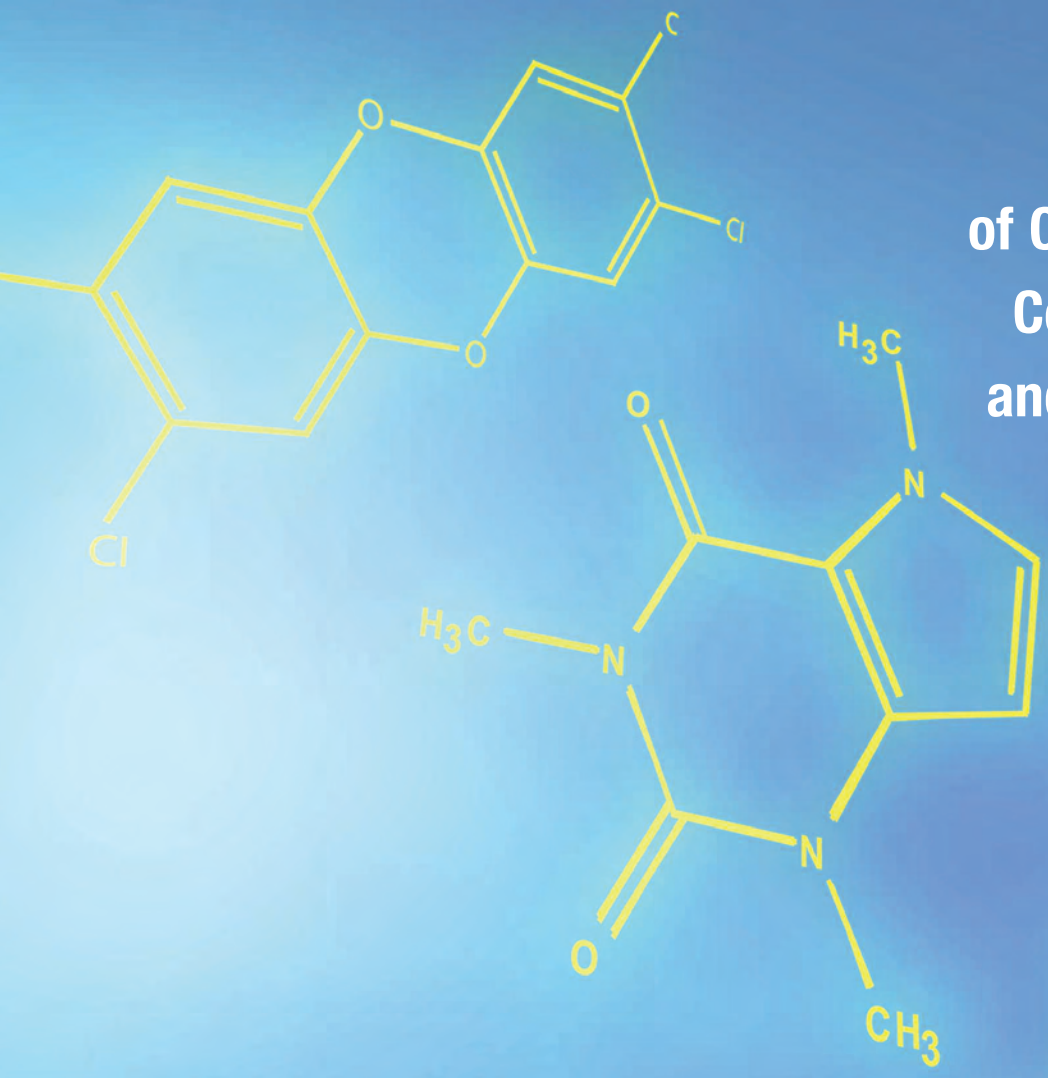
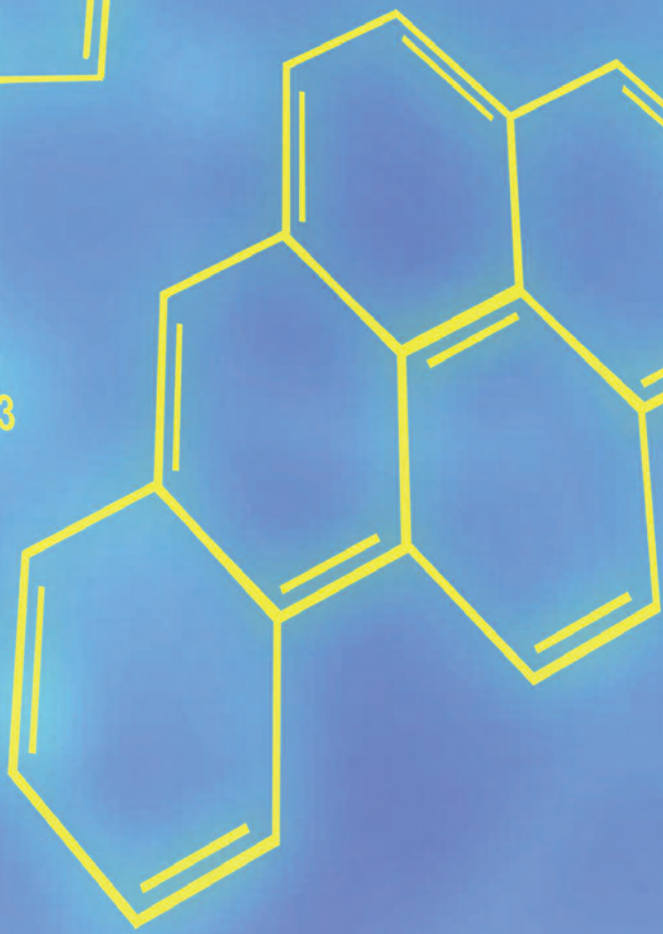
Committees on Toxicity Mutagenicity Carcinogenicity of Chemicals in Food, Consumer Products and the Environment





Committee on TOXICITY

Committee on CARCINOGENICITY

Committee on MUTAGENICITY

Annual Report 2007

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Annual Report
2007

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

About the Committees

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

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

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 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 Chair's discretion be allowed to take part in the discussion, but they are excluded from decision-making. The Code of Conduct is at Annex 2 and Annex 3 describes the Committees' policy on openness. Annex 4 has the Good Practice Agreement for Scientific Advisory Committees. Annex 5 contains a glossary of technical terms used in the text. Annex 6 is an alphabetical index to subjects and substances considered in previous reports. Previous publications of the Committees are listed at Annex 7.

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

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

COT: http://cot.food.gov.uk
COC: http://www.advisorybodies.doh.gov.uk/coc/
COM: http://www.advisorybodies.doh.gov.uk/com/

As of 2008 the COT has a new web address and where appropriate, the statements in this report have been updated to reflect the new web address.

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

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

Preface

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

2007 has been another busy year for the Committee with agreement of six statements. These cover diverse topics such as research on effects of mixtures of certain food additives on children's behaviour, on developmental effects of dioxins, potential effects of exposure to incapacitant sprays and review of the cabin air environment and ill-health in aircraft crews. Advice was also given on a number of generic issues, including nanomaterial toxicology. The Committee also held a scientific workshop on evolving approaches to chemical risk assessment, which was attended by scientists from academia and industry and other interested individuals. A report of the workshop is included in this report. The Committee's working group on variability and uncertainty in toxicology completed its task and the report was adopted by the Committee and published in March. The report on the long term health effects of the Lowermoor incident is expected to be published early in 2008.

This is my final COT report preface as I complete two 3-year terms of office at the end of March 2008. I am very grateful for the dedication and commitment of the extremely able body of experts on the Committee, with whom it has been my pleasure to work over the past 6 years. I am also indebted to my Vice-Chair, Professor Ian Rowland who agreed to extend his appointment to the Committee in order to conclude the lengthy and complex review of the cabin air environment and ill-health in aircraft crews, which he chaired on my behalf. I would like to add my sincere thanks and appreciation of the work of the administrative and scientific secretariats without out whose excellent work the Committee would not be able to function. I particularly wish to place on record the sterling support I received from Dr. Diane Benford.

Chairing the COT has been a stimulating and rewarding experience. I am pleased to have contributed to continued improvement in the Committee's ways of working, including the introduction this year of a self-appraisal process for committee members and the Chair and the establishment of the Food Standards Agency General Advisory Committee on Science. Under the Chairship of Professor Colin Blakemore, this committee will facilitate cooperation between the different scientific advisory committees that advise the Food Standards Agency, challenge the quality of that advice and also review the nature of research commissioned by the Agency. I believe the COT provides scientific advice which is robust, unambiguous and properly implemented within the framework of accepted good practice. The continuing challenges which the COT faces will be met under the able stewardship of Professor David Coggon as my successor. I wish David every success and professional fulfilment during his tenure as Chair of COT.

Professor I A Hughes (Chair)
MA MD FRCP FRCP(C) FRCPCH F Med Sci

COT evaluations

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

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

1.2 The Committee was asked to evaluate the BALPA submission and based on this and other data supplied by the secretariat, assess the risk of exposure of aircraft crews to OP's and oil/hydraulic fluid pyrolysis products in the cabin air and determine whether there is a case for a relationship between exposure and ill health in aircraft crews. The COT was also asked to provide the DfT with appropriate advice on any further research required to evaluate this subject.

1.3 At the July 2006 meeting, the COT had concluded that a priority was to consider and develop a strategy for exposure assessment in commercial aircraft and that initially this should not be restricted to any one group of compounds but should be as wide as possible. The Committee had also requested that an overview of the epidemiological data in the BALPA database be submitted. The COT had requested an independent view of the neuropsychology report on a number of pilots provided to the COT. A further review paper had been presented to the COT at its December 2006 meeting which provided information on oil and hydraulic fluid pyrolysis, an analysis of incident data provided by three airlines, an overview of published and unpublished exposure data on air contaminants in commercial aircraft, a proposed strategy for sampling and measuring air contaminants in commercial aircraft, based on a review of a number of methods which included details of the passive sampling approaches, and a proposal for a contaminant incident triggered solid phase microextraction (SPME) sampling device.

1.4 The passive SPME sampling would take place in a large number of sectors from identified airframe/engine types to provide background information on a wide range of contaminants with enough sectors monitored to identify flights where pilots reported oil odour and possibly an oil contamination incident. The COT noted that SPME technology had been applied to air monitoring under a number of conditions but would need to be validated for commercial jet aircraft.

1.5 The COT statement is included at the end of this report

Developmental effects of dioxin (TCDD) in rats

1.6 Dioxins and dioxin-like polychlorinated biphenyls (PCBs) are persistent organic pollutants that are known to cause a wide range of toxic effects in animals, some of which have been seen at very low doses. These effects may have significant consequences for human health.

1.7 In 2001, the COT assessed the risks posed by dioxins and dioxin-like PCBs. They identified a number of studies in which treatment of pregnant rats with dioxin resulted in toxicity to the developing reproductive system of male offspring. Changes in sperm quality occurred at lower doses than the

other effects of dioxin; therefore, the COT used these data to set a tolerable daily intake (TDI), which should protect humans from all the toxic effects of these chemicals. However, the Committee also noted that there were several limitations in the data, which led to uncertainties in the risk assessment.

1.8 The Food Standards Agency funded a developmental toxicity study which aimed to address some of the limitations identified by the Committee. This study examined the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on developing rats, whilst measuring the maternal and fetal levels of TCDD, termed 'body-burden'. The initial results of this work were provided to the Committee prior to publication in the scientific literature.

1.9 The Committee considered this study was valuable in clarifying some of the uncertainties in its 2001 risk assessment. In the new study, the most sensitive effect of dioxin was a delay in puberty, rather than altered sperm quality. However, this was observed at levels of dioxin exposure that were similar to those used as the basis for the 2001 risk assessment. Therefore, the Committee considered that this study provides additional evidence that the current TDI of 2 pg/kg bw/day is protective for the developing male fetus.

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

Effect of certain food colours and a preservative on behaviour in children research project results

1.11 In 2000 the COT was asked to consider the outcomes of a study conducted on the Isle of Wight investigating whether a combination of certain artificial food colours and a preservative had an effect on children's behaviour. The COT considered that the results of this 2000 study were inconclusive and further data would be required in order to draw firm conclusions.

1.12 In response the Food Standards Agency commissioned a further research project, T07040: Chronic and acute effects of artificial colourings and preservatives on children's behaviour. The new study employed an improved study design which attempted to address the limitations of the previous study. The COT was asked to review the outputs of this new research and to evaluate the significance of the findings.

1.13 The COT concluded that the study provided supporting evidence suggesting that certain mixtures of artificial food colours and a benzoate preservative are associated with an increase in hyperactivity in children from the general population. The Committee considered that, if causal, this effect could be of clinical relevance, particularly for children towards the more hyperactive end of the behaviour scales.

1.14 The Committee noted that the study did not provide an indication of a possible biological mechanism for the observed effects. In addition, a number of limitations associated with design and analysis of the study were identified that prevented further extrapolation of the results to the population level.

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

Nanoparticles used in healthcare and update on nanomaterial toxicology

1.16 In December 2005 the COT, COC and COM published a joint statement on nanomaterial toxicology http://cot.food.gov.uk/pdfs/cotstatements2005nanomats.pdf. The COT considered that information on medical applications of nanoparticles may help to identify potential areas of hazard and risk assessment for nanomaterials used in manufactured products. The Medicines and Healthcare products Regulatory Agency (MHRA) produced a review of information on the toxicology of nanoparticles used in healthcare. An addendum to the Joint COT/COC/COM statement on nanomaterial toxicology was agreed based on the MHRA review.

1.17 The COT addendum is at the end of this report.

Nickel leaching from kettle elements into boiled water

1.18 The COT has discussed nickel leaching from kettle elements on a number of occasions, dating back to 2003. Previously in 2003, Members concluded that further studies would be beneficial in order to more accurately replicate domestic kettle usage patterns for consumers.

1.19 In December 2006, the COT discussed the preliminary results of further research commissioned by the Scottish Government (then Scottish Executive). Members agreed that because the study showed no difference between the water boiled in nickel kettles and the controls, there would be no value in continuing with the study in its current form. However, Members were concerned that the study did not reflect domestic kettle usage patterns, as had been requested. The study was subsequently revised by undertaking a number of reboiling experiments and the results were discussed by the COT in May 2007. Nickel was found to leach both into tap water from Oakdale in Scotland and into deionised water, with higher concentrations in the deionised water. Similarly, the effect of standing time was greater in deionised water than Oakdale water. Prolonged standing time caused an increase in nickel concentrations and re-boiling had less of an effect. Nickel leaching from the new kettles decreased over the 5 days of the study. The highest concentration of nickel in boiled tap (soft) water was 81.5 µg/L. Using this value and assuming a 60 kg adult drinks 2L of water per day, the estimated nickel intake was 2.72 µg/kg body weight/day. This was well below the WHO tolerable daily intake (TDI) for nickel of 11 µg/kg body weight/day.

1.20 The COT agreed that the data from the re-boiling experiments addressed the previous issues that had been raised. The COT agreed on the following conclusion:

The results of this study indicate that nickel leaching from kettle elements into tap water is not a health concern for the majority of consumers. Since the World Health Organization (WHO) tolerable daily intake (TDI) is not considered protective for individuals who are pre-sensitised to nickel, a possible reaction, such as flare-ups of dermatitis, cannot be excluded for nickel-allergic individuals using new kettles with exposed nickel elements.

Reformation of PAVA (Nonivamide) as an incapacitant spray

1.21 The COT has previously provided advice in 2002 and 2004 on the health effects of pelargonyl vanillylamide (PAVA) when used as an incapacitant spray.

1.22 In 2006, the Civil Defence Supply (CDS) proposed a reformulation of the product originally assessed by the COT. The COT was not satisfied with the initial submission on the reformulation and requested additional data. The CDS supplied the additional data on the new formulation and the COT discussed it in May 2007.

1.23 Members agreed that no further evaluation of the data was required and were reassured that the data provided were sufficient and of good quality.

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

Organophosphates and human health

1.25 In 1999 the COT published a report entitled "Organophosphates", which considered whether chronic low level exposure to organophosphates, or acute exposures to levels insufficient to cause overt toxicity, can cause long-term adverse health effects. The COT report made recommendations for research in five different areas, expressed in the form of questions to be addressed. After consideration, Government was of the opinion that research projects to address two of the questions had already been commissioned prior to publication of the COT report. Subsequently, the remaining three research requirements were advertised in the Ministry of Agriculture, Fisheries and Food (MAFF) Research Requirements 2000-2001, entitled "The chronic effects of organophosphates on human health" An additional requirement to investigate the effects of organophosphates on children and unborn children was also identified and advertised at the same time.

1.26 As a result, a total of six research projects were commissioned which addressed the COT recommendations, with a seventh on the effects on children. In 2007, the COT was asked to:

- consider the research reports as they currently stand, and advise on the significance of the 0. findings reported; and

- advise on the extent to which the COT research recommendations had been addressed.

1.27 In addition, ten other research projects had been commissioned by the Government with relevance to the effects of organophosphates. Reports on these projects were also provided, and the COT was asked to advise on how these projects contributed to understanding of possible effects of organophosphate exposure on health in humans. Results were not yet available for all projects, and in these cases the Committee was asked to advise on how the projects would be expected to help to increase understanding of effects of organophosphates assuming that they achieved their objectives.

1.28 The Committee commented on the individual research projects as follows:

Research project 1

1.29 This was a survey of symptoms and organophosphate exposure histories reported in farmers self-selected as suffering ill health which they attributed to organophosphate exposure. We were informed that there was a further phase to the study, a clinical study of 70 individuals; however, publication of this phase of the study had been delayed.

1.30 The COT noted that this study could not assess causality and was not designed to do so. Its purpose was to ensure that all potentially relevant clinical effects would be identified so that they could be included in future epidemiological studies. In this respect it had served its purpose. A follow-up of respondents in the long term to gain information on the progression of symptoms would have been useful.

1.31 Previous work had shown that handling sheep dip concentrate was a major determinant of exposure. The difficulty of assessing exposure by recall was noted. For example, individuals could have exposures to sheep dip concentrate which they were not aware of. However, assessing exposure was not the main purpose of the study.

1.32 The level of long term exposure appeared to be as would be expected in farmers in general, although there was a very high number of reported acute exposure episodes. It would be useful to know if these incidents were associated with acute toxicity and, if so, how severe the toxicity was. It was observed that there had been little analysis of patterns of reported symptoms. It would also have been useful to compare the profile of reported symptoms in this cohort to those of individuals not exposed to organophosphates.

Research project 2

1.33 A limitation of this prospective cohort study of sheep dip exposure and "dipper's flu" was that it did not include ex-farmers, who might include a disproportionate number of susceptible individuals if there were variations in susceptibility from person to person. The recruitment rate was low; however, this was of less concern with respect to recruitment bias since this was a prospective study. It was noted that "dipper's flu" was not an established occupational hazard, but a phenomenon talked about by those involved in sheep-dipping. If a toxicologically mediated effect, it would be of interest since it could be an indicator of high exposure to organophosphates. However, the study did not provide evidence for a flu-like condition related to sheep-dipping or of acute organophosphate exposure being a cause of "dipper's flu". A Member advised that the results were consistent with other research which had not indicated any unusual clustering of flu-like symptoms following sheep dipping (Solomon *et al.* Occup. Med. 2007. doi:10.1093/occmed/kqm066).

1.34 An increase in endotoxin levels in sheep dip was observed after dipping, and it was noted that very high endotoxin exposures could cause respiratory effects. Organophosphate exposures in the sheep dippers appeared to be low; only three farmers showed a decrease in plasma cholinesterase activity following dipping, and this was unlikely to have been sufficient to result in ill health. This low exposure contrasted to the reports of high acute exposures in research project 1.

Research project 3

1.35 This was a cross-sectional survey of farmers identified from records of people who farmed in the 1970s. The distribution of respondents was difficult to interpret. Response may have been driven by motivation, which could mean that individuals with depression were less likely to respond. Alternatively, Members heard that the communications to farmers explained the purposes of the study, and this could also have introduced a different response bias. In addition recall bias was possible in identifying previous symptoms and exposure. However, a lack of association between ill health and sheep farming in general was observed.

1.36 A member referred to research which had indicated that neurological symptoms were more common in people who had worked with sheep dips, but that the association was not specific for working with sheep dip or insecticides (Solomon *et al.* Occup. Environ. Med. 2007, 64: 259-266).

1.37 The questionnaire to screen-identify ill health appeared to have been well validated. However, a limitation of screen-identified ill health was that people who somatise are more likely to report both current and previous ill health. This may be relevant to the association between reporting current ill health and having ever sought advice for pesticide poisoning.

1.38 The COT noted that farming practices were likely to have changed significantly since the 1970s.

Research projects 4 and 10

1.39 Research Project 4 was a case-control study comparing the prevalence of PON1 polymorphisms and diazoxonase activities in dippers with self reported ill health to healthy dippers. A member discussed the history of the assay for metabolic activity of PON1 used in research project 4. The assay had been widely used to determine PON1 activities, although it had not been developed originally for this specific purpose but rather for phenotypic assignment. It had since been discovered (research project 10) that the non-physiological conditions of the assay gave misleading results for PON1 activity with some substrates. Research project 10 had shown that the RR genotype actually conferred higher PON1 activity towards diazoxon, not lower. This had been confirmed independently in work by the researchers who had originally developed the assay. It had also been demonstrated *in vivo* in transgenic mice with human allelic variants of PON1.

1.40 The results of research project 4, taking into account the subsequent findings of research project 10, indicated that faster metabolisers of diazoxon were more likely to report chronic ill health. This was the reverse of the hypothesis being tested. A Member considered whether the multiple statistical testing could have given false associations by chance alone. However, as a prior hypothesis was being tested the concern was not as great as it would otherwise have been.

1.41 The COT agreed that the results of research project 4 may have reflected genetic differences in susceptibility to ill health, but that if so, these were unlikely to be related to organophosphate toxicity.

Research project 5i

1.42 The antibody generation to two capture antigens shown in this study was interesting, although the health consequences of this were unknown. It was thought unlikely that there would be an immunological response which would have resulted in ill health, but this was a data gap. As the study had shown an association between the antibody responses and ill health it would be useful to consult an immunologist and possibly to research this further.

1.43 It was noted that the main protein in blood is albumen, and therefore the most likely antigen *in vivo* would be organophosphate-adducted plasma albumin. This had potential for development as a long-lasting biomarker for organophosphate exposure.

Research project 5ii

1.44 The objectives of this project had been to identify novel protein targets of organophosphates. Changes in concentrations of a number of proteins had been detected at exposure levels to oxons of organophosphates which produced less than 30% brain acetylcholinesterase inhibition. These proteins were expressed at extremely low levels compared to other proteins, which had caused difficulties in identifying them. A 30 kDa protein expressed in the brain and immune system remained unidentified, as did others. Members considered whether the latest tandem mass-spectrometry techniques would help in protein identification. This was possible; however, the main difficulty was in separating the target proteins, which are expressed at extremely low levels, from other proteins which are much more abundant.

Research project 6

1.45 This was a literature review of the effects of low level exposure to organophosphates on fetal and childhood health. We discussed the authors' assumption that the diet was likely to be to be the greatest source of exposure to pesticides by young children, which contrasted to conclusions of evaluations by the US EPA that the main sources of exposure were non-dietary. It was noted that sources of exposure differed in the US from the UK because of differences in the use of pesticides in the home. We also noted that regionalisation was important as studies in the US have shown differences in pesticide exposures in rural children compared to urban children. A member considered that it might be important to distinguish between the contributions from different exposure sources in children overall and in the subset of children with the highest exposure levels.

1.46 It was observed that published studies on developmental effects of pesticides had used dose levels much higher than would be expected from the diet.

Research project 7

1.47 This project investigated whether exposure of marmosets to diazinon caused electrophysiological changes. It was considered to be a valuable study, and the endpoints assessed were considered to be clinically relevant. We agreed that medium to high acute exposures to diazinon had not produced long-term effects in marmosets in this study.

Research project 8

1.48 This was an analysis of reports of suspected adverse reactions to organophosphate sheep dips in the Veterinary Medicines Directorate's Suspected Adverse Reaction Surveillance Scheme (SARSS). It was not possible to assess causality from such data. All the data were on individuals reporting ill health, which was a necessary limitation of the database. The symptoms reported were observed to be common in the general population. It was considered that the results of this analysis should be considered in the context of research projects 1 and 3, and that the contractors should consider this further between themselves.

Research project 9

1.49 The relevance of the concentrations of diazinon used in this *in vitro* research investigating effects on differentiating neurones was questioned. The degree of acetylcholinesterase inhibition was modest despite the high concentration of diazinon used. Cytochromes P450, which would have been low in the cells tested, are required to metabolically activate diazinon to diazoxon, indicating possible non-specific effects of diazinon, unrelated to diazoxon. However, it would have been expected that some diazoxon would have been produced. Diazoxon, at concentrations which produced similar levels of acetylcholinesterase inhibition to diazinon, had a similar level of effect on neurite outgrowth. However, it was noted that commercial sources of diazoxon are unstable, so that the cells may have been exposed to compounds in addition to the oxon.

1.50 It was observed that when activated microsomes were added, the effect on neurite outgrowth was abolished, and the relevance of this was unclear. The combination of cypermethrin and diazinon had a lower than expected effect on neurite outgrowth, suggesting possible interference with cytochrome P450-mediated metabolism.

1.51 Cytoskeletal proteins are expressed at high levels. Therefore effects on these proteins are more likely to be seen than for other proteins. If neurite outgrowth is reduced, the levels of these proteins will be lower, but that did not necessarily indicate a causal relationship. It was considered that the effect should be investigated *in vivo*, though a Member recalled that diazinon had been studied *in vivo*, with no marked effects that would be consistent with the findings of this study.

1.52 It was unclear how the results of this study would extrapolate to the *in vivo* situation, where the developing neurite is better protected. Neurite outgrowth is important in the developing brain and might also be involved in learning and cognition. However, Members agreed that the health impact of any *in vivo* effect on neurite outgrowth was unknown. We considered that any proposal for follow-on work needed a clear rationale to ensure the results could be interpreted in relation to possible health consequences.

Research projects 11 and 14

1.53 This pilot study and follow-on research to assess whether organophosphates are causing gastrointestinal effects in children were considered. It was pointed out that urinary metabolites of organophosphates provided a measure of intake of both organophosphates and their metabolites, for example residues in treated sheep. Also, the levels of such metabolites would be affected both by the level of exposure and also how recently the exposure had occurred.

1.54 The purpose of the clinical study was to address concern that organophosphate toxicity might occasionally cause gastrointestinal symptoms in children which would not be recognised as they would be assumed to be due to infections. However, as planned this would be difficult to do, particularly taking into account that gastrointestinal symptoms presenting at clinics are more likely to be chronic effects than acute. Any exposure of children to organophosphates at levels sufficient to cause acute gastrointestinal symptoms was thought likely to be rare. We advised that it was important that research project 14 involved relevant epidemiological and microbiological expertise. The study design and techniques should be considered very carefully and peer reviewed.

1.55 There is a very high prevalence of gastrointestinal symptoms in children, which are mostly caused by infections, with a wide range of causal organisms. It was pointed out that gastrointestinal symptoms could affect intake of foods and excretion, and that the potential diluting effect of increased faecal volume associated with diarrhoea needed to be considered. Urinary levels of metabolites can be normalised against urinary creatinine, but this is not possible for faeces. The measurement of acetylcholinesterase in faeces was interesting. However, acetylcholinesterase was very highly expressed in parasitic worms which might importantly confound results.

Research projects 12 and 15

1.56 The literature review of pesticide exposure and risk of Parkinson's disease had been commissioned at the recommendation of the Advisory Committee on Pesticides. There was a general consistency across studies associating past exposure to pesticides in general with Parkinson's disease, although these were mostly case-control studies. Few studies had investigated associations with individual pesticides or groups of pesticides, and these had produced inconsistent results. If the relationship were causal, studies of the specific pesticides or groups of pesticides that were responsible would be expected to show substantially higher odds ratios or relative risks than studies investigating exposure to pesticides in general. We noted that further studies had been published since this literature review was completed.

1.57 It was not possible from the available epidemiological or mechanistic data to identify any specific pesticides that increase the risk of Parkinson's disease. Members discussed the plausibility of the bipyridyl herbicide paraquat being a risk factor. It was noted that paraquat was originally suspected due to its structural similarity to MPP+, a metabolite of the recreational drug contaminant MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine), which had been shown to cause acute Parkinsonism in experimental animals and humans. Paraquat had originally been thought to be unable to cross the blood-brain barrier, but there was now emerging evidence that active carrier mechanisms transport paraquat across the blood brain barrier and into cells.

1.58 Research project 15, on possible mechanisms of Parkinson's disease, involved combinations of the dithiocarbamate fungicide maneb and the bipyridyl herbicide paraquat. It did not involve any organophosphates. It was considered to have a good study design, which adequately addressed the toxicokinetics, toxicology and pathology, and should address the question on which it focused. A Member observed that there was evidence that brain exposure to paraquat following inhalation could be 2-3 times higher than would be predicted from levels in the systemic circulation, due to preferential access to the carotid blood flow to the brain. However, exposures would still be low.

Research project 13

1.59 This project investigated inter-individual differences in DNA damage produced by oxons of organophosphates and subsequent repair. The differences in DNA damage observed in lymphocytes from different individuals *in vitro* were considered marginal and very small compared to the inter-individual background variability. The level of DNA damage varied widely, which had been attributed to differences in DNA repair, but this was not convincing. It was inconclusive whether diazoxon had an effect on DNA repair. The data from the Spanish cohort occupationally exposed to organophosphates compared to a UK unexposed cohort showed a lower median level of DNA damage in the Spanish cohort, and a higher mean. Overall, it was not considered that this study provided evidence of genotoxicity of organophosphates. It was noted that this was a pilot study and the Committee questioned where it was intended that this research should lead. It was recommended that officials read the statement of the COM on biomonitoring studies of genotoxicity in pesticide applicators before considering funding any further research on this. It was not considered that there was a need to refer the project to the COM.

Research project 16

1.60 This project, collecting and analysing data from enquiries to the National Poisons and Information Service (NPIS), had been recommended by the Advisory Committee on Pesticides. It was noted to be one of a number of different sources of information through which information on acute poisoning incidents was gained. Taken together, the information from these sources was considered useful.

1.61 The Committee advised on how these projects addressed the research recommendations made in 1999 as follows:

What are the most common patterns of exposure, clinical presentation and subsequent clinical course among people in the United Kingdom with chronic illnesses that they attribute to organophosphates?

1.62 This had partially been addressed by research projects 1, 8 and 16. Information was still lacking on long-term outcomes in affected individuals.

How common is dipper's flu, and what causes it?

1.63 Some useful information had been provided by research project 2. Dipper's flu did not appear to be a specific syndrome. It was considered that this should not be a high priority for future work.

Does low-level exposure to organophosphates cause disabling neurological or psychiatric disease in a small subgroup of exposed persons?

1.64 There was evidence of long term neurological illness in persons who had used organophosphates. However, there were also associations with use of other pesticides. Whether these effects were due to toxicological mechanisms of pesticides or other mechanisms was uncertain. Other research which had been conducted in relation to this should also be considered, and research project 3 should be reconsidered when complete.

Do people with chronic disabling illness that is suspected of being related to organophosphates differ metabolically from the general population?

1.65 Research project 4 had addressed this recommendation. There was evidence of a metabolic difference but this did not correlate to enhanced toxicity of organophosphates. It was possible that this was a chance finding. It was noted that differences in susceptibility did not necessarily require a polymorphism in a metabolising enzyme; there could be a polymorphism in the biological target. Further research on the role of polymorphisms of organophosphate disposition was not considered a high priority.

Other than acetylcholinesterase inhibition, what mechanisms play an important role in the causation of adverse health effects by organophosphates?

1.66 Some interesting data had been generated - *in vitro* effects, detection of novel protein targets, developmental effects *in vivo* - but much more research would be required to fully address this question. It was considered important to better characterise exposure, since even if toxicological mechanisms other than acetylcholinesterase inhibition were identified, if these occurred at higher levels of exposure than people received, they would not be relevant to human health effects. It was noted that health beliefs and expectations and somatisation tended to predict the reporting of ill health attributed to pesticide exposure, and the possibility that part of the illness may have a non-toxicological basis should not be ignored. It was disappointing that medically unexplained symptoms had not been investigated as part of the research.

Committee Procedures

Code of Conduct for Observers

1.67 The Code of Conduct was developed to allow transparency in the Committee's deliberations whilst ensuring that the work of the Committee is not inhibited by the presence of observers. In May 2007, the Committee discussed a minor revision to the Code of Conduct to ensure that invited experts and the Secretariat were not inhibited from contributing to the work of the Committee.

1.68 It was stressed that if observers or other interested parties had information they wished to pass on to the Committee, then this should be channelled through the Secretariat as outlined in the guidance provided on the COT web pages. Observers should not approach individual Committee Members or other observers, nor address the Committee unless invited to do so by the Chair.

1.69 Committee minutes list Observers by name and affiliation and their comments are attributable. If observers consider that their comments are not accurately reflected in draft Committee minutes, this is drawn to the attention of the Committee before the next meeting to ensure factual accuracy. Observers are asked not to attribute comments to individual Members.

1.70 The Committee approved a minor revision to the Code of Conduct. Following agreement by the COM and COC Chairs the revised text was published on the committee websites.

Code of Practice for Scientific Advisory Committees

1.71 The Office of Science & Innovation (OSI) consulted with all of the scientific advisory committees on the updating of the Code of Practice for Scientific Advisory Committees. The Committee was asked if it wished to respond to the consultation, and to consider the implications of the changes for its working practices. Attention was drawn to the universal ethical code for scientists and minor changes to two of the seven principles of public life. The key proposed changes to the Code of Practice related to having access to a variety of experts; the balance between transparency and the handling of sensitive information; and the need for regular "light touch" monitoring, evaluation and spread of good practice.

1.72 The Committee saw this as an opportunity to review the adequacy of the informal induction available to new Committee members. Newer Members agreed that the opportunity to learn from experienced Members was more appropriate than a formal induction process.

1.73 The Committee reviewed the proposed changes noting that whilst the advice from scientific advisory committees should be robust, the evidence basis is frequently not. Suggestions to the OSI included that the wording could be modified to reflect that the appropriate evidence had been identified and to require a rigorous process for assessing the robustness of evidence. The Committee queried why the OSI believed some information might be withheld from a committee and considered that examples of such situations might be helpful.

1.74 It was generally agreed that COT opinions reflect consensus, rather than a collection of thoughts and opinions of individual members

Evident toxicity as an endpoint in acute toxicity testing

1.75 The UK competent authorities are leading on the development of a fixed concentration procedure (FCP) guideline (TG 433) for testing chemicals for acute inhalation toxicity within the Test Guideline programme of the Organisation for Economic Co-operation and Development (OECD). A paper on regulatory requirements for acute toxicity data and a further paper on the proposed definition of evident toxicity for acute inhalation studies on chemicals were reviewed by the COT at the September meeting in 2007. Some difficulties have been experienced with the acceptance of the principles of evident toxicity as an end-point by other OECD countries as they consider that the term has not been accurately defined.

1.76 The COT considered that describing evident toxicity for inhalation exposure might be more complex than for oral exposure, for example if a substance caused local respiratory irritation. The endpoints incorporated in the TG420 would not provide an adequate basis for deriving an acute reference dose. Lethality, or a surrogate for lethality, might be more appropriate in certain circumstances, for instance when testing highly toxic gases for the purpose of emergency planning. However, this would not be appropriate for the vast majority of chemicals tested, for instance under REACH. Members were informed that the original acute inhalation toxicity Test Guideline, which allows estimation of an LC_{50}, was not expected to be deleted but rather to be extended to include examination of the effect of exposure time as well as concentration on toxicity.

1.77 It was considered that the refined methodology would not only help in the primary objective of refining and reducing animal use, but might produce data of a more useful nature. As an example, more detailed information on acute effects of chemicals in animals could help clinicians dealing with acute toxicity in humans recognise symptoms and treat patients more effectively.

