	NONE	NONE
	Aventis	Salary		
	P & O	Share Holder		
Prof D Shuker	NONE	NONE	NONE	NONE
Prof G T Williams	Abbey National	Share Holder	NONE	NONE
	AMP Ltd	Share Holder		
	Aphton Corporation	Consultant		
	Bradford & Bingley	Share Holder		
	CGNU	Share Holder		

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

TEMPLATE FOR COC 2002

The template is designed to show the breadth of expertise available to the Committee and is intended to aide members in discussing future needs with regard to expertise necessary to fulfil the terms of reference of the COC. The compliment of COC is 13 members (10 specialists and one lay member), attendance of COM chair (ex-officio capacity) and one chair. A deputy chair has not been appointed at October 2002.



ANNEX 1

TERMS OF REFERENCE

To advise at the request of:

Department of Health

Food Standards Agency

Department for the Environment, Food and Rural Affairs

Department of Transport, Local Government and the Regions

Department of Trade and Industry

Health and Safety Executive

Pesticide Safety Directorate

Veterinary Medicines Directorate

Medicines Control Agency

Medical Devices Agency

Home Office

Scottish Executive

National Assembly for Wales

Northern Ireland Executive

Other Government Departments and Agencies

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

   a. used or proposed to be used as food additives, or used in such a way that they might contaminate food through their use or natural occurrence in agriculture, including horticulture and veterinary practice or in the distribution, storage, preparation, processing or packaging of food;

   b. used or proposed to be used or manufactured or produced in industry, agriculture, food storage or any other workplace;

   c. used or proposed to be used as household goods or toilet goods and preparations;

   d. used or proposed to be used as drugs, when advice is requested by the Medicines Control Agency, Section 4 Committee or the Licensing Authority;

   e. used or proposed to be used or disposed of in such a way as to result in pollution of the environment.

2. To advise on important general principles or new scientific discoveries in connection with toxic risks, to co-ordinate with other bodies concerned with the assessment of toxic risks and to present recommendations for toxicity testing.

ANNEX 2

CODE OF CONDUCT FOR MEMBERS OF ADVISORY COMMITTEES

Public service values

Members must at all times:

- observe the highest standards of impartiality, integrity and objectivity in relation to the advice they provide and the management of this Committee;

- be accountable, through the Chairman of the Food Standards Agency, the Chief Medical Officer, to Ministers, Parliament and the public for its activities and for the standard of advice it provides.

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

Standards in Public Life

All Committee members must:

- follow the Seven Principles of Public Life set out by the Committee on Standards in Public Life (see page 182);

- comply with this Code, and ensure they understand their duties, rights and responsibilities, and that they are familiar with the function and role of this Committee and any relevant statements of Government policy. If necessary members should consider undertaking relevant training to assist them in carrying out their role;

- not misuse information gained in the course of their public service for personal gain or for political purpose, nor seek to use the opportunity of public service to promote their private interests or those of connected persons, firms, businesses or other organisations; and

- not hold any paid or high profile unpaid posts in a political party, and not engage in specific political activities on matters directly affecting the work of this Committee. When engaging in other political activities, Committee members should be conscious of their public role and exercise proper discretion. These restrictions do not apply to MPs (in those cases where MPs are eligible to be appointed), to local councillors, or to Peers in relation to their conduct in the House of Lords.

Role of Committee members

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

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

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

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

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

The role of the Chairman

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

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

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

Handling conflicts of interests

The purpose of these provisions is to avoid any danger of Committee members being influenced, or appearing to be influenced, by their private interests in the exercise of their public duties. All members should declare any personal or business interest which may, or may be *perceived* (by a reasonable member of the public) to, influence their judgement. A guide to the types of interest that should be declared is at pages 183 and 184.