1.78 The COT advised the need to confirm the transferability of endpoints from the oral to inhalation route, and suggested that some form of validation exercise, not requiring extensive additional animal testing, should be possible.

Horizon Scanning

1.79 At the February 2007 meeting, Members were informed of a number of subjects which are either ongoing or scheduled for discussion at future meetings. Topics which the Secretariat anticipated as possible future agenda items were raised.

1.80 Developmental neurotoxicity was considered an important area and a forthcoming report of the International Life Sciences Institute (ILSI) was expected to be helpful. A workshop on this area covering, for example, rationale for using results from behavioural tests, was considered to be potentially useful.

1.81 Members were informed of FSA-funded research aiming to follow up on the research recommendations in the 2002 COT report on phytoestrogens and health. In addition to the FSA research, it was felt important to also consider the large-scale intervention studies carried out in the US focussing on sexual development and consumption of soy-based infant formula.

1.82 The FSA noted that COT advice might be required on safety of consuming some food supplements during pregnancy. The aim would not be to alter FSA advice on a balanced and varied diet to ensure adequate nutrition rather than the use of supplements but to aim to ensure that warnings, often in regard to pregnancy, are consistent on similar supplements.

1.83 Members were informed of COM discussions on transgenerational effects of methylation and agreed that a joint workshop would be appropriate.

1.84 The Committee considered a final draft report from Central Science Laboratory entitled "Horizon Scanning for Emerging Environmental Contaminants". The proposed approach would be used to facilitate discussions at a forthcoming International Conference on Analysis of Emerging Contaminants in the Environment due to be held in March 2007. In principle, there was support for the process put forward in the document.

1.85 Members raised the following items as emerging issues that may require Committee consideration:

- Appropriate testing strategies for evaluating tobacco products.
- A regular update on how the implementation of REACH is progressing.
- An update on the genetically engineered *in vitro* and humanised animal models used for toxicological studies. It was felt that systems available, their applicability (strengths and limitations) in pragmatic terms and the potential for future development would be of benefit to the Committee.
- Atypical shellfish toxins: a watching brief should be kept on this issue for incidences of unknown toxins. However, it was recognised that there is no straightforward procedure to extract, detect or evaluate such toxins.
- Lavender and Tea Tree Oil Shampoos: A recent publication reported breast development in pre-pubertal males following use of Lavender and Tea Tree oil shampoo (Henley *et al.*, 2007. Prepubertal gynecomastia linked to lavender and tea tree oils. NEJM. 356:479-485). It was proposed that the Secretariat should keep a watching brief for further reports of consumer products with *in vitro* evidence of estrogenic/anti-androgenic effects and developmental effects in humans.
- The increased use of air fresheners and essential oil burners was noted. There is concern that these may be respiratory sensitisers, asthmagens or respiratory irritants.

1.86 Members were reminded that they can draw the attention of the Secretariat to additional issues at any time.

IGHRC guidance document on chemical mixtures

1.87 The Interdepartmental Group on Health Risks of Chemicals (IGHRC) draft guidance document described the different types of mixtures for which UK government may need to conduct a risk assessment and the kinds of data that may be available for these mixtures. It described different approaches that have been used for mixture risk assessments and provided a framework in the form of a decision tree to help risk assessors consider the different issues that arise when carrying out a risk assessment for a chemical mixture.

1.88 The COT and other scientific advisory committees were asked for comments on the draft document. The COT advised on revisions to the draft, relating to further discussion of synergy, physiologically-based pharmacodynamic modelling and illustrating the framework by means of worked examples.

1.89 It was considered that the guidance would applicable to evaluations undertaken by the COT and that it would be useful to apply some of the approaches in the next few years in order to further develop experience in this area. Combined assessment of chemicals established as being part of a common mechanism group is relatively simple, whereas evaluation of dissimilar compounds is more difficult, as a clear hypothesis is needed when determining which chemicals should be assessed together.

1.90 Following revision in line with comments of COT and other scientific advisory committees the guidance document would be published in the near future.

Technical Guidance for derivation of DNELs and risk characterisation of non-threshold effects in the context of REACH

1.91 REACH (Registration, Evaluation and Authorisation of CHemicals) is a new European Regulation for industrial chemicals. It aims to: ensure a high level of protection of human health and the environment; ensure the free circulation of substances on the internal market while enhancing competitiveness and innovation; and, promote the development of alternative methods for the assessment of hazardous substances.

1.92 In order to ensure efficient implementation of the future legislation, the European Commission established a number of REACH Implementation Projects to develop guidance documents. A key area is guidance for industry on how to derive Derived No-Effect Levels (DNELs) for use in the human health hazard assessment of substances with threshold effects.

1.93 REACH defines a DNEL as the level of exposure to a substance above which humans should not be exposed. It is proposed that DNELs will be derived for relevant exposure situations by dividing the critical no (or lowest) observed adverse effect level (N(L)OAEL) by uncertainty or assessment factors (e.g. to account for inter- and intra-species differences, uncertainties in the dose-response relationship and differences in duration of exposure). For non-threshold effects REACH requires that a qualitative risk assessment be performed, but the guidance proposes that if data permit, risk managers may be aided in prioritisation by the establishment of quantitative reference levels or Derived Minimal Effect Levels (DMELs).

1.94 The Committee was asked to comment on the preliminary draft of the Technical Guidance document on calculating DNELs/DMELs for substances subject to registration and exceeding the 10 tonnes per annum manufacture/importation/usage threshold.

1.95 General comments indicated that the technical guidance would benefit from greater consideration of certain important aspects, such as the impact of study design on the assessment of NOAELs; and the choice of mathematical model used to derive benchmark doses.

1.96 There was concern that the data requirements for substances in the 10 tonnes per annum band could result in inadequate end points being analysed. The proposal to extrapolate from a 28 day study (without any information on cancer, immunotoxicity, neurotoxicity or reproductive/developmental endpoints) to a limit for lifetime exposure using assessment factors was not considered sufficiently robust.

1.97 With regard to descriptions of applying allometric scaling, it was unclear how the split in terms of toxicokinetics and toxicodynamics had been established. In addition to clarifying this split, the guidance would benefit from a more detailed explanation as to how these factors are to be applied.

1.98 Given that workers would include pregnant women, the document lacked convincing justification for treating workers differently to the rest of the population and proposing a lower intra-species assessment factor of 5.

1.99 The use of 2,3,7,8-tetrachloro-p-dioxin as an example of a receptor-mediated non-threshold carcinogen was not supported by the Committee, as dioxin-induced effects occur via a threshold mechanism. In addition, current understanding of the biology of carcinogenesis does not support the hypothesis that activation of a single receptor in a cell can result in clonal expansion of that cell.

1.100 The COT's comments were forwarded to the appropriate working groups, and the finalised guidance to industry is expected to be published shortly after March 2008.

Working Groups and Workshops

Lowermoor Subgroup

1.101 The Lowermoor subgroup was established in 2001 under the chairmanship of Professor Frank Woods to consider the health effects of the chemical exposure resulting from a water pollution incident which occurred in July 1988 in North Cornwall. The terms of reference of this subgroup were:

- To advise on whether the exposure to chemicals resulting from the 1988 Lowermoor water pollution incident has caused, or is expected to cause, delayed or persistent harm to human health; and

- To advise whether the existing programme of monitoring and research into the human health effects of the incident should be augmented and, if so, to make recommendations.

1.102 The draft report of this subgroup on the Lowermoor Water Pollution Incident was published for consultation in January 2005 and 26 responses were received. The COT discussed the consultation report in April 2005 but few comments were made.

1.103 In December 2007 the COT discussed the draft final report and provided comments for its final approval and publication. The COT agreed that the report was a thorough and balanced discussion of the available evidence, endorsed the conclusions and advised in detail on future recommendations to address uncertainties.

1.104 The COT agreed that the final revisions could be made by the subgroup for publication early in 2008.

Workshop on Evolving Approaches to Chemical Risk Assessment

1.105 On 7th February 2007 the Committee held an open workshop on "Evolving Approaches to Chemical Risk Assessment". This workshop was designed to explore in more detail some of the approaches described in the Committee's report on Variability and Uncertainty in Toxicology of Chemicals in Food, Consumer Products and the Environment.

1.106 Invited expert speakers made presentations on techniques that the Committee may wish to exploit in future risk assessments. Members participated in discussions during the workshop and subsequently. The Committee issued a statement, included at the end of this report, which summarised the presentations and the Committee's subsequent discussions.

Working Group on Variability and Uncertainty

1.107 In March 2007, the COT endorsed its report on Variability and Uncertainty in Toxicology of Chemicals in Food, Consumer Products and the Environment (VUT Report). The Report was published as a paper copy and is available for download on the FSA COT website at:
http://www.food.gov.uk/science/ouradvisors/toxicity/CPTwg/wgvut/

Ongoing work

Epidemiological studies of landfill sites and adverse birth outcomes

1.108 The COT has previously advised on the findings of epidemiological studies of landfill sites and adverse birth outcomes in 1998 and 2001. In 2007 the Environment Agency (EA) requested further advice on this issue and in July 2007 the COT considered two prepublication papers from the Small Area Health Statistics Unit (SAHSU) on reproductive health outcomes around landfill sites. In December 2007, the COT discussed its earlier statement on epidemiological studies of landfill sites and adverse birth outcomes together with the two new SAHSU studies.

1.109 The results of an exposure study sponsored by the EA were expected soon and would be presented next year. The agreed working paper will be published as a statement when the papers on both the SAHSU studies have been published.

Pyrrolizidine alkaloids in food

1.110 Pyrrolizidine alkaloids (PAs) are a large group of natural toxins which have been associated with a number of livestock diseases and cases of human poisoning following consumption of herbal remedies, or after contamination of staple foods. There is also potential for PAs to be transferred to other food products such as honey, milk, eggs and offal.

1.111 In December 2007, the Committee was asked for its view on the risk assessment of PAs in food and whether potential human exposure, particularly via honey and milk, may be of concern. The discussions will continue in 2008.

Reproductive effects of caffeine

1.112 The COT commenced a review of published research on this topic in December 2007, and will complete its review when results of research funded by the Food Standards Agency are available.

Urgent Advice

Doramectin in Lamb

1.113 Urgent advice was sought from the COT Chair by the Food Standards Agency following the discovery that lamb had entered the food chain from animals which had been treated with a veterinary medicinal product containing doramectin which had been slaughtered without the full withdrawal period for the product having been adhered to

1.114 The Chair was consulted on a risk assessment for possible intakes if a large portion of meat from the injection site was eaten. The agreed risk assessment is as follows.

Risk assessment for injection site muscle from sheep injected with a doramectin product and slaughtered after 14 and 5 days

1.115 15 mg doramectin is injected into the muscle. If all of this was consumed in meat, it would provide a dose of 250 µg/kg bw in a 60 kg adult. Since zero withdrawal period is not feasible, the maximum human adult dietary exposure would be less than 250 µg/kg bw.

1.116 Reported data showed that residues at the injection site were variable, with a mean of 2554 µg/kg 14 days after administration of doramectin at the same dose as was given to the sheep in this incident (EMEA, 2000). Information on the range of concentrations was not available.

1.117 A 60 kg adult eating a 300 g portion of meat containing 2554 µg/kg doramectin would receive a dose of 12.8 µg/kg bw. Since this was based on mean residue data, some portions of meat would result in higher doses. The dose would also be higher if the withdrawal period was less than 14 days, but available data did not allow an estimate of this.

1.118 There were no data from which to draw conclusions on a dose that would be clearly toxic to humans.

1.119 The ADI for doramectin is 1 µg/kg bw (EMEA, 2006), which would be exceeded by 12.8-fold at the average human dose with 14 day withdrawal if a 300 g portion was consumed by a 60 kg adult.

1.120 An acute reference dose (ARfD) had not been set. An NOAEL of 1.5 mg/kg bw was identified for developmental toxicity in rabbits (EMEA, 2000). Applying the default uncertainty factor of 100 indicated that 15 µg/kg bw might be appropriate as an ARfD, which was above the average human dose with 14 day withdrawal.

1.121 Studies of acute toxicity with related substances (ivermectin and abermectin) in monkeys gave a NOAEL of 1 mg/kg bw, with vomiting, mydriasis and salivation seen at higher doses (WHO, 2001). This indicated that an ARfD of 10 µg/kg bw might be appropriate if the standards 100-fold uncertainty factor was applied, or 100 µg/kg bw if humans are no more sensitive than monkeys.

1.122 Exposure from consuming one portion of lamb from the injection site at the average residue level 14 days after injection was not a concern. Some portions of lamb would contain higher levels as a result of the variability in the distribution of residues between animals and the shorter withdrawal periods for some of the animals. Available data did not allow estimates of how much higher the resulting human dietary exposure would be, but these might reach toxic levels.

1.123 Therefore there were concerns about the safety of this meat.

References

EMEA (2000). Committee for Veterinary Medicinal Products. Doramectin (pigs and sheep), Summary report (3). EMEA/MRL/186/97-FINAL. European Agency for the Evaluation of Medicinal Products.

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WHO (2001). Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 49, 2001.

Statements of the COT

COT addendum to joint statement of the commitees in Toxicity, Mutagenicity and Carcinogencity on Nanomaterial Toxicology

Background

1 In December 2005 the Committees on the Toxicity, Carcinogenicity and Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COT, COC and COM) published a joint statement on nanomaterial toxicology http://cot.food.gov.uk/pdfs/cotstatements2005nanomats.pdf

2 The objective was to provide a baseline statement on the available information on nanomaterials toxicology. The Committees suggested a systematic tiered approach to initial toxicological studies with nanomaterials. The Committees stated that there was no need to develop a new approach to risk assessment of nanomaterials but there was a clear need to provide hazard identification data on the widest possible range of nanomaterials. It was noted that in the absence of such data it was not possible to derive conclusions about the spectrum of toxicological effects which might be associated with nanomaterials. Thus it was noted that nanoparticles resistant to degradation could accumulate in secondary lysosomes, which in cells with a long survival such as neurones or hepatocytes might lead to chronic toxicity.

3 In the concluding remarks the COT indicated additional information on medical applications of nanoparticles might be important to their discussions and might be potentially relevant with regard to information on structure activity.

MHRA review

4 Following discussions between the secretariat and Medicines and Healthcare products Regulatory Agency (MHRA), the MHRA produced a review of information on the toxicology of nanoparticles used in healthcare. This MHRA review aimed to identify whether healthcare nanoparticles introduced any new toxic hazards and was based on published literature from the last five years supplemented by additional specific product information. The review excluded healthcare products where the administered product is a single large molecule or entity that just happens to fall in the nanoparticulate scale such as pro-drugs, biological macromolecules and viral transfection agents. Many publications involved *in vitro* proof of principle with incidental cytotoxicity information. The review can be found at: http://cot.food.gov.uk/pdfs/TOX-2006-28.pdf

COT discussion.

5 Based on this comprehensive review, the toxicological database to date was considered to be still inadequate to indicate whether nanoparticles have a specific form of toxicity. The apparent emphasis on an initial wide ranging *in vitro* investigation in nanotoxicology testing strategies might represent a misunderstanding of the role of *in vitro* data since animal studies remained the key hazard identification studies. The role of *in vitro* testing is as part of a tiered approach to decision making and not a means of detecting toxicity endpoints other than genotoxicity hazards.

6 Having considered the new data on healthcare nanoparticles, there were limited data on extrapolation from animals to humans and therefore the implications of such extrapolation and use of standard uncertainty factors would need further consideration as data emerged. Bioavailability and biodistribution studies have a critical role in evaluation of nanoparticles and such information is not obtained from *in vitro* studies. Common mechanisms of toxicity, for example, oxidative stress might also provide a method for prioritisation of those nanoparticles that need further testing.

7 The approach to biodegradable and non-biodegradable nanoparticles might need to be different. There is no evidence that biodegradable nanoparticles have toxicity intrinsic to their nanoparticulate state. In contrast, the evidence indicates that non-biodegradable nanoparticles can cause cell death due to their physical nature by accumulating and overloading lysosomes. Although there was an extremely limited database some studies on nanoparticles had shown evidence of potential shape-specific biological properties.

8 The information reviewed indicated there was a need to consider formulation effects which can affect surface charge and particle size and influence the resulting toxicity. Product specific assessments would be needed as well as clarity on the formulations tested. The COT was informed that this could raise difficulties for evaluating nanoparticles in cosmetics since current EU legislation does not allow *in vivo* testing on cosmetic formulations.

9 The mechanisms of toxicity seen with healthcare nanoparticles were not unique. There is a need for sufficiently sensitive endpoints to identify effects which had predictive validity for potential adverse effects in humans.

10 Conventional toxicological assessment should be sufficient to identify toxic hazards from biodegradable healthcare nanoparticles. However, it was important to ensure study designs were appropriate to the nanoparticle under investigation. Whilst the standard toxicological test batteries would detect possible effects from healthcare nanoparticles, there was as yet, insufficient information to exclude the possibility of effects not detectable by these methods. The COT was not currently aware of such effects being reported.

11 For pharmaceuticals it has been shown that incorporation into nanoparticle formulations can greatly influence the biodistribution (and hence toxicity) of included chemicals. Indeed the intention behind many such formulations is to facilitate drug delivery across tissue barriers. There is little evidence that the biodistribution of other chemicals not physically included in the original formulations, but accidentally present in the body at the same time as the nanoparticles, can be so influenced. However there is at least a theoretical possibility that freshly generated nanoparticles with reactive surfaces could significantly bind and alter the biodistribution of other xenobiotics. Such effects would not represent nanoparticle toxicity per se, but would represent a consequence of co-exposure.

12 The COT reached the following conclusions in addition to those in paragraph 12 of the joint statement on nanomaterial toxicology http://cot.food.gov.uk/pdfs/cotstatements2005nanomats.pdf

I. We wish to emphasise that the role of *in vitro* testing is part of a tiered approach to decision making and not a means of detecting toxicity endpoints other than genotoxicity.

II. We concluded that the approach to the risk assessment of biodegradable and non-biodegradable nanoparticles should be different, since the available evidence indicates that non-biodegradable nanoparticles can cause cell death due to their physical interaction with cells. In contrast, biodegradable nanoparticles are less likely to have toxicity intrinsic to their nanoparticulate state.

III. There is some limited evidence available to indicate that formulation, i.e. the matrix in which the nanomaterial is present, can affect surface charge and particle size and influence the resulting toxicity. Therefore we conclude that available evidence on formulation effects on toxicity of nanoparticles should be monitored.

COT Statement 2007/01
March 2007

FSA funded study investigating the developmental effects of Dioxin (TCDD) in rats

Non Technical Summary

1 Dioxins and dioxin-like polychlorinated biphenyls (PCBs) are persistent organic pollutants that are known to cause a wide range of toxic effects in animals, some of which have been seen at very low doses. These effects may have significant consequences for human health.

2 In 2001, the COT assessed the risks posed by dioxins and dioxin-like PCBs. They identified a number of studies in which treatment of pregnant rats with dioxin resulted in toxicity to the developing reproductive system of male offspring. Changes in sperm quality occurred at lower doses than the other effects of dioxin; therefore, the COT used these data to set a tolerable daily intake (TDI) for dioxins, which would protect humans from all the toxic effects of these chemicals. However, the Committee also noted that there were several limitations in the data, which led to uncertainties in their risk assessment.

3 The Food Standards Agency (FSA) has funded a developmental toxicity study which aimed to address some of the limitations identified by the Committee. This study examined the effect of dioxins on developing rats, whilst measuring the level of dioxin in the mother and in the fetus, termed 'body-burden'.

4 The Committee considered this study was valuable in clarifying some of the uncertainties in their 2001 risk assessment. In the new study, the most sensitive effect of dioxin was a delay in puberty, rather than altered sperm quality. However, this was observed at levels of dioxin exposure that were similar to those used as the basis for the 2001 risk assessment. Therefore, the Committee considers that this study provides additional evidence that the current tolerable daily intake (TDI) of 2 pg/kg bw/day is protective for the developing male fetus.

Background

5 The term "dioxins" is commonly used to refer to a group of 75 polychlorinated dibenzo-p-dioxin (PCDD) and 135 polychlorinated dibenzofuran (PCDF) congeners, of which fewer than 20 are considered to be biologically active. Dioxins are produced in a number of thermal reactions, including incineration of municipal waste, domestic fires and bonfires, forest fires and in internal combustion automobile engines. They are also generated as trace contaminants during the synthesis of many organochlorine compounds and during some industrial processes

6 PCBs are environmentally stable, lipophilic chemicals that were widely manufactured for a range of industrial applications between the 1930s and 1970s. Use of PCBs for industrial purposes has been discontinued but these substances may still be released to the environment during disposal of materials and obsolete equipment. There are 209 theoretically possible PCB congeners, of which 12 non-ortho or mono-ortho compounds exhibit similar biological activity to PCDDs and PCDFs, and are therefore referred to as "dioxin-like PCBs".

7 These compounds are persistent in the environment and tend to accumulate in biological systems, particularly in fatty tissues. One of the most potent and extensively studied PCDD congeners, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), exhibits a broad range of toxic effects in laboratory animals, some at very low doses. Since the toxicity of the various dioxins and dioxin-like congeners is generally accepted to be mediated by the aryl hydrocarbon receptor (AhR), and experiments using mixtures of congeners are consistent with an additive model; a system of toxic equivalency factors (TEFs) has been devised by the World Health Organisation (WHO) to enable total TCDD toxic equivalents (TEQ) to be calculated. This was initially developed in the 1980s and has subsequently been subjected to periodic review, to ensure the system incorporates newly acquired data and knowledge[1]. Estimates of dietary exposure are expressed in terms of WHO TEQ. The TEQ is defined as the sum of the products of the concentration of each congener, multiplied by the TEF.

2001 COT Evaluation

8 In 2001, the COT undertook an extensive review of dioxins and dioxin-like PCBs. The Committee on Carcinogenicity (COC) advised that TCDD should be regarded as probably carcinogenic to humans, based on the available data and, although there were uncertainties regarding the mechanism of action, it was likely that a threshold approach to risk assessment was appropriate[2].

9 The COT concluded that the available human data did not provide a sufficiently rigorous basis for establishment of a tolerable intake because:

- the epidemiological studies did not reflect the most sensitive population identified by animal studies;
- there were considerable uncertainties in the exposure assessments and inadequate allowance for confounding factors; and
- the patterns of exposure did not reflect the main route of exposure experienced in the general UK population, which is mainly from diet.

It was, therefore, considered necessary to base the evaluation on the data from studies conducted in experimental animals[3].

10 The Committee concluded that the most sensitive indicators of TCDD toxicity were the effects on the developing reproductive system of male rat fetuses exposed in utero. These endpoints had also been used to derive tolerable intakes by Joint Food and Agriculture Organisation (FAO) / WHO Expert Committee on Food Additives (JECFA), 70 pg WHO TEQ/kg bw per month[4]; and EU Scientific Committee on Food (SCF), 14 pg WHO TEQ/kg bw per week[5].

11 The key studies used different strains of rats and tended to give contradictory findings. A change in anogenital distance (AGD) was found after single oral doses given on day 15 of gestation (GD15) of 50 ng/kg bw[6], 200 ng/kg bw[7] and 1000 ng/kg bw[8]. However, the Committee considered that the data on AGD were not robust because of lack of correction for body weight or other means of normalisation, and should be regarded as an intermediate marker with no functional significance in itself. Decreases

in sperm numbers, production, reserve or morphology were found shortly after puberty (postnatal day (PND) 49-70) and in adulthood (PND120 onwards), after single oral doses of 50 ng/kg bw and above on GD15[7,8,9] and following weekly subcutaneous dosing to give a body burden of 25 ng/kg bw[10]; although these changes were not seen in one acute oral study, dosing 800 ng/kg bw on GD15[6]. Changes in the weight of the urogenital complex, including the ventral prostate were reported after an oral dose of 200ng/kg bw on GD15[6] but not at 300ng/kg bw subcutaneously[10].

12 Despite these inconsistencies, the Committee considered that the effects on sperm production and morphology represented the most sensitive effects. These were indicative of the functional adverse reproductive effects in the rat that were produced by long-term administration in a multigeneration study, at doses resulting in a 10-fold higher body burden than those in the studies of sperm production[11]. The Committee also noted that the sperm reserve in the human male is much less than that in the rat, and therefore these changes were considered relevant. The study of Faqi[10] provided the lowest LOAEL, but no NOAEL. Limitations in this study were noted but it was considered that the results could not be discounted; therefore, this was used as the basis for deriving the tolerable intake. The Committee considered that a tolerable intake based on these effects would also protect against any risk of carcinogenicity from dioxins and dioxin-like PCBs since a significant increase in incidence of tumours was only found at doses that were higher than the LOAEL in the Faqi study.

13 Several studies[6,7,8,9] reported adverse effects in male rat offspring following a single oral dose of TCDD given on GD15, and one[10] following repeated weekly subcutaneous injections. In all cases the effects were observed postnatally, although the pattern of both *in utero* and postnatal exposure would be different between single and repeat dose studies. The JECFA and SCF evaluations[4,5] used the data from toxicokinetic studies[12,13] to model the fetal body burdens on GD16, on the assumption that the postnatal effects were the result of exposure of fetal tissue at GD16.

14 The COT used a similar approach, albeit with simpler toxicokinetic modelling[3]. Derivation of a tolerable intake for humans involved: calculation of the fetal body burden of rats under the experimental conditions; correction of the corresponding maternal body burden in rats to represent chronic daily intake via the diet; the use of uncertainty factors to give an equivalent tolerable human maternal body burden; and finally, derivation of a daily intake by humans that would result in the tolerable human maternal body burden.

The FSA funded Dioxins Risk Assessment project (T01034)

15 In evaluating the available toxicity data, the Committee identified gaps in knowledge related to the risk assessment of dioxins during pregnancy. In light of this, the FSA commissioned a developmental toxicity study, conducted in accordance with Good Laboratory Practice (GLP), using Computer Assisted Sperm Analysis (CASA) for robust collection of seminology data, and using large group sizes to increase the statistical power and reliability of the analyses. This project aimed to relate dose of TCDD to maternal burden, fetal burden and biological endpoints, within the same study. In view of the complexity of TCDD toxicokinetics, it was considered essential to have both an acute dose study, so as to be directly comparable with previous studies which dosed on GD15; and a sub-chronic repeat dose dietary study where TCDD administration is more representative of human exposure.

16 The Committee was presented with prepublication drafts of the papers discussing the acute[14] and sub-chronic[15] administration studies, together with the toxicokinetic data[16].

Acute Study

17 In the acute dosing study[14], groups of 75 control (vehicle alone) or 55 (50, 200 or 1000 ng of TCDD/kg bw) pregnant female Wistar(Han) rats were dosed by oral gavage on GD15. Tissues were harvested from 25 (control) and 15 (treated groups) animals killed on GD16 and 21. Tissue levels of dioxin were determined at GD16 and GD21 using a sensitive and specific gas chromatography-mass spectrometry (GC-MS) analytical method. These tissue levels were used to determine maternal and fetal body burdens[a]. About 25 animals per group were allowed to litter.

18 During the study, 4 dams experienced total litter loss in the 1000 ng/kg dose group, compared to 1 in the control group and none in the lower dose groups. There was no statistically significant effect of maternal treatment on the sex ratio of the F1 offspring. The offspring of the 1000 ng/kg dose group showed reduced body weight throughout the study, reduced pup body weights were also seen in the 200 ng/kg dose group in the first week *post partum*.

19 There were no adverse effects of maternal treatment when the pups were subjected to a functional observational battery and no significant findings when reproductive capability was assessed. There was a significant delay in balano-preputial separation (BPS), a marker of puberty, in the offspring of the 1000 ng/kg dose group. Although body weight on PND21 had a borderline significant effect on delay on BPS; adjusting for reduced bodyweight as a covariate did not materially affect the differences between the treatment groups. Therefore, there was no evidence that the delay in BPS was related to reduced body weight.

20 Seminology was assessed on PND70 and 120. A small but statistically significant increase in sperm count was observed at PND120 in the 200 and 1000 ng/kg dose group; however, this was not seen at PND70, was within the range of historical control data, and was not reflected in testicular sperm counts. The proportion of abnormal sperm was elevated at PND70, particularly in the high dose group, although this was not seen at PND 120.

Sub-Chronic Study

21 In the sub-chronic dosing study, groups of 75 control (diet alone) or 65 (diet with 28, 93 or 530 ng TCDD/kg diet) female Wistar(Han) rats were provided with respective diets *ad libitum*. These dietary levels equated to 2.4, 8 and 46 ng/kg bw/day. Doses were selected to give hepatic TCDD concentrations approximately equivalent to the acute study, as determined by extrapolation from Hurst's toxicokinetic data [12,13]. Dosing continued for 12 weeks pre-mating (to reach steady-state), throughout mating and pregnancy, and stopped after parturition. Tissue TCDD concentrations were compared on weeks 10 and 12 in the conditioning period and on GD16 and 21, which showed that the animals had reached equilibrium. Hepatic TCDD concentrations on GD16 were approximately 50% of the acute concentrations and covered a 10-fold range in total body burdens; thus making the acute and sub-chronic studies comparable.

[a] The tissue levels, and body burdens are not quoted in this statement, so as to not prejudice subsequent publication of these results. The Committee was provided with this information in confidence during their deliberations.

22 During the study, 8 dams experienced total litter loss in the high dose group, compared to 4 in each of the lower dose groups and 3 in the control group. The level of pup death was statistically significant in the high dose group.

23 A delay in BPS was statistically significant in all three dose groups following sub-chronic maternal administration, with the greatest delay apparent in the high dose group. The delay in BPS in treated groups remained significantly different from controls when both body weight and litter were considered as covariates.

24 As with the acute study there was no statistically significant effect on F1 sex ratio; and when reproductive capacity was assessed in male F1 offspring, no statistically significant effect was seen on mating parameters or on the sex ratio in F2 offspring. No statistically significant effects were observed when F1 offspring were subjected to a functional observational battery and learning tests, with the exception of a deficit in motor activity in the high dose group.

25 Seminology was assessed on PND70 and 120. Maternal exposure had no statistically significant effects upon these parameters, with the exception that the proportion of abnormal sperm was elevated at PND70 in the high dose group, although this was not seen at PND120.

Committee Discussion

26 The Committee discussed the relevance of the delay in BPS being greater during the sub-chronic study than in the acute study. It was questioned whether this might be evidence that increased lactational exposure in the sub-chronic study was contributing to the greater delay; however, it was considered possible that exposure prior to GD15 may have increased the delay. Members considered it would have been useful to have measured AGD, since this is now a routine technique in studies of this type. Similarly measurement of hormone levels, particularly testosterone, might have yielded insights into the delay in BPS; however, the Committee acknowledged that examination of hormonal endpoints had not been essential for the initial study objectives.

27 The delay in BPS was not clearly adverse since there were no changes in fecundity in F1 generation. However, a significant delay in puberty in animals treated at doses below lethality was a matter of concern. Members considered it possible that a functional deficit resulting from BPS may manifest itself as a subtle generational effect, or may not be apparent in the endpoints examined in this study.

28 The issue of variability amongst rat strains was discussed, since it is plausible that a strain that is less susceptible to the acute toxicity of dioxins might reveal an effect on androgen synthesis or spermatogenesis. However, it was noted that the original studies by Mably *et al.* which demonstrated the potent effect on sperm count in Holtzman rats[9] could not be repeated by Ohsako *et al.* in the same strain[6]. Furthermore, the study by Faqi and colleagues observed a reduction in sperm counts in Wistar rats[10], a closely related strain to the Wistar Han rats used in the FSA funded study[14,15]. Therefore, strain differences in dioxin susceptibility to these effects were considered unlikely, although it was noted that these are out-bred strains and the potential for strain drift cannot be ignored.

Implications for the 2001 TDI

29 Previously, a LOAEL maternal body-burden of 33 ng/kg bw had been calculated for the Faqi study[10] using toxicokinetics data from Hurst *et al.*[13]. Uncertainty factors accounting for human variation in toxicokinetics (3.2) and the use of a LOAEL (3) were applied to yield a human maternal body burden. This was converted to a human maternal dietary intake using a bioavailability of 50% and a half-life of 7.5 years for TCDD. This resulted in a dietary intake of 1.7 pg/kg bw/day, which was rounded to a TDI of 2 pg/kg bw/day due to the various uncertainties in the risk assessment.

30 In the FSA funded sub-chronic study, delay to BPS was observed in the lowest dose group, hence this study also provides a LOAEL. The maternal steady-state body burden for this dose group was determined analytically[a] to be very similar to that calculated for the Faqi study[10], which was used in the 2001 risk assessment. Thus, the LOAEL body burden from the FSA funded study provides additional evidence that the current TDI of 2 pg/kg bw/day is protective for the developing male fetus.

31 Data considered during the 2001 risk assessment, indicated that GD16-21 represents a critical window of exposure in the rat. However, whilst delay in BPS was seen following acute dosing on GD15 in the FSA funded study; sub-chronic administration, at doses which gave rise to similar maternal and fetal body burdens, resulted in a greater delay in BPS. The difference in magnitude of the effect highlights uncertainty regarding the critical window of exposure in the rat.

32 The more pronounced delay in BPS in the sub-chronic study may be due to fetal exposure to the maternal body burden in *utero prior* to GD15, possible increased postnatal exposure prior to puberty, or a combination of the two. The 2001 risk assessment assumed that the effects on the reproductive system of the male offspring resulted from *in utero* exposure to the maternal TCDD body burden. However, if the critical window of exposure is post natal, the differences in toxicokinetics and relative onset of puberty between rats and humans are likely to affect the relative susceptibility of the two species. In the absence of robust data relating to the critical window of exposure, it is appropriate to assume that the effects occurred in utero, since basic modelling of rat and human TCDD toxicokinetics indicated that this would result in a more conservative risk assessment.

Committee Conclusions

33 Having reviewed the FSA funded study[14,15,16], we are content with the study design and are satisfied that the statistical power of the study, seminology and analytical data are robust.

34 We consider that the new study provides additional evidence that the current TDI of 2 pg/kg bw/day is protective for effects on the developing male fetus. Therefore, review of the TDI is not a priority on the basis of this study.

COT Statement 2007/02
May 2007

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Statement on the COT workshop on evolving approaches to chemical risk assessment

Introduction

1 On 7th February 2007 the Committee held an open workshop on "Evolving Approaches to Chemical Risk Assessment". This workshop was designed to explore in more detail some of the approaches described in the Committee's report on Variability and Uncertainty in Toxicology of Chemicals in Food, Consumer Products and the Environment (COT 2007), which has now been published.

2 Invited expert speakers made presentations on techniques that the Committee may wish to exploit in future risk assessments. Members participated in discussions during the workshop and subsequently. This statement summarises the presentations and Committee's discussions.

Presentation Summaries

The Benchmark Approach [1]

3 The Benchmark dose (BMD) is defined as the dose associated with a pre-specified (small) effect size (Crump 1984). It is estimated from a statistical model fitted to the dose-response data. To take the statistical uncertainties in the data into account, a confidence interval around the BMD is calculated. The lower 95% confidence limit is often termed the BMDL. The BMDL may serve as a Reference Point (RP), or Point of Departure (PoD) for deriving a health-based guidance level for human exposure; e.g., Acceptable Daily Intake (ADI), Tolerable Daily Intake (TDI) or Reference Dose (RfD).

4 Dose-response data can be described by a model where the dose-response parameters are simply the observed frequency of responses (in the case of quantal data), or the observed average responses (in the case of continuous data) at each dose group. In this "saturated" or "full" model the number of parameters is equal to the number of dose groups. The aim of dose-response modelling is to replace the observed dose-response data with a smoother curve, produced by a dose-response model that contains fewer parameters than the number of dose groups. On the other hand, the dose-response model should still give an adequate description of the observed dose-response, i.e. the number of parameters should not be too small. Thus, the aim is to find a dose-response model containing the minimum number of parameters that still results in a satisfactory description of the dose-response data.

5 In practice, different models with the same number of parameters can often be found that all give a satisfactory fit to the same data. In the BMD approach the BMD(L)s are calculated for all acceptable models, resulting in a range of values. The range of BMD(L) values (partly) represents the uncertainty regarding the real shape of the dose-response, and is sometimes termed 'model uncertainty'. For data that are relatively poor (e.g., few dose groups, few animals, large scatter), the range of BMDL values will tend to be wider than for data that are relatively good (e.g., many dose groups, many animals, small scatter).

[1] BMD software was demonstrated during the presentation.

6 The available software allows the BMD approach to be applied without detailed statistical knowledge: the BMDS developed by the US Environmental Protection Agency (EPA), and Proast developed by the National Institute for Public Health and the Environment in the Netherlands (RIVM). These institutions have agreed to make both packages as consistent as possible, so that the outcomes from a dose-response analysis would not depend on the software applied (as long as the same assumptions are used). The BMDS software can be downloaded from the EPA website and is easy for the non-expert to use[2]. The Proast software needs to be implemented in a statistical software environment (*S-plus* is a commercially available software package or *R* is a free alternative available under a GNU General Public License[3]) and some basic knowledge in use of S-plus or R is required to access the Proast software. The advantage of Proast is that it contains more options than BMDS, for instance the inclusion of covariates (e.g. sex) in the modelling, bootstrapping and fitting non-monotonic models. Finally, Proast allows for probabilistic hazard characterisation.

Probabilistic exposure assessment modelling

7 The purpose of probabilistic methods in risk analysis is to quantify variability and uncertainty, so that it may be taken into account in a risk assessment. Variability is real variation in factors that influence exposure and effects, e.g. dietary differences between individuals, whereas uncertainty is caused by limitations in our knowledge of factors that influence exposure and effects, e.g. chemical concentrations that are too low to be quantified. It is important to separate variability and uncertainty in risk assessment because they have different implications for risk management: variability determines the frequency and severity of effects, e.g. what proportion of the population will experience a sublethal effect, whereas uncertainty determines the accuracy and precision of our assessment, i.e. how sure we are. It is usually desirable to keep variability and uncertainty separate. This may be achieved by hierarchical simulation (2-dimensional Monte Carlo) or hierarchical analysis.

8 Variability in exposure is often expressed by estimating a percentile from distribution of exposures in the population, while the most familiar expression of uncertainty is a confidence interval. Thus one possible output from a probabilistic exposure assessment is an estimate of the 97.5th percentile exposure, together with its 95% confidence interval.

9 Dietary exposure to a chemical in food depends primarily on the frequency and amount of contaminated food that is consumed, the concentrations of chemical in the food, and body weight (because exposure is expressed relative to body weight). Body weight and food consumption vary between individuals, and concentrations vary between food items. Deterministic exposure assessments do not attempt to quantify these sources of variation; instead they provide a single, usually conservative, estimate of exposure from selected values for consumption, concentration and body weight. This can be an effective tool for screening assessments but does not describe the variation of exposure in the population.

10 Probabilistic methods quantify variation in exposure, by using distributions to describe variation in consumption, concentration and body weight, then combining these to produce a distribution for exposure. The most commonly-used methods for this are bootstrapping and Monte Carlo simulation, and software for this has been developed by several organisations. Typically, these programs use data

[2] http://www.epa.gov/ncea/bmds.htm

[3] http://www.r-project.org/

on consumption and body weight from dietary surveys, and combine them with distributions for concentration. Recently, Wout Slob (2006) has argued that this type of procedure is inadequate because it does not separate different types of variation affecting consumption: variation in the frequency of consumption (e.g. proportion of days when potatoes are eaten) and variation within and between individuals in the amount of consumption (e.g. amount of potatoes eaten). He proposed that for many (but not all) types of exposure assessment it would be preferable to use statistical models to describe variation in frequency and amount of consumption, rather than performing calculations directly with the survey data. Depending on the situation (acute or chronic assessment, and daily or less frequent intakes), the statistical models can either be used on their own or as an input to Monte Carlo simulation. In either case, the output is a distribution describing the variation of exposure.

11 Exposure assessment may be affected by many uncertainties including measurement and sampling uncertainties affecting concentrations, consumption and body weight, uncertainty about the choice of parametric distributions to describe variability, uncertainties about extrapolations used to cover data gaps, uncertainty about model structure, uncertainty about correlations or dependencies between inputs, differences in expert opinion, excluded factors, and ignorance (the possibility that unknown factors may influence exposure). Examples from different areas of exposure assessment (e.g. pesticides, packaging, etc.) have recently been reviewed in an opinion of the European Food Safety Authority (EFSA 2006).

12 The presentation illustrated some of these approaches using a practical example concerning the acute exposure of children to carbendazim in apples and apple products. Interest in this example arose from a previous modelling study by Pennycook *et al.* (2004). Bayesian methods were used to model measurement and sampling uncertainties affecting concentration data, and this was combined with survey data on consumption and body weight.

13 It is neither practical nor necessary to quantify all uncertainties. However, it is essential to consider all identifiable uncertainties at least in a qualitative way, and evaluate their potential impact on the assessment outcome, so that this can be taken into account in decision-making (risk management). The EFSA (2006) opinion proposed a tiered approach in which uncertainties are initially considered qualitatively, with the option of progressing to sensitivity analysis or probabilistic modelling for the most influential uncertainties if this appears necessary to enable risk managers to reach a decision.

14 Until now, probabilistic approaches have generally been applied to individual exposure assessments, usually for a single chemical. Another important application of probabilistic approaches is to assist in the calibration of the procedures used in deterministic screening assessments. Although screening procedures are designed to be conservative, the level of protection they offer is generally unknown. This can be estimated by comparing deterministic screening calculations either to direct measurements of exposure (e.g. duplicate diet studies) or to probabilistic estimates of exposure. The EFSA opinion on the IESTI (International Estimate of Short Term Intake) equation, which is used in acute exposure assessment of pesticides, has now been adopted and will be published on their website in due course*.

* http://www.efsa.europa.eu/en.html

Probabilistic approaches to hazard characterisation and integrated probabilistic risk assessment

15 Although probabilistic approaches in risk assessment have mainly related to exposure assessment, they have also been developed for hazard characterisation. Briefly, uncertainty or assessment factors (AF) are applied not as single numbers (such as 10 or 3), but as (statistical) distributions. These AF distributions reflect the fact that each type of factor (e.g. inter-, or intraspecies, subchronic-to-chronic) is not a constant, but varies among chemicals. The challenge is to estimate these distributions from available data. For instance, for estimating the interspecies AF distribution, both animal and human toxicity data would be required. Since human data are not available for most chemicals, data from two animal species have been used as a surrogate. A number of NTP studies were analysed where the same compounds had been tested in both rats and mice. This resulted in an estimate of the interspecies AF distribution for these two species. A subchronic-to-chronic AF distribution was established in a similar way in another study. For intra-species variation, a distribution is less easy to estimate, and so far indirect arguments must be used in postulating a specific distribution.

16 When these distributions are applied to the reference point (which could be a NOAEL or a BMD with an associated uncertainty distribution), a distribution of the potential "safe" dose for the sensitive human can be derived. A lower percentile of that distribution may then serve as a probabilistic RfD. In this way, the level of conservatism associated with the (probabilistic) RfD may be harmonised among different risk assessments (which is currently not the case for the ADI/TDI).

17 A next step is to integrate such a probabilistic hazard characterisation with a probabilistic exposure assessment into an integrated probabilistic risk assessment. Two examples were discussed. For diethylhexylphthalate (DEHP), it was assumed that the variability in the exposure assessment is much larger than the uncertainties involved; therefore, the uncertainty can be ignored. For the hazard characterisation only uncertainties were considered. This approach results in a plot with fraction of the (sensitive) human population on the x-axis, and the probability that any individual would exceed the "no-adverse-human-effect-level" on the y-axis. In this way, both variability in exposure among individuals, and scientific uncertainties (due to lack of data) are made visible in the final risk characterisation (Bosgra 2005).

18 The second example related to acephate (an organophosphate), which may be present on fruits and vegetables. Here, a further step was taken: both variability and uncertainty were accounted for in both the exposure assessment and the hazard characterisation. The approach followed was to specify the probability that a random individual from the human population would have an exposure high enough to cause a particular health effect of a predefined (but small) magnitude, the critical effect size (CES), such as a 20% decrease in acetylcholinesterase-activity. The exposure level that results in exactly that CES in a particular person is that person's individual critical effect dose (ICED). Individuals in a population typically show variation both in their individual exposure (IEXP) and in their ICED. Both the variation in IEXP and the variation in ICED are quantified in the form of probability distributions. Assuming independence between both distributions, they are combined (by Monte Carlo) into a distribution of the individual margin of exposure (IMoE). The proportion of the IMoE distribution below unity is the probability of critical exposure (PoCE) in the particular (sub)population.

Uncertainties involved in the overall risk assessment (i.e., both regarding exposure and effect assessment) were quantified using Monte Carlo and bootstrap methods, resulting in an uncertainty distribution for the probability of critical exposure (PoCE). From these calculations plots could be derived that concisely summarised the probabilistic results, retaining the distinction between variability and uncertainty.

19 The advantage of this approach (compared to the first example) was that, for any particular exposure situation, the plot shows: the fraction of the population that would exceed their (personal) critical dose, the extent of exceedance, together with the uncertainties around it. In addition, the relative contributions from the various sources of uncertainty involved could be quantified. The latter information makes clear which uncertainties in the overall risk assessment are greatest and deserve primary attention (Van der Voet and Slob 2007).

Exploring Uncertainty Using Sensitivity Analysis

20 Probabilistic risk assessments that use a mathematical model generally assume that the model is 'correct'. In reality, uncertainty from parameters and model structure propagate through to model predictions. The minimisation of these uncertainties is central to producing a meaningful risk assessment.

21 Sensitivity analysis is a tool that can focus model corroboration, direct research and prioritise additional data collection. However, sensitivity analysis describes a host of distinct techniques, each with their own strengths and applicability to the questions faced when developing and analysing a model. Whilst the more commonly used 'local' methods are computationally inexpensive and provide information on model behaviour for specific parameter combinations, their results are often misinterpreted as providing general statements to the behaviour of non-linear models. The use of a model-independent, quantitative, global sensitivity measure offers insight into model behaviour that is not provided by local methods.

22 Discussion focussed on an approach to global sensitivity analysis that uses an initial screening by the Morris method to identify the model's most influential parameters, followed by application of the Extended Fourier Amplitude Sensitivity Test (FAST) (Morris 1991, Saltelli *et al.* 1997). These methods were chosen on the basis of their applicability to diverse model structures; computational cost; complexity of their application and representation of the sensitivity. A didactic example of a sensitivity analysis performed on two physiologically based pharmacokinetic (PBPK) models was analysed. Differences between the results provided by local and global techniques were considered and methodologies determined for: model reduction, parameter estimation, model corroboration, and identification of subgroups susceptible to toxic effects within a population.

23 The methods examined could drastically reduce the time and effort involved in producing population models that predict human variability. They also provide a means for focusing research on the parameters that will provide the greatest increase in confidence in the model predictions.

Framework Approaches in Risk Assessment and Weight of Evidence Considerations

24 Structured frameworks are extremely useful in promoting transparent, harmonised approaches to the risk assessment of chemicals. There has been particular activity in developing a systematic approach to determining the mode of action of the carcinogenic effects of chemicals in experimental animals and to evaluating the potential human relevance of these effects. This work led to a publication by the IPCS (Boobis *et al*, 2006), which was an update of an earlier publication of a mode of action framework in animals (Sonich-Mullin *et al*. 2001). The first stage of the approach is to determine whether it is possible to establish a hypothesised mode of action on the basis of the experimental data. This comprises a series of key events along the causal pathway to cancer, identified using a weight of evidence approach based on the Bradford Hill criteria. The key events are then compared first qualitatively and then quantitatively between the experimental animals and humans. Finally, a clear statement of confidence, analysis and implications is produced.

25 More recently, this work has been extended to non-cancer effects. The ultimate objective is to harmonise framework approaches to cancer and non-cancer endpoints. The process for non-cancer endpoints is very similar to that for cancer endpoints. The first step is to determine whether, on the basis of experimental data, the weight of evidence is sufficient to establish an hypothesised mode of action, using an approach based on the Bradford Hill criteria (Hill 1965). This is followed by a qualitative and then a quantitative comparison of the key events between experimental animals and humans. Finally, there should be a clear statement of the conclusions, together with the confidence, analysis and implications of the findings.

26 Such frameworks enable a more transparent evaluation of the data, identification of key data gaps and a structured presentation of information that would be of value in the further risk assessment of the compound, even if it is not possible to exclude relevance to humans. For example, there may be data on the shape of the dose-response curve, identification of thresholds or recognition of potentially susceptible sub-groups, based on genetic or life stage differences, for example.

Meta-analysis and the Combination of Epidemiological and Toxicological Evidence

27 Improving the design of individual animal studies is a key strand of the NC3Rs[4] programme, but the next stage in the decision process, reviewing and combining results from individual primary studies, also needs attention; the 3Rs need to be supplemented by a 4th R: (Systematic) Review. Systematic review and meta-analysis methods (Sutton *et al*. 2000, Egger *et al*. 2001) are widely used to summarise and combine results of clinical trials, forming the basis for evidence-based medicine[5]. They are increasingly popular in epidemiology, and in health and social policy areas, but they remain relatively rare in toxicology. The talk included some results from a recent systematic review of the use of systematic review and meta-analyses in animal experiments (Peters *et al*. 2006). Low uptake of systematic review and meta-analysis in toxicological contexts may stem partly from the perception that they conflict with selection of pivotal primary studies on grounds of study quality and relevance. In fact systematic review and meta-analysis do not require uncritical pooling of all evidence, just explicit criteria for any selectivity.

[4] National Centre for the Replacement, Refinement and Reduction of Animals in Research. See http://www.nc3rs.org.uk/ (last accessed 08 January 2007).

[5] Cochrane Collaboration. See http://www.cochrane.org/ (last accessed 08 January 2007).

28 Systematic review and meta-analysis methods can contribute at two stages in the use of evidence from animal studies: for the review and combination of results i) of animal studies alone, and ii) of animal studies with evidence from humans. Application of two Bayesian synthesis approaches (extensions of the basic meta-analysis method) to the latter are briefly described here (Peters *et al.* 2005, DuMouchel and Groër 1989). The methods offer an approach to formalisation of the use of 'uncertainty factors' for inter-species effects. Transparency in a systematic review regarding the various assumptions made to identify, obtain and select relevant evidence of an appropriate quality allows reproducibility, and facilitates updating as new evidence becomes available. Quantitative synthesis of results from several primary studies offer greater statistical power and more precise estimates than the primary studies on which they are based, and provide a framework for investigation of sources of heterogeneity and quantitative sensitivity analyses.

Committee Discussion

BMD Approach

29 The BMD approach was considered to offer benefits over the NOAEL approach, since it takes the variability in the effects seen at each dose into account. There is implicit model uncertainty since the BMD approach is merely the pragmatic application of models to dose response data. It is generally appropriate to select the most conservative BMDL, having excluded biologically implausible models. Failure to fit any of the available models suggests that there are insufficient data upon which to base a robust analysis; however, Members were concerned that data available for many of the assessments conducted by COT may be inadequate for dose-response modelling.

30 The COT had used a BMDL in establishing a TDI for perfluorooctanoic acid (PFOA)[6]. This approach was taken because the lowest NOAEL of 0.06 mg/kg bodyweight (bw) per day was for hepatotoxicity in a 90-day study, whereas a 2-year study conducted in the same strain of rat indicated a much higher NOAEL of 1.3 mg/kg bw/day. Modelling both sets of data resulted in BMDL values of 0.3 and 0.74 mg/kg bw/day, respectively, hence reducing the apparent inconsistency between the two studies. It was considered premature for the Committee to adopt the BMD as the default approach until each step of the process – its theoretical basis, implicit assumptions and practical application – are thoroughly understood. It would be inappropriate to view the BMD as a superior NOAEL. Movement from the NOAEL to the BMDL would mean risks would be expressed in terms of level of effect (BMDL) rather than level of no effect (NOAEL); therefore, risk assessments might be better communicated in terms of level of protection. In addition, the acceptable risk for the critical effect size needs to be further considered.

31 The application of the BMD approach to data from two recent COT evaluations, providing examples of a rich and poor data set, might aid the Committee's deliberations. It was also noted that the European Food Safety Authority (EFSA) is also evaluating the BMD approach. The result of this evaluation should inform Committee discussions.

[6] http://www.food.gov.uk/multimedia/pdfs/cotstatementpfoa200610.pdf

Allometric Scaling

32 Allometric scaling between different animal species and humans is based on the basal metabolic rate. It may therefore be more appropriate than bodyweight scaling, for chemicals whose clearance from the body is determined by basal metabolic rate. However, many chemicals assessed by the Committee rely upon specific enzymes and protein transporters for their toxicity and/or elimination. The specificity and level of expression of these proteins can differ vastly between species and may not necessarily scale allometrically. It is not possible to predict chemicals for which allometric scaling is appropriate, which complicates the use of allometric scaling as the default method.

Assessing Uncertainty

33 The COT report on Variability and Uncertainty in Toxicology of Chemicals in Food, Consumer Products and the Environment emphasises the need for uncertainty to be taken into account. Expression of uncertainty is also required in the Science Checklist[7] recently adopted by scientific committees that provide advice to the Food Standards Agency. The Committee agreed that more consideration should be given to communication of uncertainty, qualitatively and/or quantitatively; perhaps adopting a formal method for recording the uncertainty in Committee statements. However, Members noted that quantification of uncertainty in an integrated risk assessment could lead to spurious precision which may be misinterpreted.

Probabilistic Approaches to Hazard Characterisation, Exposure and Risk Assessment

34 Probabilistic approaches offer some advantages in refinement of risk assessment, but may have greater requirements for data and resources. These higher tier methods could be used as required, on a case-by-case basis.

Framework Approaches to Risk Assessment

35 Framework approaches can offer greater transparency to the risk assessment process. They should be used, when appropriate, and follow the IPCS recommendations (Boobis *et al*, 2006).

Systematic Review and Meta Analysis

36 Guidance has been recently given to the Secretariat regarding the conduct of literature searches and reviews. This requires search terms to be included as an annex to Committee papers. The recent systematic review of fume events (TOX/2007/010, Annex 10) was cited as an example where such a review is very helpful. Bias against publishing negative results can affect the results of all review techniques, although meta analysis can make this bias more clear.

37 Formal combined analysis of epidemiological and toxicological data in a meta-analysis is appealing; however, there are a number of unresolved issues. Toxicology studies are often complex and differences in experimental protocol (such as day of dosing) may need to be taken into account during a systematic review or meta analysis. It is important that those conducting a systematic review are

[7] Annexe 4 of http://www.food.gov.uk/multimedia/pdfs/fsa060207.pdf

clear in reporting how the review has been conducted. Similarly, a common situation arises when several different sets of animal data provide conflicting NOAELs (or BMDLs). Statisticians suggest simply combining the data by meta-analysis would be unwise, and it may not be possible to provide a satisfactory explanation for differences.

38 Epidemiological data should always be interpreted in the context of available experimental data from animals, particularly when considering the plausibility of causation as an explanation for observed associations. It may be preferable to compare the results of separate reviews of human and animal data. It is important that the relative weight given to human and animal data should be clearly reported, considering sensitivity analysis where appropriate. It was suggested that meta-analysis be attempted on an example from the future COT agenda.

Overall Conclusions

39 The workshop emphasised the need to more explicitly assess and describe the uncertainty in the available data; many of the methods included in the workshop offer the opportunity to do this. The use of more transparent and reproducible methods is also important, such as framework approaches and systematic rather than narrative review.

40 Adopting new approaches should be carefully considered and only implemented if they offer a clear benefit in terms of improving the risk assessments provided by the Committee. Where possible and where appropriate, new approaches should be initially performed in parallel with existing methods, allowing for further investigation of divergent outcomes.

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Dr Martin Spendiff - Health and Safety Laboratory, Buxton, UK

Professor Alan Boobis OBE - Division of Medicine, Imperial College, London, UK

Professor David Jones - Department of Health Sciences, University of Leicester, UK

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Statement on research project (T07040) investigating the effect of mixtures of certain food colours and a preservative on behaviour in children

1 The COT was asked by the Food Standards Agency to review the results of a research project investigating the effect of two mixtures of certain artificial food colours together with the preservative sodium benzoate on behaviour in children. The study had been carried out by researchers at the University of Southampton and was funded by the Food Standards Agency. The research had been submitted for publication and the COT was provided with three draft scientific manuscripts and a commentary that had been written by the researchers, for review. The research was subsequently published as a single paper in the Lancet[1].

2 The Committee was grateful for the advice of a number of external experts which informed its discussion of this research. These were Prof. Eric Taylor and Prof. Emily Simonoff of the Institute of Psychiatry, Ms Eleanor Allan of the University of Reading Statistical Services Centre, and Prof. Ian Kimber as Programme Advisor to the Agency's T07 Food Allergy & Intolerance Research Programme, under which this project was commissioned.

Background

3 Hyperactivity, is a term that is somewhat ill defined but is used by most people to mean overactivity. To others it is associated additionally with inattention and impulsivity. Inattention, impulsivity and hyperactivity occurring together, and to a significant degree, comprise a behavioural disorder which adversely affects children's function at home and in school. This disorder is known as Attention Deficit Hyperactivity Disorder (ADHD) or Hyperkinetic Disorder (HKD). ADHD typically has onset in early childhood and is characterised by specific patterns of behaviour[2]. A review of international studies by Swanson *et al.* in 1998 suggested that the condition affects 5-10% of school age children[3]. In the UK, the best estimate of prevalence in children is 2.4%, based on data from a survey of 10,000 nationally representative children in the 1999 British Child and Adolescent Mental Health Survey[4]. The aetiology of the disorder is thought to be multifactorial, with both genetic (heritable) and environmental factors reported to be involved (the latter including for example, prematurity[5], institutionalised upbringing[6], and maternal smoking during pregnancy[7]).

4 The COT had considered the results of a previous research study known as 'the Isle of Wight Study'[8], on the effect of food colours and a preservative on behaviour in children and issued a statement on that research in 2002 (statement available at http://www.food.gov.uk/science/ouradvisors/toxicity/statements/cotstatements2002/cotfoodadditives). The Committee had reservations about interpretation of the findings in view of some aspects of the study design. The Committee noted that the results were consistent with published reports of behavioural changes occurring in some children following consumption of particular food additives. However, it was not possible to reach firm conclusions about the clinical significance of the observed effects. There had been a large placebo effect which had limited the ability to interpret and make generalisations about the results. In addition, statistically significant effects on behaviour had been observed only via parental reports of their children's behaviour, and had not been evident in the objective assessments that had been performed by independent researchers in a clinical setting.

5 Subsequently, the Food Standards Agency set up an ad-hoc Working Group of independent experts to consider the feasibility of further research on this subject and to advise on study design. The recommendations of this ad-hoc Working Group were published in 2003 and the Agency commissioned a new study via open competition in 2004, incorporating the design changes that had been recommended by the ad-hoc Working Group. It was the results of this new study that the COT was asked to review.

Study design for the new research

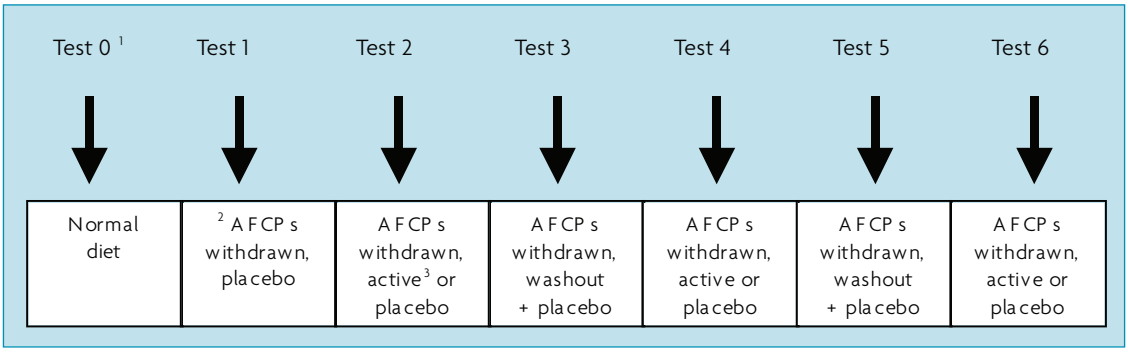
6 The primary hypothesis tested by the researchers was that mixtures of certain artificial food colours with the preservative sodium benzoate compared with a placebo increase the mean level of hyperactive behaviour of children drawn from the general population. With a minimum target sample of 80 children, the study had 80% power at alpha = 0.05 to identify an effect size of 0.32 standard deviation units (SDU) in the mean score on a hyperactive behaviour scale for the active compared with the placebo periods of the food challenge.

7 Secondary research questions addressed: whether genetic differences moderate any observed effect; whether there are effects in both pre-school and older children; whether any response to the additive mixtures is related to initial levels of hyperactive behaviour as scored on a hyperactive behaviour scale: and whether any response is seen via teacher ratings, direct observations of behaviour and computer based test performance as well as via parental ratings.

8 The researchers employed a double blind placebo controlled randomised cross-over food challenge to investigate the effect of two different mixtures of additives on the behaviour of children of both sexes and in two age groups. Children who took part in the study were selected from families volunteering from, in the case of 3 year olds, nurseries, day nurseries, preschools, playgroups and, in the case of 8 to 9 year olds, schools, in the Southampton area. Although there was a degree of self-selection in that families volunteered to take part in the study, the children that were recruited to the study (153 aged 3 years and 144 aged 8 to 9 years) from those who volunteered (n=209 and 160, respectively), were selected to represent the full range of behaviour in the general population, from normal through to high level hyperactivity. However, children who were on medication for ADHD or for whom it was considered by the researchers that the additive challenge could compromise medical treatments being given for other conditions, were excluded from the study.

9 The families were given instructions that the children should maintain, for the duration of the study, a diet that excluded the artificial food colours used in the trial and sodium benzoate used as a preservative. Compliance with the diet was monitored by means of a diary which parents completed to indicate the level of consumption of the challenge drinks and compliance with the diet over the study period. The outline design of this sub-acute challenge trial, which formed the main part of the study, is shown in the following diagram:

Fig. 1 Design of the double blind placebo controlled food challange



[1] 'Test': assessment of children's behaviour.

[2] AFCPs (Artificial Food Colours and Preservatives) withdrawn: exclusion from the diet of those artificial food colourings and of the preservative sodium benzoate, which were used in the active mixtures.

[3] 'Active': either of two specific mixtures of food colours and sodium benzoate.

10 During the 6 week challenge, children received batches of drinks on a weekly basis, one drink to be consumed on each day. Instructions to parents were that the challenge drinks should be consumed at home so that compliance could be monitored. During the wash-out weeks (weeks 1, 3 and 5) all children received a placebo drink of mixed fruit juices. During the challenge weeks (weeks 2, 4 and 6 in Fig. 1), the drinks that children received were either the placebo, or a drink of juices of identical appearance and taste containing one or other of the two additive mixtures. The order of receipt of the three drink types (Mix A, Mix B or placebo) across the three challenge weeks was allocated at random. Blinding tests conducted at the beginning and part way through the study established that two independent panels of 20 adults of similar age to the parents of the children in the study could not distinguish between the active and placebo drinks, but blinding was not assessed in children. Behaviour was assessed in each week of the study to avoid a perceived difference in treatment, but data deriving from weeks 1, 3 and 5 (the washout weeks) were not included in the analyses.

11 Behaviour was assessed using a range of different measures, including assessments by parents in the home, and by teachers and independent observers in a classroom setting. For the older children only, behaviour was additionally assessed via a computer-based attention task. For each individual measure, behaviour was scored using standardised and validated hyperactive behaviour assessment tools. Parents and teachers were asked to rate each child's behaviour over the previous week and independent assessors observed each child for 3 separate periods each week. Ratings of behaviour from each of the individual measures (teacher, parent, independent observer and computer task) were combined, un-weighted, to give an overall weekly Global Hyperactivity Aggregate (GHA) score of each child's level of hyperactive behaviour. This GHA measure of behaviour was a novel metric devised by the researchers to derive an overall outcome measure that combined both subjective and objective behavioural measures.

12 During the food challenge trial, DNA from buccal swabs collected from all children participating in the challenge was subjected to genotype analyses. The aim was to determine whether allelic variation in certain genes that have previously been implicated in ADHD influenced any observed effects of the food colour and benzoate preservative test mixtures on the children's behaviour. The genes studied included genes from the dopamine neurotransmitter system (gene catechol-o-methyltransferase, polymorphism COMT Val108Met), from the adrenergic neurotransmitter system (gene ADRA2A, polymorphism ADRA2A C1291A), and from the histamine neurotransmitter system (gene HNMT, polymorphisms HNMT T939C and HNMT Thr105Ile).

13 The primary analysis of the data from the main 6 week repeat dose challenge trial was on an intention-to-treat basis (i.e. including data obtained from the whole cohort), and was based on use of the GHA as the primary outcome measure. The researchers also carried out a number of additional post-hoc analyses on the data. These included analysis of the GHA data for a sub-set of the subjects (approximately 80% of total) who had consumed ≥ 85% of the drinks. This was a pragmatic level chosen to represent the equivalent of full consumption on 6 out of 7 days in a challenge week. A further *post-hoc* analysis of the GHA data based on another sub-set of the subjects who had consumed ≥ 85% of the drinks and for whom there were no missing data, was also conducted. Finally, the researchers conducted some analyses on the data relating to the disaggregated behaviour measures (i.e. analysis of the behaviour scores from the parental assessments, teacher assessments, independent observer assessments and from the computerised test of attention, separately) for the sub-set who had consumed ≥ 85% of the drinks and subsequently for the whole cohort. All of these analyses used data from behaviour assessments made in the baseline week (Week 0) and in weeks 2, 4 and 6 of the food challenge.

14 Details of the identity and dose of the additives in the challenge mixtures are given in Table 1. The doses were determined by the researchers based on the amount of the additives to be administered per child per day. Both additive mixtures administered to both age groups contained the same amount of sodium benzoate. For the colours, the amounts in Mix A given to 3 year olds were identical to those used in the previous (Isle of Wight) study. For 8 to 9 year olds the amounts of the colours in Mix A were increased by 25% to reflect the greater food intake by these older children. For Mix B for 8 to 9 year olds, the amounts of the colours in the mixture reflected what a child could reasonably consume in a day and were based on average consumption of foods containing colours with the assumption that the colours were included in those foods at their maximum permitted levels. Constraints regarding the maximum concentration of additives in the test drinks, which could not exceed the regulatory limits, meant that, for 3 year olds to consume equivalent amounts of Mix B colours to the older children, they would have been required to consume a 500ml drink on a daily basis. This was not regarded as feasible by the researchers and was considered likely to affect compliance adversely. Therefore, the volume of Mix B in the daily drink given to the 3 year olds was kept at 300ml which necessitated a consequential reduction in amounts of the Mix B colours that could be administered to this age group as shown in Table 1.

15 For the purposes of the COT evaluation and comparison with the Acceptable Daily Intake (ADI), the doses are also expressed on a mg/kg body weight (bw) basis in Table 1. These were calculated using average body weights for the two age groups obtained from UK National Diet and Nutrition Survey data[9,10], because the actual body weights of the children in the study were not recorded. On a mg/kg bw basis the younger children received higher doses of the additives in Mix A, whereas for Mix B the doses were comparable across the age groups.

Table 1: Composition of the food additive challenge mixtures used in research project T07040

Name of Additive (E number)	ADI[1] (mg/kg bw)	Mix A 3 year olds mg/day (mg/kg bw/day)[2]	Mix B 3 year olds mg/day (mg/kg bw/day)[2]	Mix A 8 to 9 year olds mg/day (mg/kg bw/day)[3]	Mix B 8 to 9 year olds mg/day (mg/kg bw/day)[3]
Tartrazine (E102)	7.5	7.5 (0.50)	0	9.36 (0.30)	0
Ponceau 4 R (E124)	4	5.0 (0.33)	0	6.25 (0.20)	0
Sunset Yellow (E110)	2.5	5.0 (0.33)	7.5 (0.50)	6.25 (0.20)	15.6 (0.50)
Carmoisine (E122)	4	2.5 (0.17)	7.5 (0.50)	3.12 (0.10)	15.6 (0.50)
Quinoline yellow (E104)	10	0	7.5 (0.50)	0	15.6 (0.50)
Allura Red AC (E129)	7	0	7.5 (0.50)	0	15.6 (0.50)
Total colouring per day (mg)		20	30	25	62.5
Volume of drink given daily (ml)		300	300	625	625
Concentration of colour in mg/L		66.7	100	40	100
Sodium benzoate (E211)	5	45 (3)	45 (3)	45 (1.45)	45 (1.45)

1 The ADI is an estimate of the amount of a substance in food or drink, expressed on a body weight basis, that can be ingested daily over a lifetime by humans without appreciable health risk.

2 Based on average body weight of 15kg for a 3 year old ref 9

3 Based on average body weight of 31kg for an 8 year old ref 10

16 The researchers also included a 'proof of principle' acute challenge to explore the possibility of demonstrating short term changes in hyperactive behaviour immediately post challenge. This comprised a double blind cross-over acute challenge study in a sub-set of two groups of 15 of those 8 to 9 year old boys who were considered to have responded or not responded to the additives in the 6 week sub-acute challenge trial. Mix B or placebo, was administered and the children's behaviour was then assessed over a three hour period using independent observer ratings and the specific computer based attention task.

Differences in the study design compared with the previous Isle of Wight study

17 The design of the new study had incorporated the following key changes compared with that of the previous study conducted on the Isle of Wight. A drink was administered to children daily throughout the 6 week challenge period, including the initial withdrawal period, with the aim of reducing the placebo effect that had been observed in the previous study. A second, older group of children (8 to 9 year olds) was included, in addition to conducting the trial on 3 year old children as in the Isle of Wight study. A second mixture of additives (referred to as Mix B) was included with a different combination and amount of food colours from that administered to children in the Isle of Wight study (referred to as Mix A). The inclusion of an older group of children and of a second mixture of food colours and sodium benzoate at levels that were reflective of what an average child could consume in a day was in line with the recommendations of the ad-hoc Working Group.

18 Behaviour was assessed using a wider range of measures than had been used in the Isle of Wight study. Teacher and independent observer assessments were conducted in a normal classroom setting and aggregated with the parental ratings (and for the older children only, with the results of the computer-based attention task), into the GHA score. This GHA score was the primary outcome measure for the study. It was formulated by the researchers to enable incorporation of both the objective assessments of behaviour (collected in a real life setting), and the subjective assessments of behaviour, into a single outcome measure, in order to address a concern raised in relation to the previous study that effects had only been detectable via the parental assessments and not by the more objective assessments of behaviour performed in the clinic.

Results

19 The results presented in this section are based on the statistical analyses carried out by the researchers in which the effects of certain possible confounders were adjusted for within the analysis. The factors controlled for were: week during the study; sex; base-line GHA; number of additive containing foods consumed per day in the pre-trial diet; maternal educational level and social class.

20 Table 2 summarises the results of the primary data analysis on the GHA scores (on the whole cohort), and also the results of the *post-hoc* analysis performed on the sub-group which consumed ≥ 85% of the drinks and for whom there were no missing GHA data.