(i) Declaration of Interests to the Secretariat

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

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

(ii) Declaration of Interest and Participation at Meetings

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

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

Personal liability of Committee members

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

THE SEVEN PRINCIPLES OF PUBLIC LIFE

Selflessness

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

Integrity

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

Objectivity

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

Accountability

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

Openness

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

Honesty

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

Leadership

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

DIFFERENT TYPES OF INTEREST

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

Personal Interests

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

- **Consultancies and/or direct employment** any consultancy, directorship, position in or work for industry which attracts regular or occasional payments in cash or kind;

- **Fee-Paid Work:** any commissioned work by industry for which the member is paid in cash or kind;

- **Shareholdings:** any shareholding or other beneficial interest in shares of industry. This does not include shareholdings through unit trusts or similar arrangements where the member has no influence on financial management;

Non-Personal Interests

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

- **Fellowships:** the holding of a fellowship endowed by industry;

- **Support by Industry:** any payment, other support or sponsorship which does not convey any pecuniary or material benefit to a member personally, but which does benefit their position or department e.g.:

(i) a grant for the running of a unit or department for which a member is responsible;

(ii) a grant or fellowship or other payment to sponsor a post or a member of staff or a post graduate research programme in the unit for which a member is responsible. This does not include financial assistance for students;

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

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

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

DEFINITIONS

In this Code, 'the industry' means:

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

  The Food Safety Act 1990

  The Medicines Acts 1968 and 1971

  The Food and Environmental Protection Act 1985

  The Consumer Protection Act 1987

  The Cosmetic (Safety) (Amendment) Regulations 1987

  The Notification of New Substances Regulations 1982

- Trade associations representing companies involved with such products;

- Companies, partnerships or individuals who are directly concerned with research, development or marketing of a product which is being considered by the Committees on Toxicity, Mutageneticity, or Carcinogenicity of Chemicals in Food, Consumer Products and the Environment.

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

ANNEX 3

OPENNESS

Introduction

1. The Committee on Toxicity (COT) and its sister committees the Committee on Mutagenicity (COM) and Committee on Carcinogenicity (COC) are non-statutory independent advisory committees who advise the Chairman of the Food Standards Agency and the CMO and, through them, the Government on a wide range of matters concerning chemicals in food, consumer products and the environment.

2. The Government is committed to make the operation of advisory committees such as the COT/COM/COC more open and to increase accountability. Proposals have been published in "Quangos-Opening the Doors" (Cabinet Office, July 1998). The COT/COM/COC have recently considered a number of options for greater openness of Committee business. There was a high level of agreement between the COT/COM/COC regarding the adoption of proposals for greater openness.

3. In discussing these proposals (during the course of 1999) the Committees were aware that the disclosure of information which is of a confidential nature and was communicated in circumstances importing an obligation of confidence is subject to the common law of confidentiality. Guidance is set out in the Code of Practice on Access to Government Information (second edition, 1997). Thus an important aspect of implementing initiatives for greater openness of Committee business concerns setting out clear guidelines for the handling of information submitted on a confidential basis.

General procedures for openness

4. The Committees agreed that the publication of agendas, finalised minutes, agreed conclusions and statements (subject to the adoption of appropriate procedures for handling commercially sensitive information) and appointment of a lay/public interest member to each Committee would help to increase public scrutiny of Committee business. The Committees also agreed that additional open meetings on specific topics where interest groups, consumer organisations etc could attend and participate should be held.

5. A summary of the proposals is tabulated below. A more detailed outline of procedures regarding products where confidential data has been reviewed is given in paragraphs 11-13.

6. The Committees stressed that, in view of the highly technical nature of the discussions, there was a need for all documents released to be finalised and agreed by the Committee, ie any necessary consultation with Members and Chairman should be completed before disclosure.

7. Statements and conclusions should summarise all the relevant data, such as information regarding potential hazards/risks for human health in respect of the use of products and chemicals, and any recommendations for further research.

8. The Committees will be asked for an opinion based on the data available at the time of consideration. It is recognised that, for many chemicals, the toxicological information is incomplete and that recommendations for further research to address these gaps will form part of the Committee's advice.