Table 2: Summary of analysis of changes in GHA scores following challenge with Mix A or B compared with placebo, for the whole cohort (primary analysis) and a sub-group consuming ≥ 85% of the challenge drinks and no missing data (*post-hoc analysis*)

		Mix A	Mix B
whole sample (primary analysis)	3 year olds (n = 140)	0.20 (0.01 to 0.39)*	0.17 (-0.03 to 0.36)
	8 to 9 year olds (n = 136)	0.08 (-0.02 to 0.17)	0.12 (0.03 to 0.22)*
≥ 85% consumption and no missing GHA data (*post-hoc analysis*)	3 year olds (n = 73)	0.32 (0.05 to 0.60)*	0.21 (-0.06 to 0.48)
	8 to 9 year olds (n = 91)	0.12 (0.02 to 0.23)*	0.17 (0.07 to 0.28)*

* Statistically significant (at $p < 0.05$)
Scores are expressed as mean SDU with 95% confidence intervals in parentheses

21 The researchers found a statistically significant increase in the level of hyperactive behaviour, as measured by the GHA scores, when the children were challenged with Mix A compared with the placebo in the whole group of 3 year olds. The mean increase was 0.20 SDU (95% CI 0.01 to 0.39 SDU), n = 140. In the whole group analysis for the 8 to 9 year old children, the mean increase was 0.08 SDU (95% CI -0.02 to 0.17 SDU), n = 136 which was not statistically significant. The slightly lower numbers of children included in the analysis ('n'), compared with the numbers originally recruited (detailed in paragraph 8) reflect that a few children from each age group dropped out of the study after the trial had started. Drop-outs occurred for a variety of reasons, including parental pressure of work or other commitments, medical reasons, behaviour related to the child or inadequate juice consumption. No differences were found in terms of age, gender or marital status of parents between those who dropped out and the resulting cohort.

22 The results of the whole group analyses for Mix B were rather more consistent across age groups although here, too, statistical significance was reached in only one of the age groups. A statistically significant increase in the GHA scores was reported for the 8 to 9 year olds (mean increase = 0.12 SDU, 95% CI 0.03 to 0.22 SDU). For 3 year old children, the mean change in behaviour score was of similar magnitude (0.17 SDU), but with a wider 95% confidence interval (-0.03 to 0.36 SDU).

23 Similar changes in the mean GHA scores were seen in the post-hoc analysis of the subgroup consuming 85% or more of the drinks and for whom there were no missing data. For Mix A, the mean increases compared with the placebo were 0.32 SDU (95% CI 0.05 to 0.60 SDU) in the 3 year olds and 0.12 SDU (95% CI 0.02 to 0.23 SDU) in the 8 to 9 year olds, both of which were statistically significant increases. For Mix B, the mean increases compared with the placebo were 0.21 SDU (95% CI -0.06 to 0.48 SDU) in the 3 year olds and 0.17 SDU (95% CI 0.07 to 0.28 SDU) in the 8 to 9 year olds. Here the increase was statistically significant only in the case of the 8 to 9 year olds.

24 The observed increases in the GHA scores were not statistically significantly modified by sex, pre-trial level of hyperactive behaviour, additive content of the children's pre-trial diet, maternal education level or maternal social class,

25 Based on consideration of the subgroup of children who had consumed ≥85% of the challenge drinks, the researchers found that the observed increases in the GHA scores with Mix A in 3 year olds and 8 to 9 year olds and with Mix B in 8 to 9 year olds were statistically significantly associated with differences in genotype, specifically with two genetic polymorphisms thought to impair histamine clearance (histamine N-methyltransferase, HNMT Thrlle105 and/or HNMT A939G).

26 In their draft final technical report[11] the researchers presented a post-hoc analysis of the disaggregated behaviour measures in the subgroup consuming 85% or more of the challenge drinks. Table 3 summarises the results of these analyses. The only statistically significant changes were in the parental measures for Mix A in 3 year olds and for Mix B in 8 to 9 year olds. Changes in the other measures (teacher assessments, independent observer assessments or computer based performance task) were mostly in the same direction, but were not statistically significant and the mean differences were very small.

Table 3: Summary of disaggregated analysis of changes in behaviour measures assessed following challenge with Mix A or B compared with placebo, based on subgroup consuming ≥85% of the challenge drinks

	Mix A		Mix B	
	3 year olds	8-9 year olds	3 year olds	8-9 year olds
Parental score	0.49 (0.09-0.89)*	0.03 (-0.10 to 0.16)	0.36 (-0.04 to 0.76)	0.13 (0.00 to 0.25)*
Teacher score	0.03 (-0.11-0.16)	-0.01 (-0.12 to 0.09)	0.08 (-0.05 to 0.21)	0.01 (-0.09 to 0.11)
Classroom observation score	0.10 0.07-0.27)	0.08 (-0.07 to 0.22)	-0.01 (-0.18 to 0.16)	0.05 (-0.09 to 0.19)
Computer-based task score	N.D.	0.08 (-0.16 to 0.32)	N.D.	0.20 (-0.04 to 0.43)

* Statistically significant (at $p < 0.05$)
Analyses were conducted on the data for the subgroup consuming ≥85% of the challenge drinks.
The different measures focus on differing aspects of hyperactive behaviour in differing contexts.
Scores are expressed as mean SDU with 95% confidence intervals in parentheses
N.D.: not determined

27 Subsequent analysis by the researchers of the disaggregated measures for the entire cohort indicated a smaller increase in the mean parental score for Mix A in the 3 year olds, which was not statistically significant ($p = 0.058$). For the entire cohort of 8 to 9 year old children, the increases in mean parental scores and associated confidence intervals for Mix A and Mix B were similar to those seen in the ≥ 85% consumption subgroup analysis.

28 No statistically significant differences in hyperactive behaviour were found in the acute challenge study, which was conducted on a sub-set of the older children, using Mix B only, with assessments based on independent observer ratings and computer-based tasks, but not parental or teacher observations.

Committee discussion

Design of study T07040

29 The Committee noted the changes that had been made to the design of the study compared with the previous Isle of Wight Study, which had improved the statistical power of the study to be able to detect behavioural effects. The administration of a drink daily throughout the challenge trial largely overcame the placebo effects that had been a major concern of the previous study design.

30 The dose levels of the individual additives in the two food challenge mixtures were relevant to dietary intake levels of these additives in these age groups of children, and were below the respective ADIs. The fact that the researchers had used, in one of the mixtures (Mix A) the same combination of additive colours and a preservative at the same dose as was used in the Isle of Wight study, enabled comparison with the results of that previous study. The addition of a second challenge mixture into the study design (Mix B) consisting of a combination of additive colours and a preservative more commonly found in children's foods at the time the present study was commissioned, and at higher dose levels to represent higher intake levels, represented a further improvement to the study design.

31 However, the Committee noted some limitations in the study design and analysis. The timing of the assessments of behaviour in relation to the administration of the drinks appeared to be based on an assumption that any effects would be long-lasting. The time of day the drink was to be consumed was not defined in the instructions to parents and therefore it might not have been optimal for relatively transient effects to be observed. Recording of the children's body weights would have allowed a more accurate assessment of the administered doses, and comparison with effects in individual children. The initial exclusive use of the GHA in the primary analysis did not allow assessment of the relative contributions of the parental and other more objective measures of behaviour, although results from analyses of the disaggregated measures were provided subsequently by the researchers. Analysis of the GHA scores in the wash-out weeks of the study would have provided useful information on intra-individual variability over time.

The findings of the study

32 The study showed increases in the levels of children's hyperactive behaviour when they were challenged with combinations of particular food colours together with sodium benzoate, compared with a placebo. However, the increases were not consistently statistically significant for the two mixtures or in the two age groups.

33 Based on the primary outcome for the whole unselected cohort, there was an increase in the mean GHA score associated with both mixtures compared with the placebo, for both age groups, which reached statistical significance for Mix A in the 3 year old children and for Mix B in the 8 to 9 year olds. For Mix A, the dose was slightly higher for the 3 year olds than for the 8 to 9 year olds when expressed on a body weight basis, which might have contributed to the difference in the magnitude of the increase in the GHA. For Mix B, there was no difference in dose between age groups, when expressed on a body weight basis. Influence of dose between the mixtures is more difficult to assess as two of the four food colours in each mixture were different.

34 The results of the post-hoc analyses of the GHA scores, carried out on data from a sub-set of the subjects, were broadly consistent with the primary analysis. The Committee noted that a subsidiary analysis of compliant subjects was a reasonable approach but it would have been preferable if criteria for selection of the sub-set had been defined in the original study protocol.

35 Although not all risk estimates reached statistical significance, all showed a small increase in the mean GHA score associated with consumption of Mix A or Mix B. This does not automatically lead to the conclusion that the mixtures caused an increase in hyperactivity (see paragraph 44 below). It is unclear whether the differences in response to the mixtures by the different age groups were real or, in the case of Mix A, merely reflected differences in dose on a bodyweight basis. In addition, it was noted that the individual measures that contributed to the GHA scores differed between the two age groups (there was no continuous performance monitoring using the computer based task in the younger children).

36 The researchers' findings of a significant increase in mean GHA score of 3 year old children associated with challenge with Mix A were consistent with the results reported in the previous Isle of Wight study in which the same food colours and sodium benzoate preservative mixture was used. The improvements to the protocol of the present study add weight to the previous findings.

37 The size of the observed increase in mean GHA score (which encompassed parental, teacher and independent observer assessments) associated with consumption of Mix A in 3 year olds was smaller in the present study than was observed in the Isle of Wight study, in which the quoted effect size had been based solely upon parental ratings (mean increase 0.20 SDU compared with 0.51 previously).

38 The post-hoc analyses of the disaggregated measures for both the whole cohort and the subgroup that had consumed ≥85% of the drinks, showed that the parental reports were the main contributor to the changes in the GHA score for the 3 year olds, as was seen in the Isle of Wight study. In the 8 to 9 year old children, the largest increases in hyperactive behaviour score for both mixtures were seen in the computer-based task. Parental reports were the only statistically significant discriminator of differences in children's behaviour on the challenge compared with the placebo, and, when the whole cohort is considered in the analysis, only in the case of Mix B in 8 to 9 year olds. When the same analysis was conducted on the ≥85% consumption subgroup, the differences in parental reports were statistically significant for both Mix B in 8 to 9 year olds and Mix A in 3 year olds.

39 The researchers have suggested that parents may have been more sensitive to, or more exposed to, behavioural changes in their children in this study than the independent observers or teachers, because most of the challenge drinks were consumed at home after school. The timing of consumption of the drinks was a consequence of the design of the study, as children were instructed to consume the drinks at home rather than at school, so that compliance with consuming the challenge drinks could be monitored by the families and the researchers could be relatively certain that the child had consumed the challenge drinks, as intended. However there was some uncertainty as to whether the drinks were consumed in the morning prior to school or in the evening after school and this was recognised by the COT as a complicating factor in the interpretation of the results.

40 There was no evidence of carry-over of effects on behaviour from each active challenge week to the next active challenge week (i.e. no evidence that behaviour in week 4 was influenced by the type of

challenge (artificial colour and benzoate preservative mixture or placebo) in week 2, and behaviour in week 6 by the type of challenge in week 4). However, it is not possible to say whether behavioural changes persisted from the active challenge weeks into the wash-out weeks. The study design employed the wash-out weeks to minimise the likelihood of carry-over effects confounding behaviour during subsequent active challenge weeks, and not to test the duration of any effect of the mixtures. The one week washout was chosen by the researchers on a pragmatic basis, and was the period used in the previous Isle of Wight Study. In setting the length of this period account was taken of the burdens placed on families taking part in such studies and the recognition that both subject recruitment and retention might be compromised by the use of a longer wash-out period. The duration of exposure to the additive mixtures was only 7 days and therefore it was not possible to determine whether longer term exposure would increase or decrease any potential effects on behaviour.

41 The results of the "proof of principle" acute challenge study, on a limited number of the 8 to 9 year old children with Mix B, did not demonstrate a statistically significant association between administration of the food colour and sodium benzoate mixture and hyperactivity in this group, although there was a trend towards an effect (estimate = 0.66 (95% CI -0.06 to 1.38) p = 0.072) when "responders" were compared with "non-responders". It was noted that the end point used in this acute challenge was not the same as in the main study, and that it was restricted to a small selected sub-set of boys from the main study sample.

Relevance of the findings at the individual and population level

42 The Committee was informed that, although small, the size of the reported effects on hyperactive behaviour could be of clinical relevance for individual children. The observed changes in behaviour did not obviously vary according to social or demographic factors, or to children's pre-trial level of hyperactive behaviour, pre-trial additive content of diet, or sex. The mean differences observed, if causal, could be clinically relevant. The duration of effect would be an important additional consideration, which has not been elucidated by the current study. If there are real effects of this magnitude, but they are only transient, they would potentially be of less concern. The study measured mean differences in the GHA score in the study sample, which was selected to cover the full range of behaviour in the general population, from normal through to high level hyperactivity. However, as the selection of subjects was intentionally stratified across the behaviour scale, the study sample would not have been adequately representative of the wider population.

43 Genetic factors are known to influence hyperactivity and ADHD[12,13]. The findings of the present study suggest possible differential sensitivity to the particular mixtures used in this study in relation to certain genetic polymorphisms. However, the increases in GHA scores were not limited to individuals with the specific polymorphisms measured in the study, and the observed associations between polymorphisms in the histamine N-methyltransferase gene and the difference in behaviour with Mix A in 3 year olds and Mix A and Mix B in 8 to 9 year olds compared to placebo, even if real and not merely chance effect, were not so strong that they could usefully be applied to identify at-risk groups or individuals. There were no associations between behaviour and the other genetic polymorphisms investigated in the study. These included genetic polymorphisms selected from the dopamine neurotransmitter systems, which have previously been implicated in ADHD[14].

44 The findings did not provide any information on the likely biological mechanism for the observed differences in hyperactivity. The Committee had previously considered the available data on the potential for neurotoxicity of a number of the food additives[15], including some of the colours that were used in the mixtures in the present study (quinoline yellow, sunset yellow, carmoisine, and ponceau 4R), and the preservative sodium benzoate. The limited toxicological databases that were available for the individual additives in the mixtures used in the present study did not provide positive neurotoxicological alerts at doses relevant to dietary consumption. It was considered unlikely that the colours concerned would cross the mature blood-brain barrier, although sodium benzoate might. In the absence of stronger evidence for an underlying biological mechanism of toxicity, doubt remains as to whether the observed differences in behaviour were caused by the challenge mixtures. Despite the statistical significance of some of the associations, the possibility still exists that these could have arisen by chance. Furthermore, if the associations were causal, it is not possible to determine whether specific food additives within the mixtures were responsible, or whether the association depended on the combined action of the mixture. The study did not provide any information as to whether or not any associations seen would be specific for children.

Conclusions

45 We consider that this study has provided supporting evidence suggesting that certain mixtures of artificial food colours together with the preservative sodium benzoate are associated with an increase in hyperactivity in children from the general population. If causal, this observation may be of significance for some individual children across the range of hyperactive behaviours, but could be of more relevance for children towards the more hyperactive end of the scales.

46 We note that the increases in mean levels of hyperactivity observed in this study were small relative to normal inter-individual variation and that changes in behaviour were not evident in all children in any one group and were not consistent across age groups or across the different mixtures used in the study. Therefore it is not possible to draw conclusions on the implications of the observed changes at the population level. It is also not possible to extrapolate the findings to additives other than the specific combination in the mixtures used in this study.

47 We conclude that the results of this study are consistent with, and add weight to, previous published reports of behavioural changes occurring in children following consumption of particular food additives.

48 This research has not indicated any possible biological mechanism for the observations made, which might have provided evidence of causality or of the possible effects of individual additives or of other mixtures of additives.

49 The timing and duration of any possible effects would need to be addressed by further research.

50 Further analyses of data from this study may provide additional information on intra-individual variability and the extent of any carryover from the challenge weeks into the wash out weeks.

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September 2007

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Statement: Use of PAVA (Nonivamide) as an incapacitant spray: Reformulation of Captor

Background to request for advice

1 The COT provided advice to the Home Office in 2002 and 2004 on the health effects of pelargonyl vanillylamide (PAVA or nonivamide) when used as an incapacitant spray (Captor I), and in 2006, on combined exposure to PAVA and CS gas.[1,2,3] PAVA is the synthetic equivalent of capsaicin (the active ingredient of pepper) and it is a sensory irritant. PAVA is used as a food flavour (up to 10 ppm) in Europe and in the USA where it has been given GRAS (Generally Agreed as Safe) status by the FDA. It is also used in human medicine as a rubefacient for topical application (0.012% a.i. in the UK).

2 The Civil Defence Supply (CDS) has proposed a reformulation of the product. The new formulation would contain the same amount of PAVA (0.3%) as Captor I, but with the ethanol/water (50:50) solvent replaced by a mixture of propylene glycol (72%), water (25%) and ethanol (2.7%). The instructions for application would remain the same. The new product is called Captor II. The new formulation was developed in response to requests for a formulation compatible with the use of TASER (an electroshock stun gun).

Advice requested from COT

3 The COT was asked to provide toxicological advice on the revised formulation and whether there was any increased risk to those directly or indirectly exposed to PAVA from Captor II in comparison with Captor I.

Submission October 2006.

4 CDS provided information on the purity of PAVA in the revised formulation and submitted a manufacturer's safety data sheet.[4] A further data sheet was provided to the COT for information by the secretariat. A representative from CDS attended the COT meeting to answer members' questions.

5 The COT concluded that data were required on droplet size for the reformulated product to help in the evaluation of risk on inhalation. The COT considered that the potential for systemic toxicity following dermal exposure to Captor II was low, but noted that formulation effects could be difficult to predict. CDS were asked to produce a written risk assessment regarding site of contact effects and systemic toxicity from Captor II. The risk assessment for respiratory effects would require information on droplet size to be considered. There would need to be consideration also of the potential for cross contamination. The COT asked for further information on the statements from one manufacturer's safety data sheet regarding potential skin sensitisation.

Submission May 2007

6 CDS had provided the further data requested by COT on the effect of propylene glycol on percutaneous absorption of PAVA, and on droplet size in the aerosol spray released during use of Captor II.[5,6] Representatives for CDS attended the meeting to answer questions raised by the COT.

7 The company had been able to show that the report of skin sensitisation with propylene glycol in one manufacturers' material safety data sheet was incorrect and that published data did not support a skin sensitisation hazard for propylene glycol. Members were generally reassured that the data provided were of good quality and that the new formulation was an improvement on the previous PAVA spray. The proportion of spray droplets below 10 μm emitted from Captor II and sampled following a rebound test was substantially lower than for Captor I. It was agreed that the current monitoring of PAVA use and reporting of any adverse effects (especially relating to respiratory symptoms, in particular in asthmatics) should continue, but that Captor II should present a lower risk than Captor I with regard to potential for induction of respiratory symptoms.

8 Questions were put to the representatives from CDS who had prepared the submission for the COT. One COT member noted an apparent contradiction in the submitted document between the statement that propylene glycol may increase dermal absorption and the conclusion that the new formulation is easier to wash off. The COT requested that the CDS representatives clarify the situation should the solution remain on the skin for any length of time. The representatives from CDS explained that whilst propylene glycol was more likely to cross into the skin, it was much less likely to carry the PAVA in with it. Most PAVA would remain on the skin and would be more readily removed by wiping or washing. Volatility of the new formulation was much less than Captor I. This contributed to a lower potential for cross contamination than for Captor I.

9 The COT noted that the company would be asked to provide information relevant to the Home Office Scientific Division (e.g. on product usage and standardisation) directly to the Home Office.

COT conclusion

10 The COT concluded the information submitted on the toxicological risk assessment of Captor II in relation to direct and indirect exposure, provided adequate reassurance that the risk was lower than for the previous formulation (Captor I).

11 The COT restated that monitoring of experience-in-use should be continued.

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July 2007

References

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3 COT statement on combined exposure to CS and PAVA. January 2006.

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Statement of the review of the cabin air environment, ill health in aircraft crews and the possible relationship to smoke/fume events in aircraft

This statement is an independent document and the reader is referred to the relevant discussion papers on the website for background information: http://www.advisorybodies.doh.gov.uk/cotnonfood/index.htm

Introduction

Background to the COT review

1 The COT was asked by the Department for Transport (DfT) to undertake an independent scientific review of data submitted by the British Airline Pilots Association (BALPA) due to concerns about the possible effects on aircrew health of oil/hydraulic fluid smoke/fume contamination incidents in commercial aircraft. BALPA submitted data relating to organophosphate compounds (OPs), the cabin air environment, ill-health in aircraft crews and the possible relationship to smoke/fume events in aircraft. The Department of Health (DH) commissioned the Health Protection Agency (HPA) COT Secretariat and the DH Toxicology Unit, Imperial College London, to review the BALPA submission and prepare discussion papers for the COT.

2 Throughout this document the term air contamination incident is intended to refer to incidents with internal sources (e.g. passengers, aircraft components, cleaning materials, dust, disinsection procedures and contaminants arising from aircraft systems (such as oil in engines). External contamination such as ground level air pollution is not covered in this evaluation.

Overview of BALPA submission

3 BALPA submitted evidence and met with the COT Secretariat in February 2006 (TOX/2006/21 Annex 3) to outline their views in support of their contention that acute and chronic illnesses are being induced in pilots and cabin crew through the inhalation of engine oil and hydraulic fluid, additives present in these products and pyrolysis products that may be emitted from the engines and auxiliary power unit (APU) into the air conditioning system of certain aircraft types during air contamination incidents. BALPA consider that this situation is resulting in significant flight safety issues and has unacceptable implications for the health of flight crew. BALPA are also concerned that passengers may be suffering similar symptoms to those exhibited by flight crew.

4 BALPA made two submissions of data. The first in June 2005 (denoted as submission A) consisted of 193 references and an additional submission in November 2005 (denoted as submission B) consisted of 73 references. The COT Secretariat obtained documents listed by BALPA as being part of, but absent from, the submission and complete documents where pages were missing from the documents submitted. It was noted that some data and documents were submitted more than once.

5 A large proportion of the references submitted comprised: incident details, summaries of incidents, incident reporting; airline service bulletins/lists of bulletins, statements, information leaflets, communications, messages, air quality standards; oil manufacturers' safety data sheets for oil

constituents, and photocopies of oil can labels. Also included were around 60 references relating to OPs, together with a limited number of references relating to oil and hydraulic fluid analysis, neurotoxicity/inhalation toxicity, cabin air quality testing, plus a reference to Gulf War Syndrome, multiple chemical sensitivity and aircraft disinsectants.

6 In addition, the COT Secretariat identified a further 169 relevant references and these are denoted with the prefix 'C'.

7 Although over 400 references have been identified and evaluated only a limited number of key references are quoted in this statement. A number of key references, reviews, evaluations, enquiries and conference reports have been reviewed (references A44, A67, A106, A107, A123, C162, C163) and summaries can be found in TOX/2006/21. The key themes raised in the proceedings of the BALPA air safety and cabin air quality international aero industry conference, Imperial College London, April 2005[A44] (submitted to the COT at its July 2006 meeting) and during a meeting held between the COT Secretariat and BALPA and their nominated representatives, are outlined below:

a. A range of symptoms of ill-health was described by presenters based on assessment of self-reported cases. These included acute/short-term skin, gastrointestinal, respiratory and nervous system symptoms with evidence of long-term respiratory, neurological, chronic fatigue and chemical sensitivity effects reported in some individuals. The term 'aerotoxic syndrome' was cited in the BALPA submission to describe a wide range of acute and chronic symptoms reported by aircrew ostensibly exposed to oil/hydraulic fluid smoke/fume air contamination incidents.[A4] The COT has made a general comment on the value of considering 'aerotoxic syndrome' as a diagnosis under paragraph 28 of this statement.

b. Conference presenters proposed exposure to cresyl phosphates (a type of OP), present in jet oils as anti-wear additives, as potentially responsible for some of the symptoms reported and in particular chronic ill-health. Evidence for exposure to tricresyl phosphate isomers was reported in one limited investigation of air levels in military aircraft but no data were available for commercial jet aircraft. A number of presenters reported clinical examination of pilots and neuropsychological testing that had been undertaken with two BAe 146 pilots. Evidence for neurological impairment was described in one presentation of 26 airline pilots examined clinically and by positron emission tomography (PET) scanning. Reference was made in one presentation to autonomic nervous system symptoms in pilots but no data were presented. One suggestion outlined in the BALPA conference proceedings was that single large doses or repeated small sub-clinical doses of OPs may be associated with neurological, neurobehavioral and neuropsychological consequences referred to as 'organophosphate ester induced chronic neurotoxicity' or OPICN. The COT has commented on the evidence for OPICN in paragraph 59 of this statement.

c. The BALPA conference presenters proposed that there is underreporting to regulators of contamination incidents and associated cases of illness. The COT has commented on this aspect in paragraphs 46 and 48 of this statement.

d. Possible approaches to exposure assessment were described and the practical difficulties of undertaking such investigations on working commercial jet aircraft are noted. One possible approach is the development of a biosensor based on surface plasmon resonance. A proposal for the possible development of such a device was outlined at the meeting with BALPA in February 2006 (TOX/2006/21 Annex 3).

e. Reference was also made at the BALPA conference to the Occupational Health Research Consortium in Aviation (OHRCA) project funded in the US following the publication of the National research Council (NRC) review of aircraft air quality in 2002. The proposal was to recruit 150 flight attendants in Portland and San Francisco and 120 pilots from London to undertake incident reporting and exposure monitoring (using an air sampling device fitted with a pump and filters) in a feasibility study which would then be expanded to involve ongoing monitoring. The COT has commented on possible approaches to further research in paragraphs 64-83 of this statement.

8 Versions of the BALPA database listing alleged smoke/fume incidents recorded by BALPA were submitted to the COT Secretariat on 3 occasions. The submissions were in July 2005, in February 2006 after their meeting with the COT Secretariat and then again in April 2006. The COT Secretariat had commenced analysis of the database submitted in February 2006 when the third version of the database was submitted in April 2006. The February 2006 submission listed 746 reports; the April 2006 submission listed 809 reports and covered the period 1 May 1985 – 4 April 2006. The additional entries were added throughout the database and were not limited to the period February – April 2006 and additional information was provided for some existing entries. A comparison of the April and February submissions indicated that 13 of the additional entries in the April submission were duplicates of existing entries.

Additional submissions to COT

9 In response to a request from the COT Secretariat for smoke/fume incident data, the Civil Aviation Authority (CAA) submitted the results of two searches for contaminated air events recorded on the Mandatory Occurrence Reporting (MOR) scheme database for the period 1 January 2001 – 4 April 2006. The first search comprised 262 summaries for a range of aircraft types and the second search comprised 99 summaries for the BAe 146 and B757 only. The summaries included details of health symptoms reported by pilots, cabin crew and passengers in addition to details of engineering malfunctions.

10 A short letter was received from BALPA in response to a request from the COT Secretariat for the submission of a number of pilot testimonies.

11 The COT Secretariat received a range of communications from interested parties (including reports of investigations in pilots, letters, emails and telephone calls from individuals and organisations, including campaigners and pressure groups). All information was considered and evaluated for its relevance to the review. Unsolicited information received by the COT Secretariat was also evaluated for its relevance to the submission (reference C164).

12 During the review, the COT received additional submissions of information from a number of independent external specialists including relevant information on approaches to air monitoring for complex mixtures of chemicals in air, the potential evaluation of such mixtures for sensory irritancy, background information on indoor air pollution, risk factors for sensory irritancy, a report of neuropsychological investigations in a number of self-selected pilots, and advice from an expert in aviation medicine on exposure standards for carbon monoxide at the air pressures experienced during commercial flights at high altitude and the potential response of pilots to high altitude flying. (See annexes to COT papers for full details).

13 In March 2007, the COT Secretariat again requested presenters at the BALPA April 2005 conference who had undertaken investigations of pilots to submit any updated data, but no additional information of significance for the review was returned.

14 The COT was aware that the potential exposure of aircrew to oil or hydraulic fluid combustion products is highly controversial within the air industry, and that this independent scientific review of the BALPA submission undertaken by the Committee has generated considerable interest, including some from overseas. As a result, observers attending the meetings have represented a wide range of views and expertise in this area of occupational health.

Advice requested from COT

15 The COT was asked to:

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

ii. Provide the DfT with appropriate advice on any further research required to evaluate this subject.

16 The COT considered a full discussion paper on the referral in TOX/2006/21 at its meeting on 11 July 2006 and updated discussion papers on 5 December 2006 (TOX/2006/39) and 20 March 2007 (TOX/2007/10). A meeting was held with COT epidemiologists and the DH Toxicology Unit on 2 May 2007 to further discuss aspects of health-based research identified at the 20 March 2007 COT meeting. The statement was considered at the 3 July 2007 meeting of the COT. (http://www.advisorybodies.doh.gov.uk/cotnonfood/index.htm)

Evaluation of BALPA submission

17 The COT discussion paper TOX/2006/21 was structured to review all the information submitted by BALPA in the order presented to the COT Secretariat. A number of topics were identified for further consideration and these related to:

i. Further assessment of incidents, particularly relating to those not reported to airlines or under regulatory schemes such as the CAA MOR scheme.

ii. The development of approaches to measure potential exposure to chemicals during a smoke/fume incident due to oil/hydraulic fluid contamination of the bleed air.

iii. Further assessment of the reported acute and chronic ill-health documented by pilots to include further consideration of the neuropsychological data submitted to the COT on the 11 July 2006, and the blood/fat levels of chemicals in pilots.

iv. A review of all the epidemiological data contained in the BALPA submission and additional data retrieved through literature searches.

v. A full literature search to identify published data not sourced in the BALPA submission or the initial searches undertaken by the COT Secretariat.

18 The COT discussion paper TOX/2006/39 presented information on the topics identified by COT from the July 2006 discussion. Areas for further consideration identified at the December 2006 meeting related to:

i. Further information on whether the pilots making multiple reports of smoke/fume incidents were those who also documented continuing ill-health.

ii. Identification of any further information on exposure to pyrolysed oils and hydraulic fluids.

iii. Possible approaches to investigate further the skill tests/proficiency checks for flight crew licences and ratings in relation to the neuropsychological symptoms documented in a study of self-selected pilots.

19 The COT discussion paper TOX/2007/10 presented information on the topics identified by COT in the December 2006 meeting for further consideration. n addition, a full evaluation of all the epidemiological studies (cross-sectional, case studies, case series) contained in the BALPA submission, together with additional studies retrieved by the COT Secretariat and the DH Toxicology Unit was submitted to the COT (TOX/2007/10 Annex 10).

Description of generic air conditioning system (addendum to TOX/2006/21 annex 5)

20 A brief description of a generic air conditioning system is given below to aid understanding of the physical conditions present during an oil/hydraulic fluid smoke/fume contamination incident. There are a number of differences between commercial aircraft in the design and operation of the air conditioning systems. The BAe 146 and B757 aircraft had generated more reports of contaminated air events than other airframes listed on the BALPA database. Some relevant information regarding the B757 and the BAe 146 is given below.

Bleed air

21 The air supplied to the aircraft air conditioning system is extracted from one or more stages of the aircraft engine compressor and is known as bleed air. The actual temperature and pressure of the air at the extraction point will vary depending on the ambient temperature and pressure, the stage of the

compressor used for the extraction, and the speed of the engine. These factors will produce a wide range but the maximum temperature and pressure normally could be about 300-350°C and about 60-80psi (about five times atmospheric pressure) respectively. After being ducted away from the engine, the air is usually immediately cooled to about 200-250°C and controlled to an airframe design pressure in the engine pylon of about 40psi. For the B757, the nominal temperature control is to 177°C ± 17°C /350°F ± 30°F. The cooled and pressurised air is then ducted into the aircraft and the air conditioning packs where it is further cooled and conditioned.

22 In addition to the engines, the APU on the aircraft provides bleed air for use on the ground and, for some aircraft, in flight, usually until just after take-off and from shortly before landing. Again, the actual temperature and pressure of the air supplied will be dependent on ambient conditions and the running condition of the APU. For the B757, the APU temperatures are nominally 177°C/350°F as there is no precooler present in the APU system, and for the BAe 146 aircraft 200-230°C.

Aircraft air distribution system

23 The conditioned air from the air conditioning packs and the recirculation air are ducted into a mix manifold to ensure uniform temperature. Separate supplies are then drawn for each temperature-controlled zone in the aircraft. Generally, the packs will regulate to the lowest demand and each zone supply then receives a small additional flow of hot bleed 'trim' air, extracted from upstream of the pack, to control the main supply to each zone to the appropriate temperature.

24 Most modern aircraft have recirculation systems. The cabin air volume is generally composed of 45% conditioned bleed air with 55% recirculation air.[A107] Air is drawn from the passenger cabin and ducted through filters, generally High Efficiency Particulate Air (HEPA) standard, to remove particulates. It is then blown by fans into the mix manifold downstream of the packs before the individual zone supplies are divided. Typically, air in the cabin will circulate for 2-3 minutes before being exhausted from the aircraft. In the cockpit, the air will be exchanged approximately every minute.[A107] Some aircraft systems exclude recirculated air from the flight deck air supply (e.g. B757) whilst others do not (e.g. BAe 146). Where the exclusion of recirculated air applies, the flight deck supply is extracted upstream of the mix manifold so that it comes from only one pack. In this case, the packs may run at different outlet temperatures depending on the flight deck demand. Air filtration is not required by airworthiness requirements and some aircraft, including the BAe 146, have no filtration in the recirculated air system.

25 The temperature, pressure and humidity of the air will vary between different designs and aircraft types in the air distribution system stages. Typical cabin air temperature is 22°C with a relative humidity of 10-20%.[A107] Humidity within the cabin is predominantly related to the number of people in the aircraft for that flight and is not controlled like cabin temperature and pressure. The overall range of conditions provided by the aircraft and engine systems are similar between different commercial aircraft.

Smoke/fume air contamination incidents

26 An oil/hydraulic fluid smoke/fume air contamination incident is an event in which a small quantity of oil/hydraulic fluid released into the compressor stage of the engine, due to an oil seal failure, is

extracted into the bleed air supplying the aircraft air conditioning system resulting in the formation of an oil mist or odour in the aircraft. The leaked oil/hydraulic fluid is subject to a range of temperatures within the engine and aircraft air conditioning system that might cause thermal decomposition of the oil/hydraulic fluid. Not all odours detected within the aircraft cabin originate from oil contamination of the air supply, for example, toilet and galley odours also occur, and it is not possible to define the cause of all smoke/fume air contamination incidents. It has been estimated from information provided by three airlines that overall, smoke/fume incidents associated with possible explanatory faults identified by engineers (engineering-confirmed smoke/fume incidents) occur in around 0.05% of flights (sectors) but that the incidence may be higher than this in some circumstances, depending on airframe, engine type and servicing (TOX/2006/39 Annexes 13, 14 and 18).

General comments on evidence reviewed

27 The COT recognises that any matter relating to aircrew health must be taken very seriously, both for the protection of the individuals and also to ensure the safe operation of the aircraft.

28 The COT considered as a general point prior to detailed evaluation of the submitted evidence that regardless of the cause(s) of the reported adverse symptoms, it would be prudent to take appropriate action to prevent oil or hydraulic fluid smoke/fume contamination incidents. It was noted that potential irritants may be released during oil or hydraulic fluid contamination incidents and, although on currently available evidence it was not possible to define a chemical or chemical mixtures responsible for the reported acute effects on the skin and respiratory tract, it was reasonable to consider a possible chemical causation in some instances. It was agreed that, while the term 'aerotoxic syndrome' as identified in the BALPA submission was unhelpful because the health problems described were variable between subjects, potentially multi-factorial and also not specific to this situation, it was important that the COT assessed the evidence for any harm resulting from exposure.

COT review of BALPA submission and data sourced by the COT Secretariat

Laboratory investigations of the pyrolysis of jet oils and hydraulic fluids

29 The COT has evaluated the limited number of published laboratory studies of oil pyrolysis. The thermal degradation of jet oils has been shown to form a diversity of volatile organic compounds (VOCs) including ketones, acids, aldehydes, esters, oxygen containing heterocyclic compounds, and tricresyl phosphate isomers (but not the ortho- isomer) in addition to carbon monoxide, carbon dioxide and ozone (TOX/2006/39 Annexes 10, 11 and 12). Some acids are noted to have unpleasant odours (valeric acid, isovaleric acid and caprylic acids) and some potentially irritating aldehydes can be formed (TOX/2006/39 Annex 11). It is noted that the carbon chain length of acid substituents in esters may affect the contaminants formed, and that a higher proportion of C5 or C6 acids will generally give rise to decomposition products of a higher odour. It is evident that parameters other than amount of oil released into an engine, such as bleed air temperatures and pressures in compression

chambers and airflows, would also have an impact on the chemical contaminants formed during an oil/hydraulic fluid air contamination incident. The temperatures chosen for the evaluation of oil degradation products varied between studies and ranged from 121–371°C (TOX/2006/39 Annexes 10, 11 and 12). The COT noted the theoretical formation of trimethylolpropanephosphate (TMPP) from trimethylolpropane esters and tricresyl phosphates is outlined in annex 10 of TOX/2006/39 but that this could not be demonstrated in experiments using realistic pyrolysis conditions (TOX/2006/39 Annex 11; A72). Thus, it was considered that formation of TMPP during fume contaminant incidents on commercial aircraft was unlikely, although appropriate air monitoring data were not available.

30 Comparatively few data are available on the thermal degradation of hydraulic fluids used in commercial aircraft. Skydrol 500B-4 was heated up to a temperature of 425°C, and after heating, there was no fluid and only a small amount of charred material remained. Tributyl phosphate was identified in the bulk and pyrolysed hydraulic fluid which was reduced to dryness, a low level of carbon monoxide (CO) was produced during pyrolysis, and phenol was present in the pyrolysed fluid.[A6] The thermal and oxidative degradation of triaryl phosphate-based hydraulic fluid at high temperatures leads to the formation of carbon particles, hydrogen and a variety of short chain hydrocarbons which can rearrange to form condensed ring structures.[C73] Some of these reactions can occur at low temperatures but the presence of naphthalene or phenyl acetylene in the fluid implied that most of the reactions took place at temperatures above approximately 750°C. It has been reported that the first step in the thermal degradation of all trialkyl phosphates at 200-300°C is production of phosphoric acid and olefins,[C78] while at 370°C tributyl phosphate would predominantly produce phosphoric acid, butene, 1-butanol, butyraldehyde and butyl ethers.[C79]

31 The COT commented on the high degree of variability documented in the oil pyrolysis studies summarised in annexes 11 and 12 of TOX/2006/39 with respect to the chemical species formed, their concentration in pyrolysed oils, and that there was no apparent reason for the observed variation. Attempts were made to use these data to identify potentially irritant VOCs and semi-volatile organic chemicals (SVOCs), but Members noted the variation between oils regarding the formation of such compounds. It was not possible to predict whether the concentrations and exposures to such compounds would reach a level that could result in irritancy. Hence, the COT considered that, rather than trying to predict what might be present, the best approach would be to obtain real air contamination data under actual flight conditions. The COT considered that appropriate time resolution would be required in any exposure monitoring approach to measure the levels of oil and/or hydraulic fluid pyrolysis products in cabin air during an incident. This would allow actual data on concentrations of chemical contaminants released during an incident to be evaluated. It was concluded that the pyrolysis data presented were informative but could not be used to predict which compounds to measure in exposure monitoring studies, although additional molecular modelling of pyrolysis might be helpful in this regard (TOX/2006/39 Annex 15).

Exposure monitoring

Information on chemical exposure

Engine test rig

32 One possible approach suggested for determining potential cabin air contaminants resulting from an oil/hydraulic fluid incident might be the use of an engine test rig. Whilst this option has been considered by engine manufacturers, the practical difficulties associated with introducing either a defined level of damage to an oil seal or a known volume of oil could create unrealistic situations and the evaluation of the results would therefore be difficult.

33 A published test rig study attempted to measure bleed air contaminants using a Garrett TPE 331 turboprop engine with an induced oil seal fault (TOX/2006/39 Annex 12). However, the findings would appear to have limited relevance because of the engineering differences between turboprop engines and the turbofan engines used to power commercial jet aircraft. These differences are likely to affect the conditions occurring in the engines during an oil leak e.g. airflow, degree of air compression and temperature.

34 Bleed air tests have been conducted on an ALF502R-5 turbofan engine from a BAe 146 involved in a cabin air incident.[A124,C165] When inspected on the wing of the aircraft, the engine exhibited evidence of a minor oil leak but during the bleed air tests no oil leaks were apparent. The engine was subject to two tests representing the flight profile and bleed air conditions of the incident flight as closely as possible. For each test, the engine was taken from ground idle through take-off, climb, cruise, descent, stabilisation at ground idle and then normal shutdown. Air samples were taken from both the bleed and inlet ducts at ground idle, take-off, climb, cruise and descent test points, and one set of air samples was taken over the entire test interval from initiation of ground idle through to termination of descent. The engine test air samples were analysed for aldehydes, polycyclic aromatic hydrocarbons (PAHs), VOCs, SVOCs, carbon dioxide, carbon monoxide, methane and ozone precursors. Around one hundred compounds were evaluated. Overall, the identities were established of around 90 compounds at the bleed and/or inlet ducts, including alkanes, alkenes and aldehydes. The concentration of the compounds was generally below 12 parts per billion (ppb) at the engine bleed port. Acetone, methylene chloride, carbon monoxide, methane, carbon dioxide, ethylene, 2-methylpentane, 3-methylpentane were detected at varying concentrations above this level in various phases of flight but there were marked differences between the two test runs. Assuming no significant in-cabin sources, the levels of these chemicals would be expected to be lower in cabin air. The ortho- isomer of tricresyl phosphate was not detected but total isomers of tricresyl phosphate had been detected and quantified.

Published and unpublished exposure data from studies of commercial jet aircraft

35 The House of Lords Enquiry in November 2000 concluded that the overriding research need with respect to exposure was to benchmark the air quality in current aircraft, using comprehensive measurements, with agreed methodologies, on a sufficiently large number of flights to be typical, if not fully representative.[C75] A number of observations can be made from the retrieved published studies on exposure measurements.[C56-72]

36 The available studies and reviews cited in the discussion papers reviewed by COT, in which measured exposures to VOCs and SVOCs have been reported, date from the early 1990s and refer to exposure monitoring on commercial aircraft using data from 1-45 non-smoking flights for an individual airframe.[C69] However, the majority of these studies measuring VOC/SVOC levels have examined between only one and approximately 10 flights for any particular aircraft. Thus, most studies provide relatively limited information on potential exposures.

37 No published data regarding air monitoring on the B757 were retrieved. An in-confidence report was obtained for a study undertaken to investigate potential exposure to engine oil, hydraulic fluid, APU oil and fuel using B757s on the ground (to establish methodology) and during 3 commercial flights.[C76] The approach used included photoionisation detection (PID) for real time monitoring for oil vapour and sampling with thermal desorption and analysis for identification and quantification. The B757s selected for this study had been reported previously as having problems with oil smells. The authors report that there was some limited evidence for the presence of APU oil and hydraulic fluid in cabin air on all the flights but at levels below those expected to induce mild acute symptoms. An increase in carbon monoxide was reported on Flight 1 (reported to be well below the World Health Organisation (WHO) air quality guideline) and on the return flight indicating the presence of combustion products. Around 100 individual organic compounds including VOCs and siloxanes were detected using thermal desorption. Concentrations of aldehydes were similar to those that have been found indoors in buildings.[A107] Analysis of reconstructed chromatograms suggested that oil may have been present in some samples coinciding with reports of oily smells. An increase in total VOCs was reported during cruise compared to other phases of flight.

38 Air quality measurements in a number of flights with the B777 and B747 (TOX/2006/39 Annex 12c) indicated VOC levels similar to those reported for indoor air in domestic buildings.[A107] Compounds commonly detected included toluene, limonene, a range of aliphatic hydrocarbons containing 6-7 carbon atoms (including methylcyclohexane) and some volatile oxygenated compounds, the most abundant of which was ethanol.

39 Air quality measurements have been undertaken on a small number of flights in the BAe 146,[C66,C72] and charcoal filters from the aircraft have also been analysed.[C66] Data on analysis of filters and flight deck walls from the B757 and BAe 146 for tricresyl phosphate isomers was reported to the BALPA conference in April 2005. One HEPA filter from a B757 had 930mg tricresyl phosphate isomers/4.5m2.[A44] An in-confidence report was submitted detailing a fume incident created deliberately on a BAe 146 by raising the duct temperature to a maximum. For a short time, the duct temperature exceeded 90°C, but normal operating conditions were then reinstated. The acrid smell associated with hot ducts was present during the test. Analyses of air samples taken before and after the event did not identify any SVOCs or VOCs relevant to oil/hydraulic fluid. After the report of a fume event on the same aircraft, the tests were repeated using both the APU and engine with the aircraft both on the ground and in the air. No evidence for the presence of any SVOC or VOC was reported. Nor was any evidence found in subsequent engineering inspections undertaken by the relevant airline.

40 Air quality measurements have been made in a test flight on a BAe 146 aircraft previously involved in a cabin air incident.[C166] The test flight was undertaken with replacement of the faulty engine that was fitted to the aircraft at the time of the incident. Specific flight parameters were recorded including altitude, engine bleed status, fresh/recirculated air ratios and duct temperatures. Cabin air samples taken during the flight were analysed for VOCs, SVOCs, aldehydes and ozone precursors (VOCs that are considered to contribute to the formation of ozone in the right atmospheric conditions), and the aircraft was fitted with portable carbon monoxide and carbon dioxide detectors. Low levels of a wide range of VOCs and SVOCs, aldehydes and ozone precursors were detected. The concentrations of carbon dioxide, carbon monoxide, hydrocarbons, oil degradation products and ozone were below the established CAA, Federal Aviation Administration (FAA) and contractual limits.

41 A number of observations can be made on the existing published data retrieved for the COT. None of the available studies has monitored air quality during an oil/hydraulic fluid smoke/fume incident. The methods of air sampling and analysis vary considerably between studies. A review published in 2000 which presented a consideration of the available published air monitoring studies in commercial aircraft reported that most were deficient in one or more aspects of study design or conduct.[C67] The authors commented on the need for more data using appropriate and reliable methods. The need was also noted for quality assurance of measurements, and for information on detection limits, precision and accuracy with periodic multi-point calibrations. Duplicates and field blanks should be employed.[C67] In one study that attempted to monitor bleed air and cabin air simultaneously, levels of contaminants were often lower in the bleed air than in cabin air indicating in-cabin sources of many VOCs. Where phase of flight was included in a study, levels of VOCs have often been highest on the ground and lower during the phases of flight.[C72] Where there has been some limited evidence for an elevation of exposure to a VOC that might have been related to an oil incident, levels were reported to be below odour thresholds and well below relevant occupational exposure standards. There is also the possibility for some VOCs that there are sources of exposure other than oil contamination incidents. A number of the published studies cited in annex 12c of TOX/2006/39 have investigated SVOCs including tricresyl phosphate isomers, and report no evidence for exposure in aircraft. However, tricresyl phosphate isomers and other SVOCs were extracted from the walls of cabin air supply ducts removed from BAe 146 aircraft documented in a CAA report.[A67] In-confidence information provided to the COT documented evidence for the presence of tricresyl phosphate isomers and absence of TMPP on air filters and wall swabs taken from a number of B757 aircraft. The identity of the tricresyl phosphate isomers and their concentrations was unstated (TOX/2006/21 Annex 8).

42 Overall, the dearth of available information from exposure monitoring means that no definite conclusions can be reached on the normal range of air contaminants and their concentrations in commercial aircraft during flight.

43 The available data from exposure studies presented to the COT point to the complexity of the variables that would need to be considered in any future monitoring exercise regarding the normal background range of air contaminants and also, during an oil/hydraulic fluid contamination incident. These variables include the type of oil and hydraulic fluid used, engine type and maintenance, the design and operation of the air conditioning system and the flight parameters. Overall, the COT

agreed that there was considerable uncertainty regarding the identity and levels of VOCs, SVOCs and other pyrolysis products released into the cabin air during oil or hydraulic fluid smoke/fume incidents. It was agreed that the concept of a test rig for identifying compounds that might be released into bleed air systems was potentially desirable but impractical, due to the uncertainty of the relevance of the data that would be obtained, and therefore the investigation should be undertaken on appropriate aircraft during flight (TOX/2007/10 Annex 1). Any exposure monitoring approach that was developed would need to link to data recorded by airlines with regard to the engineering status of the aeroplane and reports of odours and adverse symptoms by pilots and other crew. Further consideration of exposure monitoring is presented in paragraphs 64-72 and in the research discussion section in paragraphs 73-83 of this statement.

Information on incident reporting

Pilot reporting

44 Flight crew communicate all issues that might require engineering intervention (e.g. blown light bulbs and system failures, which are not necessarily safety incidents) to engineers using a Tech Log, or its equivalent depending on the airline. Pilots do not have to make a mandatory entry in the Tech Log regarding cabin air events and hence this system may not record all events.

45 The Air Safety Report (ASR) is a formal means of communication between the flight crew and the airline regarding any safety incident deemed worthy of reporting. The pilot can indicate that the event reported in the ASR reaches the threshold for a CAA MOR. Pilots do not necessarily have to make an ASR in relation to cabin fume events. In addition, airlines screen the ASRs they receive with regard to whether a MOR should be raised and will submit any ASR a pilot considers reaches the threshold for a MOR to the CAA. The CAA classification of MORs can be conceived as a pyramid ranging from a very small number of accidents that require major and immediate intervention through incidents, undesirable events and abnormal variations to normal variations which constitute the majority of MORs received. Cabin fume events are most likely not to reach the threshold for a MOR or, if they do, they are most likely to represent a small part of the abnormal variations/normal variations. Individual airlines would consider the threshold for submitting MORs on a case-by-case basis. Most cabin fume events would, if reported, be documented as ASRs or possibly as Tech Logs but would not necessarily generate an automated Flight Data Monitoring (FDM) record.

46 BALPA presented information that there was underreporting of the wide range of smells encountered on board aircraft, some of which might be transient oil incidents, but it was not possible to make any estimate of how many were due to oil/hydraulic fluid contaminants as opposed to food and toilet smells. The COT considered that it was unclear to what extent oil/hydraulic fluid smoke/fume events go unrecorded as no clear distinction was made between the detection of, for example, toilet/galley and oil fumes.

BALPA database analysis

47 The BALPA database relates to 770 reports between 1 May 1985 - 4 April 2006 (after removal of duplicates and reports for which no data were identified). Only limited information was entered on the BALPA database: incident date, airframe type, CAA MOR reference number and a brief note of the incident. The analysis was restricted to the BAe146/BAe AvroRJ and B757 aircraft as these airframe types had most reports of smoke/fume incidents entered on the BALPA database. An increase in the incidence of reporting is evident from around 1999/2000 onwards. The data analysis included airframe types, flight phases, odour descriptions, health symptoms and results of engineering investigations. The limited extent of reporting on the BALPA database may underestimate the incidence of adverse symptoms and a more systematic approach with objective standards set for the reporting of incidents would help to resolve the uncertainties and inconsistencies in the data. comparison was made between the BALPA smoke/fume incident and CAA MORs for the period 1 January 2001 – 4 April 2006. An attempt to estimate the total number of smoke/fume incidents in British regulated airlines was made by the COT Secretariat using a 'capture-recapture method' that has been applied to estimate the size of human populations.

48 The COT evaluated the BALPA database and data submitted from the CAA MOR database. It was noted that BALPA did not use a standardised questionnaire and thus the details provided varied considerably. It was noted that the CAA MOR database was aimed primarily at identifying aircraft malfunctions and included a series of technical questions, some of which requested information on adverse health symptoms. The COT consider that perception of smoke/fumes could have influenced a pilot's reaction to an incident and whether or not they completed an ASR. It was agreed that the reporting of smoke/fume incidents was to some extent subjective and would depend on whether individual pilots felt there was a need to report an incident.

49 It was noted that both databases were likely to be incomplete and that there were limitations in a capture-recapture analysis in estimating the total number of incidents worldwide. The Deputy Chair noted that the pilots who had written to him had all considered that underreporting of possibly minor fume incidents was widespread. It was agreed that a detailed evaluation of the extent of underreporting in the existing databases was not possible, but that objective monitoring of exposure and health would be a priority for the future.

Pilot health

Review and evaluation of epidemiological data

50 The COT agreed that even the best quality epidemiological studies evaluated in this review had not measured exposure or had investigated only a small number of cabin air contaminants. Members noted that an evaluation of incidents from the BALPA and CAA databases, including those with reported health information, had been undertaken. The Committee considered specifically a number of epidemiological studies identified as being relevant to the consideration of health symptoms reported by pilots and identified as such during the BALPA conference held in April 2005.

51 The available epidemiological studies (cross-sectional, case studies, case series) had been systematically reviewed by the DH Toxicology Unit. The Committee were satisfied with the quality of the review and agreed the evaluation of the studies undertaken. On the basis of the review, the Committee concluded that it was not possible to determine whether a causal association exists between cabin air exposures (general or following incidents) and ill-health (acute or chronic) among flight crew. The inability to reach such a conclusion was based on the lack of studies specifically designed to address this question systematically. Members considered that while there is a large body of anecdotal and descriptive evidence on possible associations of health symptoms with cabin air quality (paragraph 53), such data do not meet the standard of a properly designed and performed epidemiological study necessary to reach definite conclusions.

52 The COT concurred with the overall conclusions reached in the review, subject to the limitations of the underlying studies and their health/exposure assessment methods, and agreed that the further additional literature searching proposed was unlikely to alter the conclusions reached:

a. Some aircrew who report incidents experience a variety of health symptoms including some suggestive of irritant (eye/nose/throat/skin) symptoms.

b. Some, but not all aircrew perceive an association between their symptoms and i) cabin air quality (CAQ) in general and/or ii) CAQ incidents.

c. Aircrew report more concerns about CAQ than office workers do about air quality in buildings.

d. Symptoms have been reported more frequently among certain occupational groups than others as follows: aircrew > teachers or office workers; female aircrew > male; younger crew > older (with some exceptions).

e. Among aircrew, symptom reporting varies by employment status and occupation, with higher rates reported among current Italian flight attendants than former Italian flight attendants (based on one study), and among cabin crew than cockpit crew.

f. Symptom reporting has been higher for certain flight characteristics: longhaul > shorthaul; return flights > outbound; night flights > daytime; no humidification > humidification; no catalytic converters > converters

g. Symptom reporting may be higher for certain aircraft types: for example, B767 > DC9 (no air recirculation); jet > propeller; B747SP (flies at higher altitude) > B747.

53 A review of case reports, incidents and testimonies (TOX/2007/20 Annex 5) provided to the COT supplemented the overview of epidemiological studies (TOX/2007/10 Annex 10) and information from the BALPA database and CAA MOR evaluation reported to the COT (TOX/2006/21 Annexes 3 and 4). The following related conclusions are reached, subject to the limitations of the underlying case reports, incident reports, and testimony transcripts/submissions:

a. Aircrew experienced a variety of health symptoms in association with reported individual or repeated cabin air incidents.

b. Most reported health symptoms were acute in nature.

c. Although less frequent, some aircrew report longer-term symptoms.

d. The cabin air incidents have been collectively and generally described as odours, fumes or smoke.

54 Testimonies submitted by individuals to the COT Secretariat were considered and evaluated for their inclusion in the epidemiology overview. Overall, the Committee considered that there were a number of oil/hydraulic fluid contamination incidents with reports of plausible acute adverse symptoms, but the frequency of such incidents could not be determined.

Evaluation of BALPA database for acute and chronic health symptoms

55 An overview was undertaken of the reports in the BALPA database regarding health symptoms. Acute symptoms predominantly relating to the eyes, nose, throat, mouth, chest and the presence of nausea occurred with similar frequency in the BAe 146 and the B757 (the highest prevalence of acute symptom was approximately 8% of the evaluated reports for both aircraft types). Acute neurological symptoms of headache, dizziness, and light-headedness occurred again with roughly similar prevalence for both aircraft types. (The highest prevalence for an acute neurological effect of headache was approximately 14% of the evaluated reports for BAe 146/Avro RJ and 10% for the B757.) Chronic neurological symptoms such as tingling of limbs, tingling of the tongue, memory loss and impaired concentration were found less frequently (the highest prevalence for chronic neurological symptoms was ≥3%.) An evaluation of non-specific symptoms (e.g. 'unwell', tiredness, 'effects', 'incapacitation') and indicated that these too were less frequent (the highest prevalence was approximately 5% of evaluated reports).

56 Members noted that the types of symptoms reported in the BALPA database were common in healthy individuals as is illustrated by findings for recipients of placebos in phase one clinical trials, in studies of indoor air quality, and in surveys of the general population.[C170-C175] The evaluation of adverse health symptoms would benefit from information on comparable control populations e.g. aircrew on planes where fume incidents had not been identified, but care would be needed to take account of possible recall bias.

57 Some further consideration of possible approaches to the investigation of sensory irritation that may be experienced by pilots in commercial aircraft is given in the discussion and research section paragraphs 73-83 of this statement.

Neuropsychological investigations of pilots

58 The COT received a report of a neuropsychological evaluation of eighteen self-selected pilots, nine of whom were still flying. The Committee received independent expert advice on neuropsychology and agreed that caution was required in assessing the results of this evaluation. The COT agreed that although the pattern of neuropsychological impairment reported was not consistent amongst the pilots, overall the potential for cognitive deficits needed further consideration. Members noted that

this was a small-scale evaluation and consider that it would be necessary to conduct a further study with appropriate controls before any firm conclusions can be drawn. Members noted a peer-reviewed published reference to variation in the outcome of the Weschler Adult Intelligence Scale – Third Edition (WAIS-III) test dependent on the level of experience of the individual conducting the test.

Consideration of the neuropsychological symptoms reported in pilots and OP exposure.

59 The COT considered the evidence presented in the BALPA conference proceedings and in the additional papers submitted by BALPA. Members agreed that, on the basis of the available evidence, it was important to keep an open mind regarding the possible identity of potential risk factors and health effects in pilots. It was the view of Members that there had been an emphasis on the potential involvement of OPs in health symptoms reported by commercial airline pilots in the BALPA submission. The COT consider that there might be a number of candidate chemicals, one of which are OPs, and the Committee felt that focusing on OPs drew attention away from other potential chemical causes. The COT in 1999 concluded that the balance of evidence is not supportive of an association between chronic low level exposure to OPs and neuropsychological deficits in tests or the occurrence of OPICN. Members noted that similar patterns of symptoms have been reported in studies of other syndromes such as 'sick-building syndrome' not involving OP exposure. Members consider that, irrespective and independent of chemical exposure, the combination of odour perception, discomfort, involuntary exposure and stressful working conditions in a commercial aircraft cabin environment could lead to long-term health effects through non-toxic mechanisms in a small proportion of individuals.

Consideration of the neuropsychological symptoms reported in pilots and carbon monoxide exposure

60 The COT noted that neuropsychological symptoms attributed to carbon monoxide were either transient during moderate exposures, or seen as lasting after-effects of exposures that resulted in severe carbon monoxide poisoning and agreed that carbon monoxide was unlikely to be the cause of the reported neuropsychological impairment in pilots. However, Members noted that there was some uncertainty in that the exposures to carbon monoxide during an oil/hydraulic fluid smoke/fume incident had not been measured and also with regard to the possibility of prolonged low level exposure in commercial aircraft which needed to be examined in the proposed research. These gaps in knowledge would be addressed in the research that is proposed. Members considered that the reduced oxygen pressures in commercial aircraft at high altitude (cabin pressurisation is to 8000 feet) would have a marginal affect on the risk assessment for exposure to carbon monoxide. The COT received an independent expert opinion suggesting that lower oxygen pressure at 8000 feet would not modify the potential effects of carbon monoxide or alter the air quality standards necessary for protection of health.

Pilot skill tests and proficiency checks and their value to neuropsychological evaluation of pilots

61 The COT concurred with an independent neuropsychologist that pilot skill tests and proficiency checks were task orientated evaluations and would detect only gross neuropsychological deficits in pilots. Such deficits had been described in some individuals. Members consider that follow-up of

pilots who failed skill and proficiency tests might be a useful approach to identify retrospectively pilots for further epidemiological study. This aspect is considered in more detail in the research discussion section paragraphs 73-83 of this statement.

Evaluation of pilot blood/fat tissue bioanalyses and clinical chemistry data

62 The results of blood/fat tissue bioanalyses were submitted in a report to the COT. Twenty self-selected pilots gave blood and/or fat samples for the analysis of levels of VOCs and pesticides. Some of the pilots self-selected between blood and fat samples on the basis of whether they suspected recent exposure or were concerned about past, chronic exposure but specific time-lapse criteria were not applied to the results. The blood and fat samples were analysed by headspace gas chromatography with flame ionisation detection (GC-FID) to quantify VOC concentrations, cell-free DNA and DNA adduct formation with organic chemicals and metals.

63 The COT considered the bioanalytical methods used in the report submitted, including the presentation of results and their interpretation. Significant doubt was placed on the interpretation of reportedly increased levels of solvents in pilots due to a lack of data on method precision, and limitations identified in the origin and application of the population 'average' figures. Consequently, no analyte concentration could be derived for any of these individuals with confidence. Nor could any cause-and-effect relationship be established. The possible use of biomonitoring data is considered further in the discussion and research section paragraphs 73-83 of this statement.

COT discussion and consideration of further research

Exposure monitoring

64 The DfT specifically asked the Committee to advise on any further research required to evaluate this area as part of the referral.

65 One of the initial stages involves the determination of the identity and concentration of chemical compounds and any particulates that might be present in cabin air under normal conditions and during an oil/hydraulic fluid smoke/fume incident. The approaches and devices used in the initial stages of the strategy must fulfil certain functional requirements in terms of the range and concentration of VOCs and SVOCs monitored and compliance with air-worthiness standards (e.g. potential electromagnetic interference). It was noted that the initial methods/prototype devices must not distract pilots and crew from their duties, either during normal conditions of flight or during an incident and that, as far as possible, prototype devices should be automated or require minimal human input to set-up and operate, and be easy to access and maintain. It was noted that there are severe space restrictions in the cockpit, and that in both the cockpit and cabin environment the air inlets and outlets tend to be covered by grilles. It was noted that there is a need to reach agreement with airlines on monitoring strategies and that the size of equipment used in many of the monitoring studies might be one obstacle to obtaining airline agreement for undertaking studies involving a large number of flights.[C68]

66 The COT agreed there was considerable uncertainty regarding the identity of any VOCs, SVOCs and other pyrolysis products released into the cabin air during an oil or hydraulic fluid smoke/fume incident (paragraph 43 above and TOX/2007/10 Annex 1). Members considered that approaches to exposure measurement should cover the widest possible range of potential contaminants from oil/hydraulic fluid that could be analysed and should not focus on only a single chemical group. Also, the investigation should be undertaken on appropriate aircraft (e.g. B757s fitted with the RR535C engine identified by the COT as one possible aircraft to use) during flight (TOX/2007/10, Annex 1).

67 The COT agreed that the starting point for developing an approach to exposure monitoring should be the data from pilot reports and on the rate of engineering-confirmed oil incidents which would be informative for identifying the type of aircraft and number of flights to be included in studies. Members discussed whether it was possible to formulate a specific health related hypothesis in the exposure monitoring studies at this stage (e.g. exposure to a range of specified pyrolysis chemicals is associated with acute ill-health such as irritancy and this might be a marker for exposures associated with chronic ill-health) or whether the initial phase of exposure monitoring should be to enable hypotheses to be generated. Overall, it was felt that no specific hypothesis regarding which chemicals to monitor could be pursued at the present time and thus a staged approach to exposure monitoring and data collection from pilots (e.g. health assessment) would be most appropriate, to enable specific hypotheses to be developed and investigated.

68 The COT was provided with estimates of the number of flights that would need to be monitored for air quality in order to have a 95 percent probability of monitoring at least one flight in which an oil/hydraulic fluid smoke/fume incident occurred, assuming the underlying rate of such incidents was 1/100, 1/1000 or 1/10,000 flights. The COT agreed that there would need to be monitoring on approximately 300, 3000 or 30,000 flights respectively (per airframe with a specific engine).

69 Members agreed that the calculated incidence of oil/hydraulic fluid fume contamination was approximately 1% from pilot reports and approximately 0.05% following engineering investigation (although this might vary depending on airframe, engine type and servicing). It was noted that the estimates of incident rates and numbers of sectors required to be monitored were preliminary and should be used for initial guidance only. It was evident from this information that overall a large number of sectors would need to be monitored to have a high degree of confidence of including an engineering-confirmed oil/hydraulic fluid smoke/fume incident. Members agreed the proposed preliminary estimates of the number of flights required for exposure monitoring per airframe/engine type of more than 100 sectors for background monitoring, and of up to 10,000-15,000 sectors to assess exposures relating to engineering-confirmed oil/hydraulic fluid smoke/fume incidents, depending on the airframe and engine type, APU, rate of oil/hydraulic fluid contamination, air conditioning system operation and engine servicing. Members noted that such a study would also provide more reliable data on background exposure.

70 Members considered a wide range of analytical techniques, samplers and sensors that could be used or adapted to undertake the initial assessment of the cockpit/cabin environment (TOX/2006/39, Annex 15) in conjunction with data presented on oil content and combustion analyses, potential smoke/fume incident rates, the number of aircraft types and the number of flights/sectors that may

need to be sampled. It was agreed that time weighted solid phase microextraction (SPME) would be most practical given the large number of compounds to be detected/analysed, the cost of such devices and acceptability to commercial airlines. Members recognised that SPME would not allow monitoring of peak air concentrations during an oil/hydraulic fluid contamination incident as it produces time integrated average concentrations. Nevertheless, it would be an important step in obtaining background information on cabin air environment and could lead to more targeted studies in the future. The COT agreed that SPME devices would need validation, including calibration with chemicals that might be involved in cabin air smoke/fume incidents. The choice of chemicals for calibration should take into account the range of volatility of chemicals that might be present in the cabin environment. The analytical methods used would have to be precise, accurate, robust and fully validated. Members considered that short duration flights should be monitored to maximise the chance of monitoring an oil/hydraulic fluid contamination incident.

71 Members agreed that a two-stage approach to exposure monitoring is needed, with an initial validation of SPME technology followed by preliminary air monitoring testing using appropriate B757 and BAe 146 aircraft (defined in paragraph 67 above). These preliminary air monitoring investigations would need to collect data on pilot reports of oil/hydraulic fluid smoke/fumes from ASR forms and data on flight operations (such as Quick Access records). It would also have to take into account the often transient nature of contamination incidents. It was anticipated that the initial air monitoring studies would provide some information regarding the cabin environment in the B757 and BAe 146 linking to flight operations but it would not be possible or feasible to obtain full information to assess potential health effects from ASR forms. Such further studies would require enhanced cabin air monitoring and further consideration of health data to be collected from pilots. Members noted that molecular modelling of pyrolysis might be of use as an aid for compound identification in the initial air monitoring studies.

72 Members noted that carbon monoxide could be used as one potential indicator of burning oil and asked that monitoring of carbon monoxide be included in any exposure monitoring approach. It was noted that the DfT propose using PIDs for carbon monoxide and VOC detection in the research to be funded.

Epidemiology

73 The COT have been asked to review the BALPA submission and based on the data submitted by BALPA and that sourced by the COT Secretariat, to assess the risk of exposure of aircrew (particularly pilots) to OPs and oil/hydraulic fluid pyrolysis products in cabin air and to determine whether there is a case for a relationship between exposure and the ill-health in aircraft crews (c.f. paragraph 15i of this statement).

74 Members note the conclusion reached from the DH Toxicology Unit overview of epidemiology that it was not possible to conclude whether a causal association exists between cabin air exposures (either general or following incidents) and ill-health in commercial aircraft crews (paragraph 51). However, there were a number of oil/hydraulic fluid contamination incidents where the temporal relationship between reports of exposure and acute health effects provided evidence that an association was plausible. The Committee agreed that consideration should be given to further research investigating a

possible association between exposure to oil/hydraulic fluid pyrolysis products and ill-health in aircrews. In this respect our main focus, as specified in the referral to the COT, has been to consider further research with respect to commercial airline pilots. Thus, in relation to the provision of advice to the DfT as requested in paragraph 15ii of this statement, three research questions can be identified:

i. Are substances released into commercial aircraft via the bleed system that could potentially be harmful to health?

ii. Are exposures to such substances likely to result in acute ill-health symptoms?

iii. Are exposures to such substances likely to result in chronic health symptoms?

75 It was noted that the cabin environment on commercial aircraft represents a very specific occupational setting and that the proposed strategy for research on exposure and potential ill-health in pilots should take a staged approach to the evaluation of exposure and potential adverse health effects with consideration of the results of investigations at each stage to inform on future research questions. The COT considered that any epidemiological study would need some index of oil/hydraulic fluid air contamination incident exposure in order to be useful.

76 In relation to question 74i), regarding the potential release of harmful substances into bleed air on commercial aircraft, Members consider that the approach to exposure monitoring developed in paragraphs 64-72 of this statement represents the most appropriate and pragmatic way forward. It was noted that aircrew involved in the exposure monitoring phase of research will be asked to fill in ASRs to record reports of odours and/or symptoms. Whilst this will not systematically record all the information that could be obtained through administration of a specific questionnaire on air quality and health effects, it was considered a pragmatic approach using reporting procedures that would be regarded as normal by aircrew, and for which compliance is likely to be relatively high.

77 Members considered the general aspects of the use of biological monitoring data from pilots with alleged exposure to oil/hydraulic fluid contaminants. It was agreed that such monitoring might be performed after the proposed air sampling and in response to specific health-related questions/hypotheses. COT members considered that unless biological samples (e.g. urine, blood) were taken and analysed within an appropriate short time period, usually within12-24 hours after an oil/hydraulic fluid contamination incident, then the results of the analyses were unlikely to be informative with regard to actual exposure or in linking exposure with reported acute health effects. Members agreed that the usefulness of performing chemical analysis on tissue samples (e.g. blood, urine, adipose tissue, hair, nails) obtained from pilots would depend on the toxicokinetics of the chemicals of interest. It is possible that biological monitoring for carboxyhaemoglobin levels in pilots could be undertaken as part of a future epidemiological study, but only if the results of the air monitoring studies described in paragraphs 64-72 of this statement suggest this would be valuable.

78 In relation to question 74ii), the COT was aware that symptoms of sensory irritancy (e.g. eye and respiratory tract irritancy) were amongst the most common complaints reported in pilots (paragraph 55).[C170-C175] Members have considered a general structure-activity equation which could be used to

predict whether exposure to mixtures of VOCs and SVOCs were above or below a threshold for sensory irritancy.[C111] The COT agreed that in principle the approach could be used to evaluate measured levels of air pollution in aircraft, provided it had been properly validated. Validation could involve blind predictions of sensory irritancy thresholds for chemicals with published sensory threshold data, and predictions of sensory irritancy thresholds for chemicals followed by laboratory testing with subsequent threshold testing. The interpretation of any results derived from application of predictive algorithms for sensory irritancy would also need to take into account the large number of potentially confounding factors (such as low humidity) that might be associated with sensory irritant responses in pilots. This research would provide a crude indication/semi-quantitative measure of whether exposures in cabin air might be associated with sensory irritation.

79 The COT concluded that there was no scope for the use of animal models to explore further potential acute and chronic health effects reported to be associated with oil/hydraulic fluid contamination events in commercial aircraft, until the potential chemical exposures are better characterised.

80 With regard to the design of epidemiological studies to investigate acute and chronic health effects in pilots (questions 74ii and 74iii), Members confirm the need for objective as well as subjective measures of exposure in such studies. This could come from exposure monitoring or by use of a validated proxy measures, such as work on different types of aircraft. It was agreed that there is insufficient evidence to justify epidemiological research focusing specifically on exposure to OPs.

81 Members have considered the report of the neuropsychological evaluation in self-selected pilots and sought independent expert advice (paragraph 58). The COT agreed there was limited evidence regarding neuropsychological impairment in pilots. Overall, the Committee concluded that the available evidence, although limited, together with information from pilots supported further investigation of neuropsychological impairment in commercial pilots. However, the Committee also agreed that there was insufficient evidence to recommend any specific additional research for any other acute or chronic health effect with regard to oil/hydraulic fluid contamination incidents on commercial aircraft.