9. The release of documents (papers, minutes, conclusions and statements) where the COT/COM/COC has agreed an opinion on the available data but where further additional information is required in order to finalise the Committee's conclusions, needs to be considered on a case-by case basis. The relevant considerations include the likelihood that such additional data would alter the Committee's conclusion, any representations made by a company about, for example, commercial harm that early disclosure could cause and also the public interest in disclosure.

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

Summary of proposals for committee openness

Issue	Proposals	Comment
Open meetings on specified topics (eg invited audience, interest groups, consumer organisations, professional socieities).	Agreed. Suggestions include meeting at time of release of Annual Report. External consultation on identifying topics for such meetings.	Meetings would be on generic issues in chemical toxicology carcinogenicity, mutagenicity and risk assessment. There would be no discussion of individual commercial products.
Agenda	Agreed	Made publicly available via Internet site prior to meeting.
Papers	Agreed	Finalised papers to be made available upon request. Confidential information/annexes to be removed.
Minutes*	Agreed	Anonymised minutes made available upon request and on Internet site after appropriate consultation with members and agreement by the full committee.
Conclusions/statements*	Agreed	Agreed conclusions/statements published as appropriate including via the Internet and also made available on request.
Annual Report*	Agreed	Published in accordance with procedures for previous years.
(*Procedures for handlng confidential information outlined in para 11-13 below)		

Procedures for handling confidential information

Background

11. COT/COM/COC quite often consider information which has been supplied in confidence. For the most part this comprises information which is commercially sensitive. For example, this could include product formulations/specifications, methods of manufacture, and reports of toxicological investigations and company evaluations and safety assessments.

12. Normal procedure in the past has been to publish a summary of the Committee's advice in the Annual Report and to ask companies to release full copies of submitted reports for retention by the British Library at the completion of a review. Given the clear Ministerial commitment to the publication of detailed information regarding the activities of advisory committees, and in particular following the assessment of products which are already available to the general public, the COT/COM/COC have begun to adopt where possible a more open style of business where detailed statements have been published via the Internet soon after they have been finalised.

13. Except in cases where there is legislation under which information has been submitted and which deals with disclosure and non-disclosure, the general principle of the common law duty of confidentiality will apply. This means that any information which is of a confidential character and has been obtained in circumstances importing a duty of confidence may not be disclosed unless consent has been given or there is an overriding public interest in disclosure (such as the prevention of harm to others). The following procedure will be adopted which allows confidential information to be identified, assessed and appropriate conclusions/statements to be drafted and published on the basis of a prior mutual understanding with the companies. There is scope for companies to make representations also after submission of the information and prior to publication regarding the commercial sensitivity of data supplied and to comment on the text of statements which are to be published. However, companies would not have a right of veto in respect of such statements.

Procedures prior to committee consideration

Initial discussions

Upon referral to COT/COM/COC the Secretariat will liaise with the relevant company supplying the product in the UK to:

i) Clearly state the policy of Committee openness (as summarised above).

ii) To identify and request the information needed by the COT/COM/COC (eg test reports, publications etc).

Confidential data

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

Handling confidential data

iv) The procedures by which the COT/COM/COC will handle confidential data and the public availability of papers, minutes, conclusions and statements where reference is made to such data will be discussed with the company prior to submission of papers to the Committee(s). The general procedures for handling documents are outlined in paragraphs 4-10 above. Companies will be informed that confidential annexes to Committee papers (eg where detailed information supplied in confidence such as individual patient information and full study reports of toxicological studies) will not be disclosed but that other information will be disclosed unless agreed otherwise with an individual company.

v) The following is a suggested list of information which might be disclosed in COT/COM/COC documents (papers, minutes, conclusions and statements). The list is not exhaustive and is presented as a guide:

a) name of product (or substance/chemical under consideration),

b) information on physico-chemical properties,

c) methods of rendering harmless,

d) a summary of the results and evaluation of the results of tests to establish harmlessness to humans,

e) methods of analysis,

f) first aid and medical treatment to be given in the case of injury to persons,

g) surveillance data (eg monitoring for levels in food, air, or water).