82 There are essentially two approaches that could be used to further investigate neuropsychology in commercial pilots. The first would focus on pilots who failed the routine proficiency testing. This would have the advantage of increasing the power of the study to detect possible causes of ill-health. A disadvantage would be the possibility that pilots who failed the proficiency tests were not representative of UK commercial pilots in any association between exposure and neuropsychological deficit. The alternative approach would be a cross-sectional survey of current and past pilots, focussing initially on the findings from neuropsychological tests in relation to relatively simple, proxy measures of exposure (e.g. comparisons between pilots who flew different airframe/engine combinations and between pilots who had reported previous oil/hydraulic fluid contamination incidents and those who had not reported such events). The potential for confounding by tendency to somatise would have to be taken into consideration in such a study. There would be considerable merit in international collaboration in such research given the likely number of pilots required for such a study. To avoid missing a problem through selective exclusion of pilots who had retired because of impaired health, it would be advisable to include former pilots who had left the profession, for example within the past 5 years.

83 The proposed cross-sectional study, which would be relatively costly, would answer the following questions: to what extent do the prevalence of neuropsychological symptoms and the results of neuropsychological testing differ between pilots who have flown different airframes/engine combinations, and between pilots who report or do not report air contamination incidents, and do these associations differ by country. The study would not address whether neuropsychological symptoms occur at a different prevalence to that in the general population.

COT conclusions

84 The Committee agreed the following overall conclusions with regard to the questions posed which are reproduced below for ease of reference:

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

ii. Provide the DfT with appropriate advice on any further research required to evaluate this subject.

85 As a general point, regardless of the cause of the reported adverse effects, it would be prudent to prevent or take appropriate action to avoid oil or hydraulic fluid smoke/fume contamination incidents (paragraph 28).

86 It was not possible on the basis of the available evidence in the BALPA submission or that sourced by the Secretariat and DH Toxicology Unit to conclude that there is a causal association between cabin air exposures (either general or following incidents) and ill-health in commercial aircraft crews. However, we noted a number of oil/hydraulic fluid smoke/fume contamination incidents where the temporal relationship between reports of exposure and acute health symptoms provided evidence that an association was plausible (paragraphs 54 and 74).

Exposure

87 There was considerable uncertainty regarding the identity of VOCs, SVOCs and other pyrolysis products released into the cabin air during an oil/hydraulic fluid smoke/fume incidents (paragraph 43). Approaches to exposure measurement should address the widest possible range of potential contaminants from oil/hydraulic fluid that could be analysed and should not focus on only a single chemical group or compound (paragraph 66).

88 No specific hypothesis regarding which chemicals to monitor could be pursued at the present time and thus a staged approach to exposure monitoring and data collection from pilots (e.g. health assessment) would be most appropriate, to enable specific hypotheses to be developed and investigated (paragraph 67).

89 Available options for exposure monitoring and passive sampling of a large number of flights on appropriate aircraft represent the best initial approach (paragraphs 69 and 70). A two-stage approach to exposure monitoring needed to be undertaken, with validation and calibration of SPME technology

followed by preliminary air monitoring testing using appropriate B757 and BAe 146 aircraft (paragraph 71). he preliminary air monitoring investigations would need to collect data on pilot reports of oil/hydraulic fluid smoke/fumes and data on flight operations. Molecular modelling of pyrolysis could be used as an aid for compound identification in the initial studies (paragraphs 31 and 71).

90 Any exposure monitoring approach that is developed would need to link to data recorded by airlines with regard to engineering status of the aircraft and reports of odours and adverse symptoms by pilots. It would also have to take into account the often transient nature of contamination incidents (paragraph 71).

91 Carbon monoxide could be used as one potential indicator of burning oil and thus the measurement strategy should include monitoring for carbon monoxide exposure (paragraph 72).

Health

92 In order to address concerns about incident-related acute irritation, a general structure-activity equation might, in principle, be used to evaluate the acute sensory irritancy thresholds of mixtures present in cabin air incidents after independent validation of the approach to sensory irritants (paragraph 78). The outcome of this research would provide an indication/semi-quantitative measure as to whether exposures in commercial aircraft cabin air might be associated with sensory irritation. Overall, there was insufficient evidence available to the COT to recommend additional epidemiological research on any acute health effects (paragraph 81).

93 We confirm the need to obtain objective measures of exposure in epidemiological studies (paragraph 75). These could come from exposure monitoring or through use of validated proxy measures of exposure. There was insufficient evidence to justify epidemiological research focusing specifically on OPs (paragraphs 80 and 82).

94 The available evidence, although limited, together with information from pilots supported further investigation of neuropsychological impairment in commercial pilots (paragraphs 58 and 81). However, there was insufficient evidence to recommend any specific additional research for any other acute or chronic health effect with regard to oil/hydraulic fluid contamination incidents on commercial aircraft (paragraph 81).

95 The most appropriate epidemiological approach to research on neuropsychological status in commercial pilots would be a cross-sectional study to investigate how the prevalence of reported neuropsychological symptoms and the results of neuropsychological testing differ between pilots flying different airframes/engine combinations and between pilots who report, or do not report, air quality incidents, and whether associations differ between countries. Such a study would need the development and use of a validated proxy exposure approach for oil/hydraulic fluid contamination exposure in order to determine whether there is an association between oil/hydraulic fluid smoke/fume contamination and neuropsychological effects (paragraph 83).

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2007 Membership of the Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment

CHAIR

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Professor and Head of Department of Paediatrics
University of Cambridge

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Reader in Molecular Toxicology, University of Nottingham

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Professor of Pharmacoepidemiology, University of Bath

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Chair of Panel of Examiners for the Toxicology Specialty, Royal College of Pathologists

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Head of Public Health Nutrition research group, Rowett Research Institute

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Professor of Pharmacology and Physiology, Associate Dean for Research, Hull York Medical School

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Head of Applied Neuroscience Group, University of Nottingham Medical School

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Professor of Human Nutrition and Director of Northern Ireland Centre for Diet and Health (NICHE),
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University of Leicester

Dr L Stanley BA PhD (upto 31 March 2007)
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Dr D Tuthill MB BCh MRCP MRCPCH (from 1 April 2007)
Consultant Paediatrician, Children's Hospital for Wales

Miss A Ward BA
Public Interest Representative

Mrs A Williams OBE
Public Interest Representative

SECRETARIAT

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Mrs J Shroff BA	*Administrative Secretary*
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Dr D Gott BSc PhD	
Ms R Harrison BSc MSc	
Mrs F Hill BSc MSc	
Dr D Mason BSc PhD	
Mr B Maycock BSc MSc	
Ms C A Mulholland BSc	
Mr D Renshaw BSc EurBiol CBiol MIBiol	
Dr C Tahourdin BSc PhD	
Dr N Thatcher BSc PhD	
Miss T Gray BA	
Miss J Murphy BA	

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

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor Ieuan Hughes (Chair)	BP Amoco	Shareholder	Archives of Disease in Childhood	Associate Editor
	BP Amoco	Daughter is an employee of this Company	Academy of Medical Sciences	Fellow
			Society for Endocrinology	Member
			Royal College of Paediatrics and Child Health	Fellow; Senior Examiner; Regional Academic Advisor
			Medical Research Council	Member of Advisory Board
			Pfizer Aventis NovoNordisk Diabetes UK Wellcome Trust Juvenile Diabetes Research Fund	Funds received from all these sources for Departmental research and education in medicine and health related topics
Dr David Bell	Alliance & Leicester BAA BG Centrica HBOS Plc International Power National Grid RT Group Rolls Royce Scottish Power Thus Transco United Utilities	Shareholder	FSA Astrazeneca Dow Aptuit Inc	Research Contract BSRC CASE studentship Research Grant Consultancy

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor Alan Boobis OBE	Bank Santander Barclays	Shareholder	GlaxoSmithKline	Support by Industry
	BG Group BT Group Centrica Halifax		FSA Department of Health	Research Contract
	National Grid Transco Scottish Power Astellas Pharma	Consultancy	ILSI HESI Elsevier	Unpaid member of Board of Trustees Editor-in-Chief; Food and Chemical Toxicology
			JMPR JECFA (vet drugs) EFSA PPR Panel (Panel on Plant Protection Products and their Residues)	Member
Dr Phillip Carthew (membership ceased 31 March 2007)	Unilever	Salary	NONE	NONE
Professor David Coggon (membership began 1 April 2007)	Halifax Standard Life	Shareholder	Colt Foundation British Occupational Health Research Foundation Faculty of Occupational Medicine	Trustee Trustee Trustee (and President Elect)
Dr Rebecca Dearman	Syngenta CTL AstraZeneca Research Institute for Fragrance Materials, (RIFM)	Shareholder Consultancy	Unilever Syngenta European Chemical Plasticizers Industry (ECPI) American Chemical Council (ECPI)	

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Dr Corrine de Vries	NONE	NONE	Schering AG Yamanouchi	Research Grant
Dr John Foster	AstraZeneca	Shareholder	NONE	NONE
Professor David Harrison (membership began 1 April 2007)	The Forensic Institute Crusade Laboratories	Shareholder Consultancy (dormant)	NONE	NONE
Dr Joy Hinson	GlaxoSmithKline	Shareholder	Society for Endocrinology	Council member and Education Advisor
			Journal of Endocrinology	Member of the editorial board
			Current Opinions in Endocrinology and Diabetes.	
Dr Peter Jackson	Mitchell & Butler Intercontinental Hotels Marks & Spencer Ecofin Dana Petroleum BHP Bilton St Ives Venture Production Ventus	Shareholder	Boehringer Ingelheim Johnson & Johnson Bayer	Departmental Research Funding
Professor Justin Konje (membership began 1 April 2007)				
Professor Joseph Lunec (membership ceased 31 March 2007)	NONE	NONE	Scilucent LLC USA	Funding research group to investigate toxicology of soya -bean oil implants
Dr Geraldine McNeill	Smith & Nephew Diageo Café Direct	Shareholder	World Cancer Research Fund	Grant panel member

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor Ian Morris	Takada Pharmaceuticals	Consultancy		Son is a student fellow of British Heart Foundation
	Society for Endocrinology Society for Medicines Research Society for study of fertility British Society for Toxicology	Membership		
Dr Nicholas Plant (membership began 1 April 2007)			Xenobiotica British Toxicology Society Pfizer GlaxoSmithKline AstraZeneca	Associate Editor Member of Education sub-committee Research Grant CASE PhD Award CASE PhD Award
Dr David Ray	Medical Research Council	Employer		
	ZLB Behring (Switzerland)	Consultancy		
	Bayer AG (Germany)			

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor Ian Rowland	Alpro Foundation European Natural Soybean Association Glanbia Danone Clasado	Consultancy	ILSI Europe Kelloggs Cereal Partners	Partner in EC funded project
	Woolwich Halifax	Shareholder	Geest Vitacress Yakult UK Unilever Masterfoods Scottish Crops Research	Funds received from these sources for Departmental research
Dr Lesley Rushton (membership ceased 31 March 2007)	Transport and General Workers Union	Consultancy – completed	European Silica	Ongoing Cohort Study Contract to IEH – completed
	Friends Provident	Shareholder	International Manganese Institute	Contract to IEH to prepare criteria document – completed
	Northern Rock		American Chemistry Council	Contract to IEH for systematic review and meta analysis – completed
	Unilever	Consultancy – advice on design of an epidemiological survey relating to dermatitis	CONCAWE	Contract to Imperial College for research study: pooled analysis and update of case -control studies of benzene and leukaemia – ongoing

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Dr Lesley Stanley (membership ceased 31 March 2007)	CXR Biosciences	Salary	Alizyme	Company Contract
	Agan Procter & Gamble Toxel	Company Contract	Arrow Bayer Cyclace	
	Association of Plastics Manufacturers, Europe Eurochlor European Council for Plasticisers and Intermediates Halogenated Solvents Industry Association	Consortium Client	Entremed Etiologics Ferring Grupovita Guerbet Ionix Nestle	
	AstraZeneca GlaxoSmithKline NovoNordisk Pfizer Wyeth	Research Collaboration	Neuroseach Oncosense Serono Stiefel Strakan Yamanouchi	
Professor Stephan Strobel (membership ceased 31 March 2007)	NONE	NONE	NONE	NONE

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Dr David Tuthill (membership began 1 April 2007)	Cardiff & Vale NHS Trust	Salary	Royal College of Paediatrics and Child Health Welsh Paediatric Society British Society of Paediatric Gastroenterology, Hepatology and Nutrition Paediatric Research Society British Association of Parenteral and Enteral Nutrition Nutrition Society British Society of Clinical Allergy and Immunology	Fellowship
	SMA Nutricia Milupa	Consultancy		
Miss Alison Ward	NONE	NONE	Farm Animal Welfare Council	Member
Mrs Alma Williams	NONE	NONE	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 independent authoritative advice on potential mutagenic hazards and risks is required. Frequently recommendations for further studies are made.

During 2006, the Committee provided advice on a wide range of topics including genotoxicity of acrylamide (germ cell effects), benzimidazoles (as a common mechanism group), ethaboxam (partial review), formaldehyde (evidence for systemic mutagenicity), potassium sorbate and sodium benzoate (consideration of potential for genotoxicity to mitochondria) and terephthalic acid (used in food contact materials).

The COM also undertook a further consideration of test strategies and evaluation of chemical mutagens which included a presentation of the proposed GADD45a gene screen for genotoxins and a review of the progress towards the development of an OECD test guideline for the *in-vitro* micronucleus assay.

The COM has initiated an ongoing full review of acrylamide and use of toxicogenomics in mutagenicity evaluation. A review of the mutagenicity of mixtures of chemicals has been completed and a COM statement is in preparation.

The COM agreed to initiate a full review of its guidance document which had been published in December 2000.

Professor P B Farmer Chair
MA DPhil CChem FRSC FBTS

Acrylamide

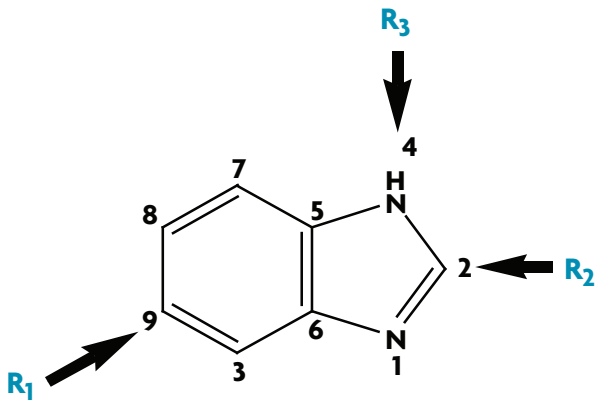
2.1 HSE had asked for an opinion on the evidence regarding germ cell mutagenicity of acrylamide and the evidence regarding a threshold for germ cell mutagenicity with this chemical. In view of the need to provide advice to HSE rapidly on the submitted data, the COM undertook a review of evidence provided by HSE using a postal consultation process. The Chair wrote to HSE in February 2008 and concluded that there were many uncertainties with regard to extrapolation of the available mutagenicity data on acrylamide for risk assessment, particularly as acrylamide (and glycidamide) seem to be clastogenic in both somatic and germ cells. Any attempt should take into account the genotoxic effect on the most sensitive cell, i.e. early spermatozoa. The default recommendation from COM is to assume no threshold.

2.2 A copy of the letter (COM statement 07/02) is appended at the end of this annual report. The COM agreed to conduct a full review of acrylamide which is ongoing (see paragraph 2.34 of this annual report).

Benzimidazoles: Consideration of a common mechanism group

2.3 The COT published its report on Risk Assessment of Mixtures of Pesticides and Similar Substances in September 2002. One of the recommendations of the report was that a scientific and systematic framework should be established to decide when it is appropriate to carry out combined risk assessments of exposures to more than one pesticide and/or veterinary medicine. One group of substances highlighted in the 2002 COT report as requiring further consideration as a possible common mechanism group was the benzimidazoles. These are substances used as pesticides (fungicides) and/or as veterinary medicines (mostly anthelmintics), which contain the benzimidazole ring (Figure 1). Additionally some pesticides and veterinary medicines are pro-benzimidazoles, i.e. they do not contain the benzimidazole ring but are metabolised *in vivo* to benzimidazoles. These compounds are considered here together with the benzimidazoles

Figure 1: Molecular structure of the benzimidazole ring



2.4 For the purposes of this review, the COM considered that aneugenicity was the toxic effect that the substances may have in common, as a result of inhibition of tubulin polymerisation.

2.5 The Committee considered a review of findings in genotoxicity studies and other data relevant to assessments of aneugenicity. On the basis of the reviewed data, there were good correlations for these compounds between inhibition of mammalian tubulin polymerisation and evidence for *in vitro* aneugenicity in mammalian cells, and between *in vitro* aneugenicity in mammalian cells and *in vivo* aneugenicity for these compounds. The Committee considered that evidence for a common functional effect of benzimidazoles on tubulin would be important for an assumption of dose additivity. The Committee considered that a decision tree would be useful to aid assessing inclusion of a benzimidazole in a common mechanism group. A flow diagram is shown in the COM statement appended at the end of this report. It was considered that genotoxicity data for benzimidazoles must be sufficient to demonstrate that mutagenic effects observed are solely due to aneugenicity.

2.6 In order to test the assumption additivity and exclude the possibility of other, less-predictable, combined effects the COM recommended that several example pairs of benzimidazole compounds which pass through the decision tree be tested alone and in combination in the *in vitro* micronucleus assay. The COM also recommended that possible combined effects of benzimidazoles with other aneugens which interact with tubulin should be considered in any research and testing strategy.

2.7 The COM statement is appended to this report.

Ethaboxam- Partial Review

Evaluation October 2006

2.8 The COM were asked for advice by PSD on a new pesticide active ingredient which is undergoing evaluation through the independent Advisory Committee on Pesticides (ACP). The referral statement is as follows; "PSD have asked for advice from the COM on the most appropriate strategy to be taken with regard to the testing for aneugenicity of ethaboxam *in vivo* and the potential for risk of site of contact *in-vivo* aneugenicity effects and their likely significance for risk assessment. This is not a full referral of the mutagenicity data to the COM." Ethaboxam is a new fungicide and is formulated as a 100 g/L suspension concentrate for control of grapevine downy mildew caused by Plasmopara viticola.

2.9 The data holder (LG Life Sciences) and representative attended the COM meeting of the 12 October 2006 to make a short presentation and to answer COM queries regarding the evaluation of ethaboxam for aneugenicity. The data holder and representative submitted reasoned arguments concerning the COM conclusions and additional investigations for polyploidy from the *in vitro* cytogenetics assay in human peripheral blood lymphocytes on 1 November 2006.

COM consideration and conclusions: October 2006

2.10 The COM considered that an appropriate investigation of polyploidy as an indicator for potential aneugenicity had been undertaken in the *in vitro* chromosomal aberration study in peripheral blood

lymphocytes given the predominant effects of ethaboxam on cell division. The COM agreed that more evaluation of the mouse lymphoma data to predict potential for aneugenicity might have been possible.

2.11 The COM agreed that a further evaluation for aneugenicity and clastogenicity should be undertaken *in vitro* to describe appropriate dose-response data and to determine the NOELs. The COM noted that the potential NOEL for non disjunction might be lower than for chromosome loss or gain and this end point should also be investigated.

2.12 The COM agreed the NOEL for MN induction in PBLs *in vitro* was likely to be lower than the value of 5 µg/ml reported in the existing study in PBLs but probably above the tested concentration of 1.0 µg/ml.

2.13 The COM considered that it would be difficult to undertake an evaluation of site of contact aneugenicity *in vivo* for ethaboxam given the current knowledge of potential approaches. A pragmatic *in vivo* testing strategy should include a re-test using intraperitoneal dosing in mice with evaluation of MN in the bone marrow to include dose response, multiple sampling times and an evaluation of whole chromosomes. Members agreed that consideration should also be given to investigating MN formation in other tissues in this study such as the liver, germ cells and splenocytes. Members considered it was important to measure plasma and tissue concentrations of ethaboxam. The COM considered that the results of the repeat *in vivo* BM MN assay in mice would be important for regulatory decision making with regard to ethaboxam.

Update Evaluation May 2007

2.14 The data holder's representative submitted an interim report of the additional studies requested by COM on 16 April 2007. The COM discussed these data at its meeting of the 15 May 2007. The data holder's representative made a short presentation of the results of the additional studies during this meeting. Additional data on the results of studies for non disjunction were submitted during the COM meeting of the 15 May 2007.

2.15 The COM considered a number of questions in deriving conclusions:

i **The interpretation of new *in vitro* MN data in PBLs**: The COM agreed that NOELS were slightly lower than the submitted test data reported; approximately 4 µg/ml (24 hour PHA stimulation) and 2 µg/ml (48 hour PHA stimulation).

ii **The need for additional *in vitro* data on aneuploidy and chromosome disjunction**: Members noted no evidence for non disjunction had been reported. The COM noted that ethaboxam treatment resulted in a greater effect in mononucleate compared to binucleate cells. The mechanism of ethaboxam induced effects *in vitro* was unclear and needed to be resolved.

iii **The interpretation of the new *in vivo* MN data in mice given ethaboxam via intraperitoneal dosing**: The small statistically significant increase in MNPCEs seen in the repeat study at high doses (2 x 300 mg/kg bw in 1% methylcellulose/0.1%Tween 80) resulting in severe toxicity and increased mortality at both the 24 hour and 48 hour post dose bone marrow sampling times is a similar

result to the first intraperitoneal study where small statistically significant increase in MN in the bone marrow was reported in mice (following intraperitoneal dosing of 2 x 486 mg/kg bw (in aqueous 1% methylcellulose) with sampling at 48 hours post dose). Severe toxicity and increased mortality were also reported in the first intraperitoneal bone marrow mouse MN assay with ethaboxam. The COM noted the evidence for aneugenicity of ethaboxam *in vitro* and considered that there was no adequate explanation for the small increases in bone marrow micronuclei seen in the intraperitoneal *in vivo* tests with ethaboxam. The COM could not exclude a direct aneugenic effect of ethaboxam in these studies. The COM did not agree that the increase in MNPCEs seen in these studies could definitely be related to a toxic-stress response. Thus overall the top dose level in the repeat intraperitoneal bone marrow MN assay of 300 mg/kg bw should be regarded as a LOAEL. The COM agreed that further evaluation of the slides from the repeat bone marrow MN assay at the top dose level with additional procedures to identify whole chromosomes should be undertaken if sufficient micronuclei could be identified.

iv **The need for additional *in vivo* MN data in other tissues (eg spleen)**: The COM agreed that further information could be provided for other tissues but this would require additional *in vivo* studies in mice using intraperitoneal dose levels below 2 x 300 mg/kg bw. There would need to be additional toxicokinetic evaluation undertaken. The COM considered that such a request would need to come from the Advisory Committee on Pesticides (ACP).

v **The use of tissue concentration data for risk assessment**: The COM did not agree from the data submitted, that ethaboxam concentrations in testes could be used as a surrogate for bone marrow. The COM noted the absence of bone marrow tissue levels from the repeat bone marrow MN assay in mice. The COM suggested that in this instance an initial risk assessment could be undertaken using the peak plasma levels of ethaboxam at the LOAEL for MN induction in bone marrow reported in the repeat intraperitoneal bone marrow MN assay, although further evaluation of the suitability of this approach for risk assessment of site of contact aneugenicity would need to be considered by the ACP.

2.16 The COM agreed two statements on ethaboxam (COM/07/S1 and COM/07/04) which were forwarded to the ACP and are reproduced at the end of this Annual report.

Formaldehyde: Evidence for systemic mutagenicity

2.17 Formaldehyde is produced worldwide on a large scale and is used in the production of phenolic, urea, melamine and polyacetal resins. Formaldehyde is also used as an intermediate in the manufacture of industrial chemicals and as an aqueous solution (formalin) as a disinfectant and preservative in many situations. The COM was asked to consider the evidence for systemic mutagenicity from animal experiments and biomonitoring studies of workers exposed to formaldehyde. The objective was to consider the potential for systemic mutagenicity following inhalation exposure, the predominant route of exposure in the occupational groups for which evidence of leukaemia had been reported by IARC.

2.18 The COM were aware of a number of recent internationally recognised reviews which had reported on both carcinogenicity and mutagenicity and agreed there was no need to specifically review all of the

mutagenicity data on formaldehyde. This included a recently published evaluation of the mode of action (MOA) for nasopharyngeal cancer. The COM acknowledged that formaldehyde is a direct acting *in vitro* mutagen in bacterial and mammalian cells (including rodent and human cell lines). Mutagenic effects reported included point mutations, chromosome aberrations, sister chromatid exchanges, DNA strand breaks and UDS in rat nasal turbinate cells. With regard to the MOA for rat nasopharyngeal tumours, most reviewers had considered the formation of formaldehyde DNA-protein cross links (DPX) with a similar dose-response to the formation of nasal tumours in rats, with consequent marked local effects on cytotoxicity, cell proliferation and local site of contact mutagenic effects as key elements in the proposed MOA. The magnitude of the formaldehyde induced local site of contact cell proliferation had been emphasised in the available reviews. The Committee agreed to focus its initial discussion on the available peer reviewed scientific literature regarding toxicokinetics of absorbed formaldehyde and the evidence in the published literature for systemic *in vivo* mutagenicity.

2.19 The COM statement is appended to this report.

COM conclusions

i The COM concluded that the amount of formaldehyde systemically available following inhalation exposure at the occupational exposure standard would be negligible.

ii The COM was aware that formaldehyde was a direct acting *in vitro* mutagen. The COM concluded that there was no convincing evidence from *in vivo* mutagenicity studies in experimental animals and from biomonitoring studies of genotoxicity in workers exposed to formaldehyde for a direct *in vivo* systemic mutagenic effect of inhaled formaldehyde. A secondary mechanism might be involved in the genotoxic effects documented in peripheral blood lymphocytes in the biomonitoring studies reviewed.

iii The COM concluded that there was no reason to consider that direct systemic mutagenicity would be involved in the mechanism of formaldehyde induced systemic tumourigenicity.

iv The Committee concluded that for occupational and environmental exposure to formaldehyde, the pattern of metabolism and distribution of formaldehyde indicate that a threshold for *in vivo* systemic mutagenicity is likely.

Sodium benzoate and potassium sorbate

2.20 Sodium benzoate (E211) and potassium sorbate (E202) are two examples of organic acid food preservatives based on benzoic and sorbic acids. Benzoic acid and its sodium, potassium and calcium salts and sorbic acid and its potassium and calcium salts are permitted for use in a wide range of foods in the EU. These preservatives have been subject to a risk assessment by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).[1,2]

[1] Joint WHO/FAO Expert Committee on Food Additives (1996) Benzyl acetate, benzyl alcohol, benzaldehyde and benzoic acid and its salts. Available at: http://www.inchem.org/documents/jecfa/jecmono/v37je05.htm

[2] Joint WHO/FAO Expert Committee on Food Additives (1974) Toxicological evaluation of some food additives, including anti-caking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. Available at: http://www.inchem.org/documents/jecfa/jecmono/v05je18.htm

2.21 In 1999, a study was published in Free Radical Biology and Medicine by Professor Peter Piper, then from University College London, which raised the possibility that these preservatives may be mutagenic to the yeast mitochondrial genome.[3]

2.22 This study used genetically modified yeast cells in an *in vitro* system to demonstrate the effects of potassium sorbate and sodium benzoate on the respiratory capabilities of the cells. Yeast superoxide dismutase (SOD) mutant S. cerevisiae cells were incubated with the two preservatives and the effects observed using a halo assay. The author concluded that the test substances produced an increased number of respiratory-deficient yeast cells under aerobic conditions which indicates that damage was occurring to the mitochondrial DNA in the yeast cells.

2.23 Using a postal consultation, COM members were asked by the Food Standards Agency to comment on the paper by Professor Piper whilst taking into account the large package of other toxicological data available on these preservatives.

2.24 Members were interested in the hypothesis presented by Professor Piper but were of the opinion that direct extrapolation of these results from SOD mutant yeast cells to mammalian cells *in vivo* was not possible. Members considered that mammalian mitochondria *in vivo* have sufficient anti-oxidant and DNA repair mechanisms to deal with any oxidative stress that may be attributed to the action of these preservatives in addition to that normally seen through the normal respiratory activities of the cell. The SOD mutant cells used in the study by Professor Piper have a significantly attenuated anti-oxidant and DNA repair response and therefore had a greater susceptibility to oxidative DNA damage.

2.25 In conclusion, COM members noted the evaluation of sorbates and benzoates by JECFA and were aware of the large package of toxicology data, including rodent carcinogenicity studies. COM members concluded that the study by Professor Piper did not suggest a need for a full re-evaluation of the mutagenicity data on benzoates and sorbates. On the basis of this conclusion, no further *in vivo* mutagenicity testing of these two preservatives was considered necessary at this time.

Terephthalic acid

2.26 Terephthalic acid is used as a starting material in the manufacture of polyethylene terephthalate (PET). PET may be used to coat the internal surface and welded joints (side stripes) of food cans. PET can also be used to manufacture beverage bottles.

2.27 In 2001, the Committee considered that the limited *in vitro* mutagenicity data package and absence of toxicokinetic data in the *in vivo* micronucleus assay were insufficient to determine the mutagenic potential of terephthalic acid. Therefore, the Committee recommended that an adequately conducted *in vitro* cytogenetics test in mammalian cells was needed before any definite conclusions could be reached which would indicate that the bladder tumours in the rat carcinogenicity bioassay arose from a non-genotoxic mechanism.

[3] Piper P.W. (1999) Yeast superoxide dismutase mutants reveal a pro-oxidant action of weak organic acid food preservatives. Free Radical Biology and Medicine. 27 (11/12) 1219-1227

2.28 In May 2006, the COM was presented with a submission of data that BP Chemicals Ltd had commissioned, following the 2001 meeting. A mouse metabolism study was submitted to address concerns regarding bone marrow exposure at the doses selected for the mouse micronucleus study, which had been reviewed at the 2001 meeting. The Committee considered that this metabolism study was not helpful in demonstrating target tissue exposure had been achieved in the micronucleus test. A second *in vivo* study was submitted to supplement the micronucleus study. A single oral dose of terephthalic acid (2000 mg/kg bw, >99.9% purity w/w) was assessed for its ability to induce UDS in the liver of male Alpk:APfSD rats. Members agreed that this unscheduled DNA synthesis (UDS) study had been adequately conducted and was negative. As per the original COM data request, *in vitro* cytogenetics data using human lymphocytes to assess the clastogenicity of terephthalic acid were provided. This assay was limited to 500 µg/ml at which the pH of the culture medium was reduced from 7.10 to 6.74. under the conditions of this initial study, terephthalic acid was found to be clastogenic. A second study was submitted using the sodium salt of terephthalic acid, sodium terephthalate (99% purity w/w). In this study, no reduction in pH was observed when tested up to 2100 µg/ml, the maximum concentration stipulated by the protocol for this assay (10 mM). The author of the study report concluded that sodium terephthalate was not clastogenic under the conditions of this study.

2.29 The Committee agreed that the two *in vivo* studies were adequate and negative, indicating that terephthalic acid is not an *in vivo* mutagen. The available evidence supported the previous COM conclusion of a non-genotoxic mechanism for the bladder tumours seen in the rat carcinogenicity study.

2.30 A full statement is appended at the end of this Annual report.

Horizon Scanning

2.31 The annual horizon scanning exercise was intended to provide an opportunity for members and advisers from Government Departments/Agencies to discuss and suggest topics for further work. Considerable progress on the items identified in the 2006 horizon scanning exercise had been made, although it was noted that the review on mutational spectra had not been initiated and this would be carried over to next year's work programme. The 2007 literature search used PUBMED and initially indicated several thousand publications in 2006/7, which could be potentially relevant. The search strategy subsequently focused on a number of areas such as, genotoxicity test strategy, novel mutagens, mutagens in the environment, genotoxicity biomonitoring, high potency mutagens, potency of genotoxins, mutagen spectra/spectrum and chemical mutagens. Members were asked for their views on the identified areas and for additional suggestions for further work.

2.32 With regard to specific chemicals, phenol was another chemical that had been identified for further work. The COM had reviewed unpublished data in 2003, which suggested a plausible mechanism for secondary indirect effect for positive mutagenic results seen in the bone marrow i.e. hypothermia occurring at dose levels associated with positive micronucleus assays. Members had agreed that before definite conclusions could be drawn on the significance of such data they would need to see a peer reviewed published report of the study. A publication on these data was now available. The committee agreed that it could review this hypothesis i.e. the potential for false positive effects due to hypothermia or hyperthermia using phenol as one example compound.

2.33 The committee agreed that the review of the approaches to the genotoxicity testing of mixtures should be completed. Members agreed to initiate an update of the current COM guidance on mutagenicity testing, but noted that this would be a long process. Members' suggestions for other areas for future work included the significance of aneuploidy, its causes and possible approaches to risk assessment, also mitochondrial mutation and its potential involvement in various diseases, and epigenetics. Members agreed a focused review of the utility of the Ames test for evaluation of low levels of mutagenic impurities in test materials would be valuable.

Test Strategies and Evaluation

2.34 The COM has an ongoing remit to review and provide advice on mutagenicity testing strategies. During this year, the COM heard a presentation from Dr R Walmsley (University of Manchester) on the TK6 GADD45a GFP genotoxicity assay which had been proposed as an early screening asay to be undertaken prior to *in-vitro* mutagenicity testing. The COM considered this to be an interesting presentation and that initial results had suggested both good specificity and sensitivity. Members were interested to see the results when the dataset had been expanded, including further exogenous activation results. Members asked for more information on the validation of the exogenous metabolising fraction to be used in the GADD 45a GFP assay. This would involve studies on source of exogenous metabolising fraction (e.g. rat or hamster liver) and the level of incorporation in the assay and effect of exogenous metabolising fraction on the growth of TK cells in the assay and influence on the flow cytometry method. The Committee looked forward to reviewing more information on this developing approach to genotoxicity screening.

2.35 The COM discussed the ongoing development of an OECD guideline for the *in vitro* micronucleus test at the May 2007 meeting which was attended by representatives of the Industrial Genotoxicity group (IGG). The COM advice was passed to U.K. representatives to take forward at an OECD working group to be convened in October 2007.

Ongoing Reviews

2.36 *Acrylamide*; The HSE have requested a further evaluation from the COM regarding the information cited by the PPG in its letter to the chair of COM (dated 8 May 2007) (COM statement 07/02). The Food Standards Agency have also requested that a consideration be given to all available genotoxicity data on acrylamide by COM. The COM has agreed that the ESR review completed by HSE (EU Risk Assessment report 2002) could be used as a basis for the review. A submission of data from the Polyelectrolyte Producers Group (PPG) was reviewed at the October 2007 meeting. Further consideration of the genotoxicity data on acrylamide will be undertaken by the COM during 2008.

2.37 *Toxicogenomics*; The COT/COC/COM held a joint symposium on the issue of genomics and proteomics in October 2001 and published a joint statement in December 2004 on the use of Toxicogenomics in toxicology. This was based on literature review of 50 studies and included information from the International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI) collaborative programme of research. This topic was identified during the 2006 horizon

scanning exercise for an updated review. The DH Toxicology unit drafted a short overview of a number of new relevant *in vitro* studies, which included data on gene expression changes in studies on DNA adducts and mutagenicity for the October 2007 meeting. A large number of papers had been retrieved, but those selected for review were specifically chosen with the aim of identifying any advancement in the field, which may affect the conclusions drawn in the last statement. The COM noted that further information was available which would be considered during 2008.

2.38 *Chemical Mixtures*; The COM expressed an interest in the evaluation of the mutagenicity of chemical mixtures during the 2005 and 2006 horizon scanning exercises. One important recommendation was to consider the possibility occurrence of synergistic interactions regarding mutagenic effects of chemical mixtures, the possible mechanisms for any synergistic effects and the implications of such a finding for risk assessment. The COM considered two discussion papers during 2007 and a draft working paper is in preparation for the February 2008 meeting.

Statements of the COM

Statement on partial review of Ethaboxam

COM/07/S1 - February 2007

Introduction

1 The COM have been asked for advice by PSD on a new pesticide active ingredient which is undergoing evaluation through the independent Advisory Committee on Pesticides (ACP). The referral statement is as follows; "PSD have asked for advice from the COM on the most appropriate strategy to be taken with regard to the testing for aneugenicity of ethaboxam *in-vivo* and the potential for risk of site of contact *in-vivo* aneugenicity effects and their likely significance for risk assessment. This is not a full referral of the mutagenicity data to the COM."

2 Ethaboxam is a new fungicide and is formulated as a 100 g/L suspension concentrate for control of grapevine downy mildew caused by Plasmopara viticola. The proposed pattern of use is for up to 5 applications per season, with an interval of 7 to 10 days depending on weather conditions and disease pressure. Thus the key sites of contact for evaluation are the upper respiratory tract (in particular the nasal passages) and possible skin contact to concentrate and in-use dilution.

3 The data holder (LG Life Sciences) and representative (Huntingdon Life Sciences) attended the COM meeting of the 12 October 2006 to make a short presentation and to answer COM queries regarding the evaluation of ethaboxam for aneugenicity.

COM consideration of areas for discussion

4 The Committee considered the submitted data which included the draft PSD evaluation which presented information on structure, use, ADME studies, general toxicology, mutagenicity, carcinogenicity and reproduction,[1,2] and copies of the mutagenicity studies relevant to the evaluation of aneugenicity which had [1,2] been submitted to PSD.[3-8] (Some information on a possible approach to the assessment of aneugenicity *in-vivo* in the gastrointestinal tract and the evaluation of aneugenicity data was also presented to members.[9,10]) Members derived an evaluation of these studies and considered the areas of mutagenicity evaluation to raise with the data holder and their representative.

5 The areas of discussion related to:

i the evaluation of the data from the available *in-vitro* chromosomal aberration assay in human peripheral blood lymphocytes (PBLs) and the mouse lymphoma assay (MLA) with regard to identifying biological alerts for potential aneugenicity *in-vitro*.[3,4]

ii the evaluation of the data from initial *in-vitro* study of effects of ethaboxam on cell division in cytochalasin B blocked human PBLs[5] and the subsequent *in-vitro* micronucleus (MN) assay in human PBLs and evaluation of a NOEL for MN formation.[6]

iii the evaluation of data from the *in-vivo* oral bone marrow MN assay in rats[7] and the intraperitoneal bone marrow MN assay in mice.[8]

6 The primary objective of the COM was to consider an appropriate testing strategy for aneugenicity which could be recommended to the ACP.

Data holder presentation

7 The data holder (LG Life Sciences) and representative (Huntingdon Life Sciences; HLS) were asked to make a short presentation to the COM and to answer members queries. All comments were made by the data holders representative.

8 HLS noted that some of the fungicidal activity of ethaboxam was due to interference with the fungal cytoskeleton possibly by inhibition of tubulin subunits. The negative findings in the Ames and MLA tests indicated no potential for gene mutagenicity. The *in-vitro* cytogenetics and MN tests in PBLs were considered to be positive in the absence of exogenous metabolic activation. However two negative bone marrow MN assays were available in the rat (oral dosing) and in the mouse (intraperitoneal dosing). In addition following a request from the ACP, a re-analysis of sections from the gastrointestinal tract from the rat 2 year chronic toxicity/carcinogenicity assay had not indicated any effects on mitosis. HLS also noted that no adverse toxicity had been reported in a 28 day dermal toxicity study in the rat. It was also considered that potential site of contact concentrations (skin and via oral ingestion) would be low.

COM questions for data holder and representative

9 A summary of the response given by the data holder's representative on the areas for discussion (see paragraphs 5, 7 and 8 above) is given below.

10 HLS noted that the initial strategy had placed a weight of evidence on the mouse lymphoma assay and the available *in-vivo* MN assays and thus the initial *in-vitro* study in PBLs had not specifically incorporated an investigation of MN formation. The data holder's representative noted the comments made by the COM and evaluation of the *in-vitro* chromosomal aberration assay and the mouse lymphoma assay. HLS noted the evaluation of the COM with regard to the *in-vitro* MN assay in PBLs regarding the assessment of dose response for aneugenicity and clastogenicity and the possible effects of adding exogenous metabolic activation systems. HLS considered that the use of 0.5% carboxymethyl cellulose was a relatively normal approach to oral dosing in *in-vivo* bone marrow MN assays. The COM considered that oral absorption would have been limited in comparison to that seen in the oral rat kinetics study (up to 60%) using radiolabelled ethaboxam which had used 1% CMC and Tween 80 as a surfactant to aid solubility in the dosing vehicle. HLS noted the interpretation of the *in-vivo* bone marrow MN assays in rats and mice reached by the COM.

11 The data holder and HLS withdrew from the meeting so that the COM could derive its conclusions.

Additional data submitted by industry: LG Life Sciences (1 November 2006)

12 The data holder and representative submitted reasoned arguments concerning the COM conclusions and additional investigations for polyploidy from the *in-vitro* cytogenetics assay in human peripheral blood lymphocytes.[4]

13 The COM noted the new data but agreed that it would still be appropriate to investigate the aneugenicity and clastogenicity *in-vitro* to describe appropriate dose-response data and to determine the NOELs. The COM noted the data holder had agreed to undertake a further *in-vivo* BM MN assay in mice using intraperitoneal administration. Members agreed that this study should include dose-response, multiple sampling times, and an evaluation of whole chromosomes. The COM considered that the results of the repeat *in-vivo* BM MN assay in mice would be important for regulatory decision making with regard to ethaboxam.

COM consideration and conclusions

14 The COM considered that an appropriate investigation of polyploidy as an indicator for potential aneugenicity had been undertaken in the *in-vitro* chromosomal aberration study in peripheral blood lymphocytes given the predominant effects of ethaboxam on cell division. The COM agreed that more evaluation of the mouse lymphoma data to predict potential for aneugenicity might have been possible.

15 The COM agreed that a further evaluation for aneugenicity and clastogenicity should be undertaken *in-vitro* to describe appropriate dose-response data and to determine the NOELs. The COM noted that the potential NOEL for non disjunction might be lower than for chromosome loss or gain and this end point should also be investigated.

16 The COM agreed the NOEL for MN induction in PBLs *in-vitro* was likely to be lower than the value of 5 µg/ml reported in the existing study in PBLs but probably above the tested concentration of 1.0 µg/ml.

17 The COM considered that it would be difficult to undertake an evaluation of site of contact aneugenicity *in-vivo* for ethaboxam given the current knowledge of potential approaches. A pragmatic *in-vivo* testing strategy should include a re-test using intraperitoneal dosing in mice with evaluation of MN in the bone marrow to include dose response, multiple sampling times and an evaluation of whole chromosomes. Members agreed that consideration should also be given to investigating MN formation in other tissues in this study such as the liver, germ cells and splenocytes. Members considered it was important to measure plasma and tissue concentrations of ethaboxam. The COM considered that the results of the repeat *in-vivo* BM MN assay in mice would be important for regulatory decision making with regard to ethaboxam.

February 2007
COM/07/S1

References

1 General information on ethaboxam volume 1 submission 91/414/EEC (draft in-confidence PSD evaluation) 2006.

2 Detailed evaluation volume 3 91/414/EEC (draft in confidence PSD) 2006.

3 Mouse Lymphoma Assay. In confidence Huntingdon Life Sciences project LKF 038, 21 December 2001. (in confidence report).

4 *in-vitro* Chromosome aberration assay in human peripheral blood lymphocytes. Huntingdon life Sciences project LKF/039, 24 October 2001. (in confidence report).

5 In vitro investigation of toxicity using Cytochalasin B in human peripheral blood lymphocytes. Huntingdon Life Sciences project LKF/087, 12 February 2003.

6 *in-vitro* MN assay in human PBLs. Huntingdon Life Sciences report on LGC-30473. Draft in confidence report July 2006.

7 Rat bone marrow MN assay. Huntingdon Life Sciences project LKF/046, 15 November 2001. (in confidence report).

8 *in-vivo* mouse micronucleus test. Huntingdon Life Sciences project LKY24, 13 November 1996. (in confidence report).

9 Rosefort C *et al* (Mutagenesis, 19, 277-284, 2004).

10 Vanhauwaert A *et al* (Mutagenesis, 16, 39-50, 2001).

Statement on Acrylamide

COM/07/S2 - February 2007

Request for advice on Germ Cell Mutagenicity of Acrylamide

Text of letter from COM Chair to Health & Safety Executive (HSE)

Thank you for your enquiry which was received by the secretariat on the 5 January 2007. HSE has asked for an opinion on the evidence regarding germ cell mutagenicity of acrylamide and the evidence regarding a threshold for germ cell mutagenicity with this chemical. I have consulted with a number of members by post and have also asked for a view from Professor David Phillips (chair of COC) who has agreed the contents of this letter. The need to reply to you before the 1 February 2007 COM meeting has meant that it is not possible to consider this at a formal meeting of COM.

You provided a number of documents for members to consider. These included a copy of the EU Risk Assessment Document. A Submission from Polyelectrolyte Producers Group May 2006: Acrylamide: Recent Scientific Data relating to carcinogenicity, mutagenicity and human exposure. You also submitted 7 published references selected by HSE. (References 1-7, see end of this letter). The secretariat drafted a briefing note for COM members, who also had access to the evaluation from the Polyelectrolyte producers and references 1-7.

I give below agreed answers to the questions you have raised:

i) Does the new evidence confirm that acrylamide is a germ cell mutagen?

There is overwhelming evidence in the EU RAR that acrylamide is a male germ cell mutagen. The additional references submitted support this conclusion.[1,2,7] The possibility exists that acrylamide is also a female germ cell mutagen.

ii) Can a threshold be identified for the end point?

No. The mechanism for acrylamide induced germ cell mutagenicity is not fully understood. There is evidence to show that metabolism of acrylamide to glycidamide, a DNA reactive epoxide, is a key step in the *in-vivo* mutagenic responses to acrylamide. There is no evidence to support a threshold mechanism for acrylamide or glycidamide induced mutagenicity available in the submitted papers. The default approach recommended by COM is to assume no threshold for what appears to be a clastogenic mode of action. (http://www.advisorybodies.doh.gov.uk/com/comivm.htm). The submitted references which evaluated dosimetry for mutagenic response of acrylamide can neither prove nor disprove a threshold.[1,3] It is noted that the submitted data is consistent with a linear dose-response for mutagenicity.[3]

iii) Is there sufficient evidence to show that both somatic and germ cell mutagenicity are dependent upon metabolism of acrylamide to its epoxide glycidamide?

There is evidence from the EU RAR for DNA binding in germ cells following dosing of rodents with acrylamide. An abolition of acrylamide induced MN formation DNA damaging effects was noted in CYP2E1 mice which lack the ability to metabolise acrylamide to glycidamide.[5] These data taken with a review of existing germ cell mutagenicity data[7] suggest that glycidamide is a critical metabolite in the *in-vivo* mutagenicity of acrylamide including the germ cell mutagenicity.

iv) Is there sufficient evidence to show that humans metabolise acrylamide to glycidamide to a lesser extent than rodents (with humans < rats < mice)? In view of iii) and iv) what can be said about risks to human health?

Fennell showed that overall AUC values for glycidamide formation in humans and rats from absorbed acrylamide were similar.[6] The rate constants for acrylamide and glycidamide reaction with haemoglobin reported in this paper were slightly higher in humans compared to rats. Paulsson *et al* reported a higher level of acrylamide metabolism and protein adduction in mice compared to rats.[4] Overall mice metabolise acrylamide to a greater extent than humans, but rats appear to be relatively similar to humans regarding extent of metabolism. Its not possible to extrapolate these data to predict mutagenic risk to humans. The default COM approach in such situations where there is insufficient information to allow a conclusion regarding a threshold approach to risk assessment is to assume a no threshold approach to risk assessment.

v) Assuming that a threshold for germ cell mutagenicity is not supported, what are the views of the committee concerning the use of a toxicological reference point or dose descriptor on the dose-response relationship as a basis for assessment of risks (calculation of Margins of Exposure) taking into account interspecies differences in metabolism? In this regard, it is noted that the Allen paper derives a toxicological reference point (claimed to be a threshold for mutagenicity) from combining the dose-response relationship for somatic cell mutagenicity and that for germ cell mutagenicity. As the data appear to indicate that acrylamide is a more "potent" germ cell mutagen than a somatic cell mutagen, how valid is this approach? Are there any robust dose-response data for germ cell mutagenicity of acrylamide (in the totality of the available database) from which to identify a dose descriptor to use for risk assessment purposes? What are the uncertainties involved in such an approach?

COM members cautioned the dose-response modelling undertaken by Allen *et al*, and considered that the interpretation of the results of the modelling was problematic. COM members agreed it was too premature to derive any conclusions regarding genotoxic potency on the basis of this paper.

I would therefore consider there are too many uncertainties with regard to extrapolation of the available mutagenicity data on acrylamide for risk assessment, particularly as acrylamide (and glycidamide) seem to be clastogenic in both somatic and germ cells. Any attempt should take into account the genotoxic effect on the most sensitive cell, ie early spermatozoa. The default recommendation from COM is to assume no threshold.

I hope the answers we have provided are useful to HSE in its consideration of acrylamide. Please do not hesitate to contact me or the secretariat if you have any further questions.

February 2007
COM/07/S2

References

1 Allen B *et al* Regulatory Toxicology and Pharmacology, 41, 6-27, 2005.

2 Marchetti F *et al*, Environmental and Mol Mutagen, 30, 410-417, 1997.

3 Abramsson-Zetterberg L *et al* Mutation Research, 535, 215-222, 2003.

4 Paulsson B *et al* Mutation Research, 516, 101-111, 2002.

5 Ghanayem B *et al* Mutation Research, 578, 284-297, 2005.

6 Fennell TR *et al* Toxicological Science, 84, 1-13, 2005.

7 Favor J and Shlelby MD. Mutation Research, 580, 21-30, 2005.

Statement: Benzimidazoles: An approach to defining a common Aneugenic Grouping

COM/07/S3

Introduction

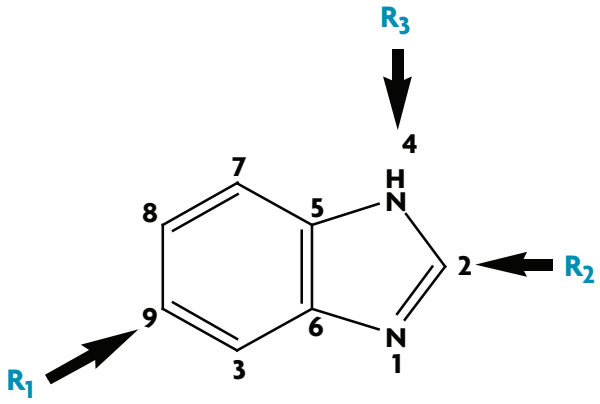
1 The COT published its report on Risk Assessment of Mixtures of Pesticides and Similar Substances in September 20021. One of the recommendations of the report was that a scientific and systematic framework should be established to decide when it is appropriate to carry out combined risk assessments of exposures to more than one pesticide and/or veterinary medicine. The COT recommended that the default assumptions in risk assessments of combined exposure should be that chemicals with different toxic action will act independently, and that those with the same toxic action will act additively[1]. The COT has observed that where groups of chemicals with similar modes of action have been studied, additivity (or less than additivity) has been demonstrated rather than synergy. Examples include endocrine disrupters that act by agonism of oestrogen receptors, dioxin-like compounds and organophosphates[1,2].

2 Under dose additivity (also called simple similar action or simple joint action) the effect of a mixture is obtained by summing the doses of the individual compounds after adjustment for differences in their potencies[1]. Because it occurs across the full dose-response curve, it is relevant to low doses. In contrast, if compounds have independent toxic actions, combined effects may be seen at high doses (e.g. due to effect addition or pharmacokinetic interactions), but would not be expected at doses below thresholds for the individual chemicals1.

3 A new draft regulation proposed by the European Commission concerning the placing of plant protection products on the market (to replace Directive 91/414/EEC) requires additive and synergistic effects of pesticides to be taken into account in the approvals process when the methods to assess such effects are available[3]. The US EPA is already required by the 1996 Food Quality Protection Act in the USA to assess risks from exposures to combinations of pesticides.

4 One group of substances highlighted in the 2002 COT report as requiring further consideration as a possible common mechanism group was the benzimidazoles. These are substances used as pesticides (fungicides) and/or as veterinary medicines (mostly anthelmintics), which contain the benzimidazole ring (Figure 1). Additionally some pesticides and veterinary medicines are pro-benzimidazoles, i.e. they do not contain the benzimidazole ring but are metabolised *in vivo* to benzimidazoles. These compounds are considered here together with the benzimidazoles.

Figure 1: Molecular structure of the benzimidazole ring



5 The mechanism of action of benzimidazole compounds as fungicides is widely considered to be binding to free tubulin, particularly β-tubulin at the colchicine binding site, disrupting microtubule formation and thereby inhibiting mitosis[4,5,6,7]. The primary mechanism of action of benzimidazoles as anthelmintics is also considered to be by binding to free β-tubulin and inhibiting its polymerisation[8,9], as a result affecting microtubule-dependent glucose uptake[10,11]. A number of benzimidazoles have been shown to also inhibit mammalian tubulin polymerisation and to be aneugenic *in vivo*.

Definition of "common mechanism" of toxicity

6 The COT report did not specifically define "common mechanism" in its 2002 report. However, it variously referred to chemicals which would be in a common mechanism group as "similarly acting", having "the same" toxic action, or having a "common mode of action"[1]. The US Environmental Protection Agency (EPA), which is required by the 1996 Food Quality Protection Act in the USA to assess risks from exposure to combinations of pesticides, has defined a common mechanism group as consisting of "Two or more chemicals or other substances that cause a common toxic effect(s) by the same, or essentially the same, sequence of major biochemical events (i.e., interpreted as mode of action)." (http://www.epa.gov/pesticides/glossary/index.html#common_mechanism, accessed 27 November 2006).

7 For the purposes of this review, aneugenicity was considered to be the toxic effect that the substances may have in common, as a result of inhibition of tubulin polymerisation.

Risk assessment of individual benzimidazoles

8 The COM has previously advised that it is reasonable to assume that aneuploidy-inducing chemicals (particularly those that function by damaging the cell division apparatus) have a threshold of action[12]. In 1993 the COM provided advice on methodology for identifying thresholds for aneugens acting by spindle inhibition[12]. In 1996, the Committee considered the results of experiments undertaken with the benzimidazoles benomyl and carbendazim and concluded that the studies had been satisfactorily

conducted and the data indicated No Observed Effect Levels (NOELs) for these two chemicals[13]. The Committee also saw similar data for thiophanate-methyl[14]. The Committee advised in 2000 that there is a sound scientific basis to assume that these chemicals have a threshold of action in both somatic and germ cells[15]. Other committees have drawn similar conclusions for other benzimidazoles where genotoxicity data have provided evidence for aneugenicity.

Review of the data on benzimidazoles

9 The Committee considered a review of findings in genotoxicity studies and other data relevant to assessments of aneugenicity, both from the regulatory assessments of authorised benzimidazoles (and pro-benzimidazoles) and from additional relevant papers identified in the published peer-reviewed literature. Since this was a consideration of a general approach to grouping these compounds, the Committee was not asked to evaluate the individual compounds. The findings are summarised in Table 1.

10 The data indicate that helminth tubulin binding is a relatively poor predictor of *in vivo* aneugenicity, and the Committee considered that aneuploidy in fungi could also not be considered to be a reliable predictor of *in vivo* aneugenicity in mammals. However, there were good correlations for these compounds between inhibition of mammalian tubulin polymerisation and evidence for *in vitro* aneugenicity in mammalian cells, and between *in vitro* aneugenicity in mammalian cells and *in vivo* aneugenicity for these compounds.

11 Results in the mouse lymphoma assay did not appear to be a good predictor of results in the *in vivo* micronucleus assay (data not shown in Table 1). For 4 compounds which were positive in an *in vivo* micronucleus assay and were studied in the mouse lymphoma assay, two were negative in the mouse lymphoma assay, one was clearly positive, and one was equivocal as there was not a clear dose-response relationship.

Additional relevant data

12 Two non-benzimidazole substances which both reduce tubulin polymerisation but by binding to different sites of tubulin (dilanatin and vinblastine) were reported to have dose-additive inhibitory effects on mammalian microtubule assembly when tested in combination[16].

In contrast possible synergy in the antiproliferative effects on mammalian cells was reported for two compounds which have different effects on tubulin (paclitaxel and vinorelbine), one reducing polymerisation and the other stabilising polymerised tubulin, preventing microtubule disassembly which is necessary for completion of cell division[17]. The Committee considered that evidence for a common functional effect of benzimidazoles on tubulin would be important for an assumption of dose additivity.

Table 3: : Summary of positive and negative results indicating action on tubulin and *in vitro* and *in vivo* aneugenicity. Note that some of the results are from studies reported in the peer-reviewed literature, which have not been critically assessed by this Committee.

Chemical	Binding to helminth tubulin	Inhibition of mammalian tubulin polymerisation *in vitro*	Aneugenicity in non-mammalian cells†	In vitro aneugenicity in mammalian cells‡	In vivo aneugenicity (e.g. bone marrow micronucleus assay)
Albendazolea	Positive	Positive	ND	Positive in micronucleus assay. No kinetochore staining	Positive in bone marrow micronucleus assay. No kinetochore staining
Albendazole oxidea	Positive	ND	Positive	Positive in micronucleus assay. No kinetochore staining	Positive in bone marrow micronucleus assay. No kinetochore staining
Benomylb	ND	Positive	Positive	Positive	Positive
Carbendazimb	ND	Positive	Positive	Positive	Positive
Febantelc	ND	ND	ND	ND	Negative
Fenbendazolec	Positive	ND	ND	ND	Negative
Flubendazole	Positive	Positive	ND	Polyploidy and cell transformation	Negative
Fuberidazole	ND	ND	ND	Inhibition of mitosis	Negative
Mebendazole	Positive	Positive	Positive	Positive	Positive
Netobimina	ND	ND	ND	ND	Positive in bone marrow micronucleus assay. No kinetochore staining)
Omeprazole	ND	ND	ND	Positive in micronucleus assay. No kinetochore staining	Positive in micronucleus study in hepatocytes. No kinetochore staining
Oxfendazolec	Positive	Positive	ND	ND	ND
Oxibendazole	Positive	Positive	ND	Polyploidy and metaphases of abnormal morphology	Negative
Thiabendazole	Positive	Positive	Positive	Positive	Positive
Thiophanate methylb	ND	ND	ND	ND	Positive
Triclabendazole	No	ND	ND	ND	Negative

ND: No data identified

a Netobimin is metabolised to albendazole which is metabolised to albendazole oxide

b Benomyl and thiophanate-methyl are metabolised to carbendazim

c Febantel is metabolised to fenbendazole, which is metabolically interconvertible with oxfendazole

† Tests for mitotic aneuploidy in yeast and *Aspergillus nidulans*

‡ Includes studies in human lymphocytes, human/mouse hybrid cell line R3-5, human ovarian granulosa cells, Chinese hamster LUC2 and DON:Wg3h cells, Chinese hamster primary cells, rat primary heptocytes, with additional data from CHO cells and mouse embryo fibroblast C3H/10T1/2 clone 8 cells.

Decision tree for inclusion in a common mechanism group

13 The Committee considered that a decision tree would be useful to aid assessing inclusion of a benzimidazole in a common mechanism group. Key information required would include the chemical structure, results from *in vitro* studies in mammalian cells and/or *in vivo* micronucleus assays, and data to indicate that compounds act via a common functional effect on mammalian tubulin, i.e. inhibition of polymerisation (See Figure 2). It was not considered essential for a benzimidazole to have been studied *in vivo* for it to be included in the common mechanism group since the available data indicated that *in vitro* aneugenicity is a good predictor of *in vivo* aneugenicity for the benzimidazoles.

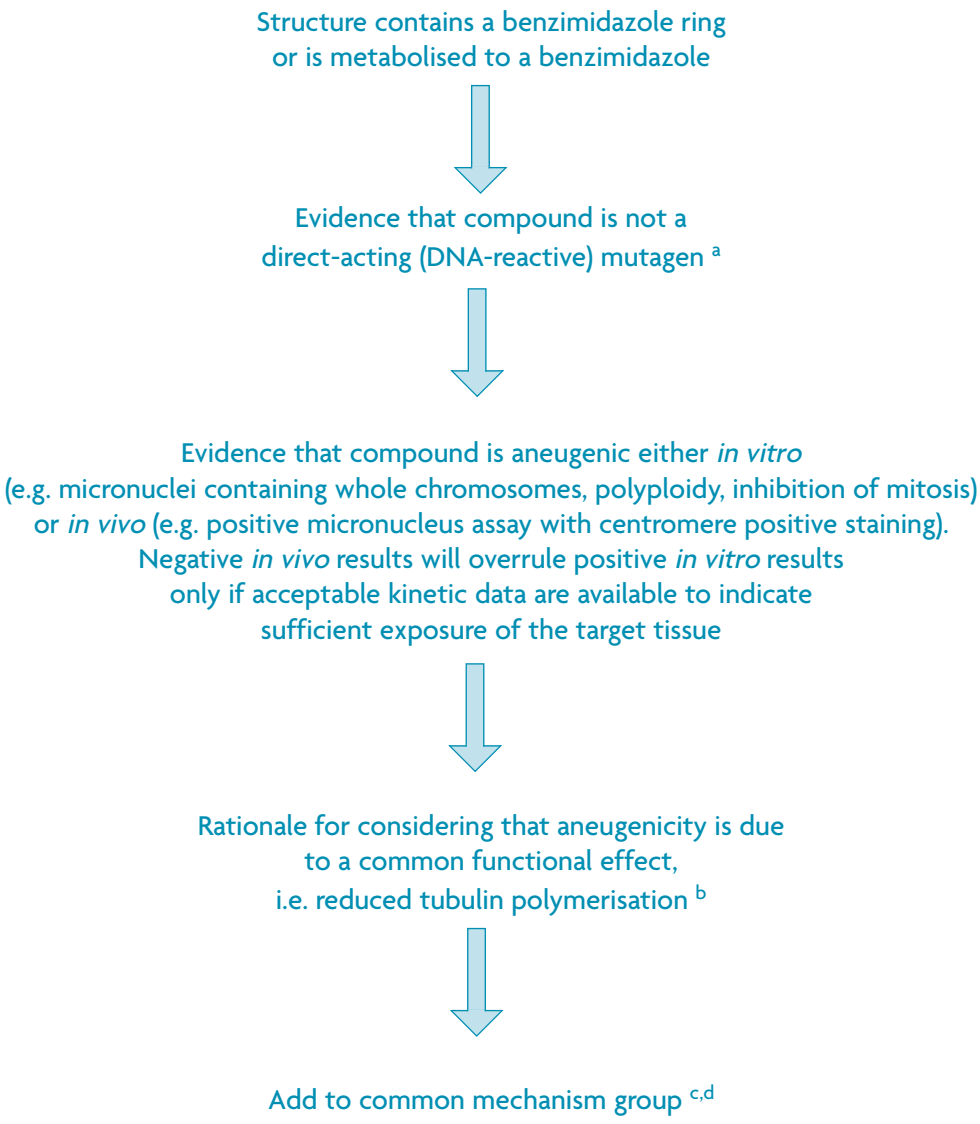
14 Since aneugens may also be clastogens, or may produce clastogenic metabolites, it was considered that genotoxicity data for benzimidazoles must be sufficient to demonstrate that mutagenic effects observed are solely due to aneugenicity, and this is reflected in the decision tree.

Research recommendations

15 The Committee recognised that an assumption of dose addition from combined exposure is pragmatic and is supported by data previously seen by the COT for compounds that act by various common modes of (non-genotoxic) action. However, in order to test the assumption and exclude the possibility of other, less-predictable, combined effects the COM recommended that several example pairs of benzimidazole compounds which pass through the decision tree be tested alone and in combination in the *in vitro* micronucleus assay.

16 We recognise that there is also a need to consider possible combined effects of benzimidazoles with other aneugens which interact with tubulin. We recommend that a suitable approach would be to test pairs of benzimidazoles and other aneugens which interact with tubulin in a similar manner as benzimidazoles (inhibition of tubulin polymerisation). In addition, any identified aneugens to which there may be co-exposure with benzimidazoles but which interact with tubulin in a different manner (e.g. by enhancing tubulin polymerisation) should also be tested in pairs with benzimidazoles.

Figure 2: Decision tree for including a compound in a benzimidazole common mechanism group for aneugenicity



Notes:

a In some instances there may additionally be a need to assess risks from combined exposure with other benzimidazoles which are aneugens even if the compound may also be a direct acting mutagen, in which case the substance may continue through the procedure.

b Any *in vitro* data showing reduced polymerisation of mammalian tubulin would be suitable

c Mixtures of selected benzimidazoles assigned to the common mechanism group should be studied *in vitro* in order to test the default assumption of dose additivity and exclude the possibility of greater than additive effects (i.e. synergy).

d Non-benzimidazoles which are considered to be aneugens may also be added to the common mechanism group if dose addition with at least one benzimidazole in the common mechanism group is demonstrated.

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Updated Statement: Partial review of Ethaboxam Consideration of initial data from studies requested by COM in statement COM/07/S1

COM/07/S4 - October 2007

Background

1 The COM has been asked for advice by PSD on a new pesticide active ingredient which is undergoing evaluation through the independent Advisory Committee on Pesticides (ACP). The referral statement is as follows; "PSD have asked for advice from the COM on the most appropriate strategy to be taken with regard to the testing for aneugenicity of ethaboxam *in-vivo* and the potential for risk of site of contact *in-vivo* aneugenicity effects and their likely significance for risk assessment. This is not a full referral of the mutagenicity data to the COM." Ethaboxam is a new fungicide and is formulated as a 100 g/L suspension concentrate for control of grapevine downy mildew caused by Plasmopara viticola. The proposed pattern of use is for up to 5 applications per season, with an interval of 7 to 10 days depending on weather conditions and disease pressure. Thus the key sites of contact for evaluation are the upper respiratory tract (in particular the nasal passages) and possible skin contact to concentrate and in-use dilution.

2 The Committee considered the submitted data at the October 2006 meeting which included the draft PSD evaluation which presented information on structure, use, ADME studies, general toxicology, mutagenicity, carcinogenicity and reproduction and copies of the mutagenicity studies relevant to the evaluation of aneugenicity which had been submitted to PSD. (Some information on a possible approach to the assessment of aneugenicity *in-vivo* in the gastrointestinal tract and the evaluation of aneugenicity data was also presented to members.) The COM heard a presentation from the data holder (LG Life Sciences) and representative (Huntingdon Life Sciences).

3 The COM agreed that a number of additional studies should be undertaken to further evaluate the potential for *in vitro* and *in vivo* aneugenicity as summarised from statement MUT/07/S1 below:

i. The COM agreed that a further evaluation for aneugenicity and lastogenicity should be undertaken *in-vitro* to describe appropriate dose-response data and to determine the NOELs*. The COM noted that the potential NOEL for non disjunction might be lower than for chromosome loss or gain and this end point should also be investigated.

ii. The COM considered that it would be difficult to undertake an evaluation of site of contact aneugenicity *in-vivo* for ethaboxam given the current knowledge of potential approaches. A pragmatic *in-vivo* testing strategy should include a re-test using intraperitoneal dosing in mice with evaluation of MN** in the bone marrow to include dose response, multiple sampling times and an evaluation of whole chromosomes. Members agreed that consideration should also be given to investigating MN formation in other tissues in this study such as the liver, germ cells and splenocytes. Members considered it was important to measure plasma and tissue concentrations of ethaboxam. The COM considered that the results of the repeat *in-vivo* BM*** MN assay in mice would be important for regulatory decision making with regard to ethaboxam.

*= No Observed Effect Level, ** MN = micronucleus, *** = Bone Marrow

Introduction to current review

4 HLS submitted an interim report of the additional studies requested by COM on 16 April 2007. The COM discussed these data at its meeting of the 15 May 2007. HLS made a short presentation of the results of the additional studies during this meeting. Additional data on the results of studies for non disjunction were submitted during the COM meeting of the 15 May 2007.[2]

COM consideration of areas for discussion

5 The COM considered the new data submitted.[1,2] There were a number of questions relating to the interpretation of the *in vitro* MN assay with regard to the derivation of NOELs in binucleate and mononucleate cells and the appropriate statistical level of significance to apply. With regard to the repeat intraperitoneal *in vivo* bone marrow MN assay in mice, members considered that the justification of the top dose needed further explanation as there was evident excessive toxicity and mortalities reported at the top dose of 300 mg/kg bw in the main study. Members also agreed that HLS should be questioned on the selection of tissue levels in the testes as a surrogate for the bone marrow and whether *in vitro* data on non disjunction were available (subsequently submitted during the COM meeting of 15 May 2007).[2]

Presentation to COM from HLS

6 HLS reported the data from the repeat *in vitro* MN assay in peripheral blood lymphocytes (PBLs) using both 24 hour and 48 hour PHA stimulation. FISH analysis using probes for chromosomes 1,8,11,17 and 18 in binucleate cells was undertaken. Overall NOELs after 24 hour and 48 hour PHA stimulation were interpreted to be 7 µg/ml and 4 µg/ml respectively. There was no evidence for non disjunction.

7 With regard to the repeat *in-vivo* bone marrow MN assay in mice (groups of 7 male mice dosed intraperitoneally twice at 50 mg/kg bw, 150 mg/kg bw or 300 mg/kg bw (24 hours apart) with bone marrow sampling 24 hours and 48 hours after the last dose), a small statistically significant ($P<0.01$) increase in the group mean incidence of micronucleated polychromatic erythrocytes (MNPCEs) was reported at the 24 hour and 48 hour sampling times after the second intraperitoneal dose of 300 mg/kg bw ethaboxam (in 1% methylcellulose/0.1% Tween 80). The trend analysis was statistically significant at the 48 hour sampling time point. A statistically significant reduction in percentage of polychromatic erythrocytes (PCEs) had been documented at both 150 mg/kg bw and 300 mg/kg bw ethaboxam ($P <0.001$ or <0.0001) with a statistically significant trend ($P<0.001$). The increase in MNPCEs at the top dose of 300 mg/kg bw reported at both the 24 hour and 48 hour sampling time points was not considered biologically relevant as it was only seen at the high dose where there was significant systemic toxicity and mortality reported, the individual and group mean data were within the historical control ranges and the low vehicle control MNPCE rate increased the statistical significance.

8 The toxicokinetic analysis for ethaboxam revealed evidence of exposure in the plasma, liver, spleen and testes. Tissue concentrations had not been measured in the bone marrow due to the small amount of tissue. HLS considered that tissue concentrations of ethaboxam were at least equivalent to testes and this was supported by evidence of bone marrow toxicity (reduced PCE frequency). Overall HLS suggested ethaboxam did not induce biologically significant increases in MNPCEs and therefore no subsequent FISH analysis for whole chromosomes had been undertaken.

9 HLS concluded that the request for additional data from COM had been adequately fulfilled. NOELs for binucleate PBLs were interpreted to be 6 µg/ml (24 hour stimulation) 3 µg/ml (48 hour stimulation). There was no evidence for induction of non disjunction. Ethaboxam was not mutagenic in a repeat intraperitoneal bone marrow MN assay in mice with additional toxicokinetic data in directly and indirectly exposed tissues. Overall HLS concluded there was no evidence for genotoxicity *in vivo*.

COM questions for HLS

10 In response to a question from the COM on the evaluation of NOELs for the *in vitro* MN assay, HLS reported that the dose spacing in the study was very small and the size of increase in MN frequency at low concentrations was small.

11 In answer to questions on the conduct of the repeat *in vivo* bone marrow mouse MN assay. HLS considered the process had been adequate and the top does level (300 mg/kg bw) satisfactory. The COM considered the level of toxicity seen at the top dose level was excessive. Members asked for an explanation of the cause of the small increase in MNPCEs seen in the study. HLS noted the response in animals was within the historical control range, and the response had been seen at highly toxic doses at the limit of the MTD and could be interpreted as a toxic-stress related response. Members asked if further investigation using procedures to identify whole chromosomes would be useful. HLS considered there were too few MNPCEs on slides at the top dose level for any meaningful analysis for the presence of whole chromosomes.