Procedures during and after Committee consideration

vi) The timing of release of Committee documents (papers, minutes, conclusions and statements) where the item of business involved the consideration of confidential data would be subject to the general provisions outlined in paragraphs 4-10 above. Documents would not be released until a Committee – agreed conclusion or statement was available.

vii) The most important outcome of the Committee consideration is likely to be the agreed statement. Companies will be given an opportunity to comment on the statement prior to publication and to make representations (for example, as to commercial sensitivities in the statement). The Chairman would be asked to consider any comments provided, but companies would not be able to veto the publication of a statement or any part of it. Companies will continue to be asked to release full copies of submitted reports for retention by the British Library at the completion of a review.

ANNEX 4

GLOSSARY OF TERMS

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

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

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

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

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

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

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

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

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

Allele: Alternative form of a gene.

Allergen: Substance capable of stimulating an allergic reaction.

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

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

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

Aneugenic: Inducing aneuploidy (qv).

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

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

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

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

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

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

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

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

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

– Strength – The stronger the association the more likely it is causal. The COC has previously noted that the relative risks of (3 need careful assessment for effects of bias or confounding.

- Consistency – The association has been consistently identified by studies using different approaches and is also seen in different populations with exposure to the chemical under consideration.

- Specificity – Limitation of the association to specific exposure groups or to specific types of disease increases likelihood that the association is causal.

- Temporality – The association must demonstrate that exposure leads to disease. The relationship of time since first exposure, duration of exposure and time since last exposure are all important in assessing causality.

- Biological gradient – If an association reveals a biological gradient or dose-response curve, then this evidence is of particular importance in assessing causality.

- Plausibility – Is there appropriate data to suggest a mechanism by which exposure could lead to concern? However, even if an observed association may be new to science or medicine it should not be dismissed.

- Coherence – Cause and effect interpretation of data should not seriously conflict with generally known facts.

- Experiment – Can the association be demonstrated? Evidence from experimental animals may assist in some cases. Evidence that removal of the exposure leads to a decrease in risk may be relevant.

- Analogy – Have other closely related chemicals been associated with the disease?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Dominant lethal assay: See Dominant Lethal mutation.

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

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

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

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

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

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

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

Erythrocyte: Red blood cell.

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

Exogenous: Arising outside the body.

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

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

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

Forestomach: (See glandular stomach).

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

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

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

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

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

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

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

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

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

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

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

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

Genomics: The study of genes and their function.

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

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

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

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

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

Hepatic: Pertaining to the liver.

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

Hepatotoxic: Causing toxicity to the liver.

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

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

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

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

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

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

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

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

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

Intraperitoneal: Within the abdominal cavity.

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

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

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

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

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

Ligand: A molecule which binds to a receptor.

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

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

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

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

Malignancy: See 'tumour'.

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

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

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

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

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

Metabolite: Product formed by metabolism of a compound.

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

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

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

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

Micronucleus test: See Micronuclei.

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

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

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

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

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

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

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

Mycotoxin: Toxic compound produced by a fungus.

Neoplasm: See 'tumour'.

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

Nephrotoxicity: Toxicity to the kidney.

Neurobehavioural: Of behaviour determined by the nervous system.

Neurotoxicity: Toxicity to the nervous system.

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

Non-genotoxic: See 'carcinogens'.

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

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

Null allele: inactive form of a gene.

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

Oedema: Excessive accumulation of fluid in body tissues.

Oestrogen: (See estrogen)

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

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

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

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

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

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

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

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

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

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

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

Polymorphism: (see genetic polymorphism)

^{32}P postlabelling: A sensitive experimental method designed to measure low levels of DNA adducts induced by chemical treatment.

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

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

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

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

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

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

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

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

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

Renal: Relating to the kidney.

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

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

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

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

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

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

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

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

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

TDI: See 'Tolerable Daily Intake'.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Xenobiotic: A chemical foreign to the biologic system.

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

ANNEX 5

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

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ANNEX 6

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Guidance on a Strategy for Testing of Chemicals for Mutagenicity. Department of Health (2000)

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment: Risk Assessment of Mixtures of Pesticides and Similar Substances, Food Standards Agency (2002)

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