12 The COM asked for more explanation regarding the assertion that tissue concentrations in the testes could be regarded as equivalent to bone marrow. HLS considered that testes were representative of peripherally exposed tissue and hence similar to bone marrow. HLS noted that higher tissue levels in liver and spleen might in part represent surface retention of ethaboxam following intraperitoneal dosing. Members asked if tissues had been specially prepared to avoid such contamination by intraperitoneally dosed ethaboxam. HLS reported that standard operating procedures had been used. COM members considered that surface retention of ethaboxam in spleen and liver was unlikely at 24 hours post dose. Overall the time course seen for all tissues and plasma suggested a reduction in tissue concentrations at 6-12 hours post dose, a peak at between 12-24 hours post dose, with a relatively slow elimination (compared to plasma) for liver, spleen and testes. Overall COM members were not convinced that testes could be selected as a representative tissue of bone marrow.

13 The data holder, HLS and JSCI withdrew from the meeting so that COM could derive conclusions.

COM consideration and conclusions

14 The COM considered a number of questions in deriving conclusions:

i. **The interpretation of new *in vitro* MN data in PBLs**: The COM agreed that NOELS were slightly lower than the submitted test data reported; approximately 4 µg/ml (24 hour PHA stimulation) and 2 µg/ml (48 hour PHA stimulation).

ii. **The need for additional *in vitro* data on aneuploidy and chromosome disjunction**: Members noted no evidence for non disjunction had been reported. The COM noted that ethaboxam treatment resulted in a greater effect in mononucleate compared to binucleate cells. The mechanism of ethaboxam induced effects *in vitro* was unclear and needed to be resolved.

iii. **The interpretation of the new *in vivo* MN data in mice given ethaboxam via intraperitoneal dosing**: The small statistically significant increase in MNPCEs seen in the repeat study at high doses (2 x 300 mg/kg bw in 1% methylcellulose/0.1%Tween 80) resulting in severe toxicity and increased mortality at both the 24 hour and 48 hour post dose bone marrow sampling times is a similar result to the first intraperitoneal study where small statistically significant increase in MN in the bone marrow was reported in mice (following intraperitoneal dosing of 2 x 486 mg/kg bw (in aqueous 1% methylcellulose) with sampling at 48 hours post dose). Severe toxicity and increased mortality were also reported in the first intraperitoneal bone marrow mouse MN assay with ethaboxam. The COM noted the evidence for aneugenicity of ethaboxam *in vitro* and considered that there was no adequate explanation for the small increases in bone marrow micronuclei seen in the intraperitoneal *in vivo* tests with ethaboxam. The COM could not exclude a direct aneugenic effect of ethaboxam in these studies. The COM did not agree that the increase in MNPCEs seen in these studies could definitely be related to a toxic-stress response. Thus overall the top dose level in the repeat intraperitoneal bone marrow MN assay of 300 mg/kg bw should be regarded as a LOAEL. The COM agreed that further evaluation of the slides from the repeat bone marrow MN assay at the top dose level with additional procedures to identify whole chromosomes should be undertaken if sufficient micronuclei could be identified.

iv. **The need for additional *in vivo* MN data in other tissues (eg spleen)**: The COM agreed that further information could be provided for other tissues but this would require additional *in vivo* studies in mice using intraperitoneal dose levels below 2 x 300 mg/kg bw. There would need to be additional toxicokinetic evaluation undertaken. The COM considered that such a request would need to come from the Advisory Committee on Pesticides (ACP).

v. **The use of tissue concentration data for risk assessment**: The COM did not agree from the data submitted, that ethaboxam concentrations in testes could be used as a surrogate for bone marrow. The COM noted the absence of bone marrow tissue levels from the repeat bone marrow MN assay in mice. The COM suggested that in this instance an initial risk assessment could be undertaken using the peak plasma levels of ethaboxam at the LOAEL for MN induction in bone marrow reported in the repeat intraperitoneal bone marrow MN assay, although further evaluation of the suitability of this approach for risk assessment of site of contact aneugenicity would need to be considered by the ACP.

COM/07/S4
October 2007

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Formaldehyde: Evidence for Systemic Mutagenicity

COM/07/S5 - November 2007

Introduction

Background to COM consideration of Formaldehyde

Exposure

1 Formaldehyde is produced worldwide on a large scale and is used in the production of phenolic, urea, melamine and polyacetal resins. Formaldehyde is also used as an intermediate in the manufacture of industrial chemicals and as an aqueous solution (formalin) as a disinfectant and preservative in many situations. Formaldehyde also occurs as a natural product in most living systems and in the environment. There are also a number of non occupational sources of exposure including vehicle emissions, from building and household materials, from food and cooking and from tobacco smoke. Most formaldehyde released to the environment is rapidly degraded and human exposure from environmental sources is most likely to occur when there is a continuous source present.[1,2]

Toxicokinetics

2 Formaldehyde is a normal intermediary metabolite in humans. It is estimated that endogenous blood concentrations of formaldehyde are approximately 0.1 mM. Absorbed formaldehyde can be metabolised to formate and enter the one carbon pool for incorporation in DNA, RNA and proteins. Other pathways of metabolism include oxidation to carbon dioxide. Absorbed formaldehyde is rapidly excreted. A small proportion of material may be metabolised by a number of non saturable pathways at the site of contact to form cross links with proteins and a small amount to DNA. The Committee agreed that a consideration of the toxicokinetics of formaldehyde would be important with regard to consideration of potential for systemic mutagenicity.

Mutagenicity and Carcinogenicity of Formaldehyde

3 Formaldehyde has recently been considered by IARC and placed into group 1 (carcinogenic to humans).[1] There is sufficient evidence that formaldehyde causes nasopharyngeal cancer in humans. There was limited evidence that formaldehyde causes sinonasal cancer in humans. The working group concluded that '.. there is strong but not sufficient evidence for a causal association between leukaemia and occupational exposure to formaldehyde. Increased risk for leukaemia has consistently been observed in studies of professional workers and in two of three of the most relevant studies of industrial workers. These findings fall slightly short of being fully persuasive because of some limitations in the findings from cohort and garment workers in the USA and because they conflict with the non-positive findings from the British cohort of workers'. This conclusion stimulated some discussion in the published literature regarding the possible mechanism of formaldehyde induced increased risk of leukaemia.[3] The IARC working group commented on the possible mechanism and '..noted evidence for clastogenic damage to circulatory cells, but overall since there was no good

animal models for acute myeloid leukaemia, did not reach a conclusion with regard to the mechanism of acute myeloid leukaemia reported in epidemiological studies'.[1]

Introduction to COM discussion

4 The COM was asked to consider the evidence for systemic mutagenicity from animal experiments and biomonitoring studies of workers exposed to formaldehyde. The objective was to consider the potential for systemic mutagenicity following inhalation exposure, the predominant route of exposure in the occupational groups for which evidence of leukaemia had been reported.

5 The COM were aware of a number of recent internationally recognised reviews which had reported on both carcinogenicity and mutagenicity and agreed there was no need to specifically review all of the mutagenicity data on formaldehyde.[1,2,4] This included a recently published evaluation of the mode of action (MOA) for nasopharyngeal cancer.[4] The COM acknowledged that formaldehyde is a direct acting *in vitro* mutagen in bacterial and mammalian cells (including rodent and human cell lines). Mutagenic effects reported included point mutations, chromosome aberrations, sister chromatid exchanges, DNA strand breaks and UDS in rat nasal turbinate cells.[1,24] With regard to the MOA for rat nasopharyngeal tumours, most reviewers had considered the formation of formaldehyde DNA-protein cross links (DPX) with a similar dose-response to the formation of nasal tumours in rats, with consequent marked local effects on cytotoxicity, cell proliferation and local site of contact mutagenic effects as key elements in the proposed MOA. The magnitude of the formaldehyde induced local site of contact cell proliferation had been emphasised in the available reviews.[2,4]

6 The Committee agreed to focus its initial discussion on the available peer reviewed scientific literature regarding toxicokinetics of absorbed formaldehyde and the evidence in the published literature for systemic *in-vivo* mutagenicity.[1-30]

The kinetics of absorbed formaldehyde

Studies in experimental animals[3,14-16]

7 The COM considered information from published inhalation studies in rats and Rhesus monkeys (using either single exposure to 6 ppm for 6 h or repeated exposure to the same concentrations 5 days/week for 4 weeks with sampling after the last exposure) and agreed the majority of absorbed formaldehyde was incorporated into intermediary metabolism (≥91%) with small amounts bound as DPX (ca ≤9%).[3,14,15] Importantly there was no evidence for an increase in blood formaldehyde concentrations in these studies. In some relatively old published experiments the metabolic incorporation and covalent binding of formaldehyde was investigated in nasal tissue and bone marrow following inhalation exposure of rats to formaldehyde (dual labelled ^{3}H- and ^{14}C-) at a number of concentrations up to 15 ppm for 6 h. There was evidence for covalent binding of formaldehyde to rat nasal DNA but no evidence for binding to bone marrow.[15] In a further study using a similar inhalation exposure rats were pretreated with an intraperitoneal injection of phorone designed to deplete non protein sulphydryl levels and hence increase the potential for DNA binding of absorbed formaldehyde. Metabolic incorporation into rat nasal tissue and bone marrow were significantly decreased, but there was no evidence for covalent binding to bone marrow DNA.[16]

Modelling of toxicokinetics in humans

8 The committee noted that a number of research groups had attempted to model the systemic uptake and distribution of formaldehyde in humans exposed to formaldehyde at exposure levels close to or at the UK occupational exposure standard of 2 ppm for 8 hours.[2,3,5,29] These studies had concluded that the systemic blood levels of formaldehyde resulting for such exposures would be ≤0.1% of the endogenously formed blood concentrations of formaldehyde (ie approximately 0.0001mM compared to an endogenous level of approximately 0.1 mM).

9 The Committee concluded that systemic exposure to formaldehyde at potential cancer target organs resulting from inhalation exposure would be a negligible amount compared to endogenously formed formaldehyde. Members acknowledged that there was limited information on the proportion of free and bound formaldehyde and the potential for release of adsorbed formaldehyde bound to macromolecules but overall considered that the potential for redistribution of formaldehyde was very small compared to endogenously formed formaldehyde.

Potential for *in vivo* systemic mutagenicity

Studies in experimental animals[6-13]

10 The COM noted that a number of test materials had been used in *in vivo* studies in experimental animals which included aqueous solutions (30-50%) stabilised with 10% methanol. Alternatively paraformaldehdye hydrolysed using sodium hydroxide might be used as a source of formaldehyde generation. Formaldehyde is intrinsically reactive and thus unstabilised solutions may be oxidised to formic acid and at low temperatures a precipitate of trioxymethylene may be formed.[2] The precise composition of the test material used was not clear in many of the studies reviewed but was presumed to be predominantly formaldehyde. Members agreed the presence of methanol would complicate the evaluation of *in vivo* genotoxicity studies.

11 The COM agreed that the available *in vivo* tests for micronucleus induction and chromosome aberrations in bone marrow in rodents using inhalation exposure or intraperitoneal administration were predominantly negative.[7-11] A slight and apparently dose related increase in micronucleated cells/1000 PCEs was noted in mice given two intraperitoeneal doses of formaldehyde (derived from paraformaldehdye at 6.25, 12.5 or 25 mg/kg bw) separated by 24 hours at two samplings (16 h and 40 h post final dose).[8] It was noted that the study authors had not considered that a statistically significant increase had been documented in this study. The Committee considered a recently published *in vivo* comet assay in rats exposed by inhalation to up to 10 ppm 6 h/day for 5 days/week. An apparent dose-related increase in comet tail moment had been reported following examination of 50-100 cells/animal in peripheral blood lymphocytes and in the liver. It was noted that in principle a cross linking agent such as formaldehyde might be expected to reduce comet tail moment and it was possible that the effects might have been due to oxidative damage and possibly apoptosis. The COM noted that formaldehyde induced disturbances of protein and lipid oxidation in this study.[13]

12 The Committee considered the evidence for a dominant lethal effects and noted two positive results had been reported. Thus a single intraperitoneal dose of formaldehyde (50 mg/kg bw) administered to Q strain mice with separate matings each week for seven weeks resulted in increased embryonic death

in the 1st and 3rd weeks.[6] There was no evidence for a clastogenic effect on spermatocytes in this study. The COM concluded it was unlikely that the effects reported resulted from a systemic mutagenic effect of formaldehyde.

13 In a separate investigation an increase in the number of abnormal spermatozoa and evidence for a dominant lethal effect was reported in a study using isogenic University of Lagos rats given intraperitoneal doses of 0.125-0.5 mg/kg bw of formaldehyde.[12] These doses were reported to be between 1/16 and ¼ of the intraperitoeneal LD50 in this strain of rat and are very much lower than the dose levels used in other *in-vivo* mutagenicity studies with formaldehyde. The dominant lethal study examined matings 1-7 days, 8-14 days and 15-21 days post dosing. A significant increase in the number of dead implants was reported in the period 1-7 days post dose which was accompanied by a reduction in sperm counts and abnormal spermatozoa.[12] Members noted that the use of methanol to stabilise formaldehyde could have contributed to the observed effects on spermatozoa morphology reported in rats.

14 The COM concluded that the mechanism by which formaldehyde could have induced the observed effects in the dominant lethal studies was unclear but did not involve a direct systemic mutagenic response.

Other site of contact *in vivo* mutagenicity studies

15 The Committee noted clear evidence for an increase in micronucleated cells of the basal epithelium of the stomach in a study in rats where a single oral dose of 200 mg/kg formaldehyde was administered.[11] In a separate study using repeated inhalation exposure of rats to formaldehyde (up to 15 ppm 6 h/day for 1 or 8 weeks), lung lavage samples in addition to bone marrow samples were examined for chromosome aberrations. There was no evidence for a clastogenic effect in bone marrow samples but a statistically significant increase in chromosome aberrations was noted in lung lavage samples.[10]

16 The COM agreed that formaldehyde was a site of contact *in-vivo* mutagen. Members noted that the site of contact effects in the gastrointestinal tract might not all be due to formaldehyde reacting directly within the target cells as the effects extended down the gastrointestinal tract further than would be expected for a highly reactive chemical.

Biomonitoring studies of formaldehyde exposure[17-28]

17 Biomonitoring studies of genotoxicity in workers exposed during a variety of activities including manufacture of formaldehyde and use of formaldehyde in mortuary and anatomy departments and in paper impregnation were evaluated. A further group used in biomonitoring studies were dialysis patients where the dialysis equipment was sterilised with formaldehyde. A recent study of volunteers exposed to formaldehyde at levels below the occupational exposure standard was also retrieved.[31] A number of these studies had reported evidence for an increase in MN or DPXs in PBLs.[17-28]

18 The COM considered these data with regard to the evaluation of data from toxicokinetic studies and *in-vivo* genotoxicity studies in experimental animals which suggested there would be no biological rationale for a direct systemic mutagenic effect of formaldehyde in biomonitoring studies. None of the studies collected the appropriate information previously identified by COM to assess background variation in results. Thus members noted that the quality of the biomonitoring studies was poor with limited account for confounding factors, including age, and also considered that the method for determination of DNA-protein cross links (SDS separation of protein-linked DNA) in PBLs had not been adequately validated. The COM suggested that a secondary mechanism could be responsible for the positive findings in peripheral blood lymphocytes.

19 The COM considered that no definite conclusions could be reached with regard to the small increases in DNA-protein cross links reported in the studies published by Saham *et al.*[22,26]

20 The Committee considered if any of the available studies was of sufficient quality to draw conclusions with regard to systemic mutagenicity of formaldehyde. Members noted the study by Ye and colleagues[27] clearly showed an increase in micronuclei in nasal mucosal cells in workers exposed to formaldehyde during manufacture whilst no concurrent increase in micronuclei in peripheral blood lymphocytes was noted.[27] The increase in SCE formation in peripheral blood lymphocytes in this study may have resulted from a secondary mechanism following oxidative DNA damage.

21 Members commented on the publication by Orsiere T *et al* (2006).[28] The apparent increase in micronuclei with centromeres was not consistent with the proposed mechanism of formaldehyde effects cross linking DNA and proteins. It was noted the protocol was not optimal for identification of aneuploidy, and that individual data were not available. Members considered that no definite conclusions could be reached on the data presented in this publication.

22 There was no evidence for an increase in micronuclei in buccal smears from volunteers exposed for up to 4 h/day for 10 working days to levels of formaldehyde below the UK occupational exposure limit (ie < 2 ppm).[31]

23 The Committee concluded that there was no convincing evidence regarding direct systemic mutagenic effects of formaldehyde from the available biomonitoring studies. The COM agreed a secondary mechanism might be involved with regard to the genotoxic effects documented in peripheral blood lymphocytes in the biomonitoring studies reviewed.

Additional *in vitro* study of formaldehyde induced DPX

24 The Committee noted that recent *in vitro* studies had demonstrated that DPXs formed from formaldehyde were effectively removed at concentrations up to 100 μM.[30]

COM conclusions

25 The COM concluded that the amount of formaldehyde systemically available following inhalation exposure at the occupational exposure standard would be negligible.

26 The COM was aware that formaldehyde was a direct acting *in vitro* mutagen. The COM concluded that there was no convincing evidence from *in vivo* mutagenicity studies in experimental animals and from biomonitoring studies of genotoxicity in workers exposed to formaldehyde for a direct *in vivo* systemic mutagenic effect of inhaled formaldehyde. A secondary mechanism might be involved in the genotoxic effects documented in peripheral blood lymphocytes in the biomonitoring studies reviewed.

27 The COM concluded that there was no reason to consider that direct systemic mutagenicity would be involved in the mechanism of formaldehyde induced systemic tumourigenicity.

28 The Committee concluded that for occupational and environmental exposure to formaldehyde, the pattern of metabolism and distribution of formaldehyde indicate that a threshold for *in vivo* systemic mutagenicity is likely.

COM/07/S5
November 2007

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Statement on the Mutagenicity of Terephthalic Acid

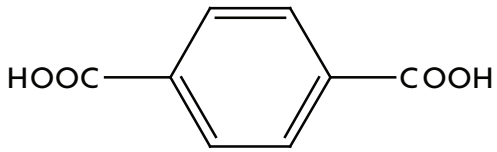
COM/07/S6 – December 2007

Background

1 Terephthalic acid (Figure 1) is used as a starting material in the manufacture of polyethylene terephthalate (PET). PET may be used to coat the internal surface and welded joints (side stripes) of food cans. PET can also be used to manufacture beverage bottles.

2 Terephthalic acid has been found to migrate from food contact materials at concentrations below 0.7 mg/kg food[1,2]. Migration from food contact materials is specifically controlled by European Regulation, which stipulates a specific migration limit (SML) for terephthalic acid of 7.5 mg/kg food.

Figure 1: Terephthalic acid



Previous Committee Evaluations

3. In October 2000, the Committee on Toxicity (COT) considered the health effects of terephthalic acid in the context of a survey on the migration of this compound from can coatings into food [1]. The COT concluded that the concentrations of terephthalic acid that had been determined in foods analysed in the survey were not of concern for public health on the basis of the then available information. However, the COT requested that, in the light of the bladder tumours occurring in rats fed the highest dietary concentration of terephthalic acid (5% in the diet, equivalent to 2.5 g/kg bw/day) in long-term studies, the view of the COM be sought on the potential *in vivo* genotoxicity of this compound [3].

4. In November 2001, the COM considered the mutagenicity of terephthalic acid based on a limited data set. *In vitro* assays included several bacterial mutagenicity assays that, although finding terephthalic acid to be negative, were either poorly reported or had inadequate protocols [4,5,6]. Overall, the Committee accepted that the evidence from the bacterial studies suggested that terephthalic acid is not mutagenic in a limited number of Salmonella typhimurium strains. An *in vitro* cytogenetics test in lung fibroblasts was also considered by the Committee [7]. Although terephthalic acid was found to be negative when tested at a concentration of 2 mg/ml using an exposure period of 48 hours, the study did not address the influence of an exogenous metabolic activation system. In addition, the effect of shorter exposure periods were not investigated. Finally, members reviewed a negative *in vivo* micronucleus assay conducted with terephthalic acid in ICR mice [8]. This was conducted to current standards but lacked toxicokinetic data, so gave no direct measurement of bone marrow exposure.

However, signs of toxicity were reported which suggested that the test material had been absorbed into the systemic circulation and thus dose selection had been adequate.

5 The Committee considered that the limited *in vitro* mutagenicity data package and absence of toxicokinetic data in the *in vivo* micronucleus assay were insufficient to determine the mutagenic potential of terephthalic acid. Therefore, the Committee recommended that an adequately conducted *in vitro* cytogenetics test in mammalian cells was needed before any definite conclusions could be reached which would indicate that the bladder tumours in the rat carcinogenicity bioassay arose from a non-genotoxic mechanism[9] .

6 Subsequently the COT has evaluated a multi-generation reproductive toxicity study. Whilst dietary administration of up to 20 g/kg diet terephthalic acid for two successive generations did not result in any alterations in reproductive performance, histopathological changes in the urinary bladder and the kidney were reported at this dose[10]. Further histopathological examination was conducted at the request of the COT. These changes comprised transitional epithelial hyperplasia, cystitis, inflammatory or mononuclear cell infiltration and haemorrhage. This expert report concluded that these treatment related changes indicated an irritant effect of the compound on the bladder mucosa at this dose level at 20 g/kg diet; however, no changes were observed in the bladder of animals receiving lower doses[11,12]. The COT were satisfied with this analysis, determining a NOAEL of 425 mg/kg bw/day for this study, equivalent to the 5 g/kg diet dose group. However, the COT decided that a final statement should not be issued until the additional mutagenicity data on terephthalic acid had been evaluated by the COM[13].

Discussion of Submitted Data

7 In May 2006, the COM was presented with a submission of data that BP Chemicals Ltd had commissioned, following the 2001 meeting.

Mouse Metabolism Study

8 A mouse metabolism study was submitted to address concerns regarding bone marrow exposure at the doses selected for the mouse micronucleus study, which had been reviewed at the 2001 meeting. The test material for this study was prepared from unlabelled terephthalic acid (756.2 mg, 99.9% purity w/w) mixed with [^{14}C]-terephthalic acid (4.51 MBq = 0.25mg, 99.2% purity w/w) in aqueous carboxymethylcellulose (8.69g, 0.5% w/w). Seven groups of three male Crl:CD-1TM(ICR) BR mice were administered this test material (800 mg terephthalic acid/kg bw) via the intraperitoneal route. This dose, strain and administration route were selected to be consistent with those used in the micronucleus study. Groups of mice were sacrificed at 2, 4, 6, 12, 24 and 48 h post treatment and the excreta of the 48 h dose group were collected for the duration of treatment.

9 The administered dose was extensively absorbed into the systemic circulation, widely distributed and rapidly excreted. Greater than 70% of the administered dose was excreted in the urine by 24 h. The highest mean tissue concentration was found in the kidney 2 h post administration (563 µg equiv/g, representing 1% of the administered dose) followed by bone (74 µg equiv/g). Difficulties in isolating murine femur bone marrow were experienced and bone marrow could be extracted from two of the three mice in the high dose group, with one below the limit of detection and the other 92 µg equiv/g, compared to blood (167 µg equiv/g) and plasma (221 µg equiv/g). Radioactivity declined rapidly in all

tissues, with levels in most tissues below the limit of detection by 48 h. Analysis of the urine found a single peak, which was reported to comprise the parent compound and a sulphate conjugate of terephthalic acid (based on mass spectrometry data).

10 It was noted that there was considerable variation in tissue levels of terephthalic acid between the three animals used in each dose group, which was most apparent at 2 hours. Members also noted that the vehicle was different to the one that had been used in the micronucleus study, further complicating interpretation of the data.

11 The Committee considered that this metabolism study was not helpful in demonstrating target tissue exposure had been achieved in the micronucleus test. However, members noted there was evidence of systemic toxicity in the preliminary toxicity study and in the micronucleus study. Also, that there had been a reduction in the Polychromatic/Normochromatic erythrocyte ratio (P/N ratio) in the micronucleus test, which suggested that target tissue exposure to terephthalic acid, had occurred.

Unscheduled DNA Synthesis Study (UDS)

12 A second *in vivo* study was submitted to supplement the micronucleus study. A single oral dose of terephthalic acid (2000 mg/kg bw, >99.9% purity w/w) was assessed for its ability to induce UDS in the liver of male Alpk:APfSD rats. Groups of three rats were sampled at 2 and 16 h post administration. Assessment of the mean net nuclear grain counts and percentage of cells in repair indicate terephthalic acid did not induce UDS at either time point. This study was performed to GLP, adhering to OECD guideline 486 (1997). Negative (vehicle) and positive (N-nitrosodimethylamine, 10 mg/kg bw) controls behaved as expected in this assay.

13 Members agreed that this unscheduled DNA synthesis (UDS) study had been adequately conducted and was negative.

In Vitro Cytogenetics Studies

14 As per the original COM data request, *in vitro* cytogenetics data using human lymphocytes to assess the clastogenicity of terephthalic acid were provided. In the first study, concentrations of 50, 250 and 500 µg/ml terephthalic acid (99.9% purity) were applied in the presence and absence of S9 metabolic activation. This assay was limited to 500 µg/ml at which the pH of the culture medium was reduced from 7.10 to 6.74. At 1000 µg/ml the pH of the medium was reduced to pH 6.00.

15 Following the standard protocol, two independent experiments were performed; Experiment 1 assessed the clastogenicity of terephthalic acid following 3 h incubation in the presence and absence of S9 metabolic activation and Experiment 2 assessed terephthalic acid following 3 h incubation in the presence of S9 and 20h in the absence of S9. All cultures were harvested 20 h after dosing (68 h after culture initiation).

16 A dose related reduction in mitotic index was observed in both experiments of this study. Statistically significant increases in the percentage of aberrant cells were observed following 20 h incubation in the absence of S9 metabolic activation ($p<0.01$ at 250 and 500 µg/ml). In addition, there were small increases in the percentage of aberrant cells following 3 h incubation in the presence and absence of S9 metabolic activation. Therefore, under the conditions of this initial study, terephthalic acid was found to be clastogenic.

17. A second study was submitted using the sodium salt of terephthalic acid, sodium terephthalate (99% purity w/w). In this study, no reduction in pH was observed when tested up to 2100 µg/ml, the maximum concentration stipulated by the protocol for this assay (10 mM). Therefore, concentrations of 1000, 1500 and 2100 µg/ml were examined. As before, independent experiments assessed the clastogenicity of sodium terephthalate following 3 h incubation in the presence and absence of S9 metabolic activation and 3 h in the presence of S9 and 20 h in the absence of S9. All cultures were harvested 20 h after dosing (68 h after culture initiation).

18 Small but statistically significant increases in the percentage of aberrant cells were observed, when compared to the vehicle control, following 3 h incubation in the presence and absence of S9 metabolic activation. These were not concentration related and were within the range of the historical control. Treatment did not reduce the mitotic index. No statistically significant increases in aberrant cells were observed in cultures incubated for 20 h in the absence of S9 metabolic activation. The author of the study report concluded that sodium terephthalate was not clastogenic under the conditions of this study. It was also argued that the positive finding for terephthalic acid in this study should be taken in the context of the negative result for sodium terephthalate in the second study. The author suggested that the clastogenicity observed in the initial study was not associated with the terephthalate anion itself.

19 Both studies were performed to GLP, adhering to OECD guideline 473 (1997). Negative (vehicle) and positive (mitomycin C, 0.5 µg/ml; cyclophosphamide, 50 µg/ml) behaved as expected in both studies.

20 Members were concerned that a relatively small reduction of 1 pH unit could not fully account for the clastogenicity observed in the first study. Whilst the criteria for a positive response had not been fulfilled in the second study, the low incidence of aberrations in the control cultures meant it was not possible to determine that terephthalic acid produced no effect. It was noted that 100 metaphases had been scored in the controls and at each dose group level. Members agreed that this should be increased to 200 to aid interpretation of the data.

21 These additional counts were provided for the May 2007 meeting. Members considered that the additional metaphase counts had reaffirmed the previous result, which suggested a weak clastogenic effect *in vitro*, and that the mechanism for this effect was unclear. Interpretation of this response was complicated by the incidence of aberrations in the control cells, which was much lower than the historical control. However, this study did not meet the criteria for a positive effect.

Conclusions

22 The Committee agreed that the two *in vivo* studies were adequate and negative, indicating that terephthalic acid is not an *in vivo* mutagen. The available evidence supported the previous COM conclusion of a non-genotoxic mechanism for the bladder tumours seen in the rat carcinogenicity study.

COM/07/S6
December 2007

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12 TOX/2005/15 Terephthalic acid: multigenerational reproduction study additional histopathological examinations. http://www.food.gov.uk/multimedia/pdfs/TOX-2005-15.pdf

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2007 Membership of the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment

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Dr D Benford BSc PhD	*Joint Scientific Secretary – Food Standards Agency*
Mrs J Cleverly MAAT	*Administrative Secretary – Health Protection Agency*
Dr L Hetherington BSc PhD	*Scientific – Health Protection Agency*
Ms F Pollitt MA DipRCPath	*Scientific– Health Protection Agency*
Mr S Robjohns BSc MSc	*Scientific – Health Protection Agency*

Declaration of COM members interests during the period of this report

	Personal Interest		Non Personal Interest	
MEMBER	COMPANY	INTEREST	COMPANY	INTEREST
Professor P B Farmer (Chair)	Santander Bradford & Bingley Foreign & Colonial Friends Provident Health Effects Institute Torotrak ILSI HESI	Shareholder Shareholder Shareholder Research Committee Member Shareholder Committee Member	American Chemistry Council CEFIC	Research support and conference attendance expenses. Research Support
Dr C Allen	NONE	NONE	NONE	NONE
Dr B Burlinson	Huntingdon Life Sciences	Salary Employee Share Option Holder	NONE	NONE
Dr G Clare	Covance Allied Domecq AstraZeneca Diageo HBOS Marks & Spencer	Salary Shareholder Shareholder Shareholder Shareholder Shareholder	NONE	NONE
Dr J Clements	Covance	Salary Share Option Shareholder	NONE	NONE
Dr B M Elliott AstraZeneca	Syngenta	Salary Share Option Holder Shareholder	NONE	NONE
Dr D Gatehouse	Covance Friends Provident GlaxoSmithKline Share Option Holder Shareholder	Salary Consultant Shareholder Pension	NONE	NONE
Mrs R Glazebrook	BT Group Lloyds TSB National Grid	Shareholder Shareholder Shareholder	NONE	NONE

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor N J Gooderham	Banco Santander	Shareholder	FSA	Research contract
	CENES	Shareholder	GlaxoSmithKline	CASE studentship
	Silence Therapeutics	Shareholder	FEMA (USA)	Research support
	Hargreaves Lansdown	Shareholder		
	Proctor & Gamble	Consultant		
Dr D P Lovell	National Grid Transco	Shareholder	AstraZeneca	Spouse Shareholder
			National Grid Transco	Spouse Shareholder
	Pfizer	Shareholder Share Options Pension		
Dr I Mitchell	Kelvin Associates	Associate Consultant		
	IM Enterprises	Director/Creditor		
	Chilfrome Enterprises	Director Pensioner		
	GlaxoSmithKline	Option and Shareholder Consultant	NONE	NONE
	Allergy Therapeutics	Shareholder		
	BG	Shareholder		
	Cadbury Schweppes	Shareholder		
	GEC	Shareholder		
	GSK	Shareholder		
	ICH	Shareholder		
	Mitchell & Butler	Shareholder		
	Pfizer	Shareholder		
	Real Good Food	Shareholder		
	Renishaw	Shareholder		
	Royal Dutch Shell	Shareholder		
	RTZ	Shareholder		
	Unilever	Shareholder		
	Vedanta	Shareholder		
	BP	PEP Holder		
	Centrica	PEP Holder		
	Green King	PEP Holder		
	Scottish & Southern	PEP Holder		
Dr E M Parry	Invesco	PEP Holder		
	Fleming	PEP Holder	NONE	NONE
	Legal & General	PEP Holder		
	Quintiles	Consultancy		

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Prof D H Phillips	Aviva Banco Santander BG Group Bradford & Bingley Centrica National Grid ECETOC Servier Butler Jeffries (solicitors)	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Honorarium Honorarium Honorarium	NONE	NONE

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

Preface

The Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC) evaluates chemicals for their human carcinogenic potential at the request of UK Government Departments and Agencies. The membership of the committee, agendas and minutes of meetings, and statements are all published on the internet (http://www.advisorybodies.doh.gov.uk/coc/index.htm).

During 2007, the Committee provided advice on a range of interesting topics. It carried out a number of reviews and gave further consideration to the issue of folic acid fortification and carcinogenesis.

Among the generic issues considered by the committee, it gave further consideration to the issue of risk communication. In this context, it assessed the use of the Margin of Exposure (MOE) approach, which is being developed in international fora for risk communication and risk prioritisation of non-threshold carcinogens. At the request of the Health and Safety Executive, the committee provided technical guidance on the draft guidance for assessment of the carcinogenicity of chemicals in the context of new European legislation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

Chairing the COC continues to be a challenging but rewarding task. I thank the members and secretariat for their support during the past year and look forward to working with them on the challenges ahead.

Professor David H Phillips
BA PhD DSc FRCPath

Assessing the risks of acute or short-term exposure to carcinogens

3.1 The Department of Health and the Health Protection Agency are occasionally asked to provide advice on the health risks of acute or short-term exposure to genotoxic carcinogens. In 2006, they sought the advice of the COC on how this might be done and, after discussion, members decided that it would not be appropriate to use an acute T25 approach. One member had also informed the COC that there were some papers in the literature which might indicate a way forward and three of these were discussed by the committee at a meeting in 2007.

3.2 An underlying principle in the approaches suggested by two of the papers was that tumour incidence is linearly related to the cumulative dose of a chemical. However, the COC disagreed with this principle, which it assumed had derived from observations about the relationship between radiation dose and cancer risk. In the case of chemicals, it was noted that DNA repair processes could be significant at low doses, a non-linear response could occur due to the complexity of the carcinogenic process, and genotoxic carcinogens may have different effects eg at high doses some genotoxic carcinogens could also promote cancer via a cytotoxic mechanism. The relationship could also be affected by latency. It was noted that there were few relevant epidemiological data on chemicals but it appeared that, for smoking, the risk was sublinear with time, at least for lung and bladder cancer.

3.3 One paper had considered whether exposure of 1-10 days to genotoxic carcinogens may contribute to tumour development and, if so, whether this contribution to cancer risk could be quantified. A pragmatic approach was proposed, which used the premise that tumour incidence is linearly related to the cumulative dose of a chemical and incorporated the principle of the Virtually Safe Dose (VSD) associated with an "acceptable" risk level. The approach then applied factors to scale up from low level exposure daily over 70 years to the dose which might be acceptable if exposure was only for 1 day, or 2-10 days. The COC noted that the paper was mainly theoretical and used mathematical modelling to develop risk estimates from animal carcinogenicity data (an approach that is not recommended by the COC). Although there were areas of uncertainty and a number of assumptions in the proposed approach, members considered that extrapolation from lifetime exposures to short-term (up to 10 days) exposures for low doses would probably overestimate the cancer risk. They considered that, although there was an assumption that certain subpopulations would be more susceptible than others, there was no suitable data to allow any quantitative estimation and associated adjustment to cancer risk estimates. However, it was suggested that it may be possible to adapt the method by using the Margin of Exposure (MOE) approach and that this might provide a pragmatic approach to the risk assessment of short-term exposures to genotoxic carcinogens, although there would be some associated degree of uncertainty.

Betel quid, Pan Masala and areca nut

3.4 This item was considered in 2006 but not included in the 2006 Annual Report.

3.5 Areca nut is an ingredient of betel quid or pan masala, which is chewed as an aid to digestion and as a stimulant. It also may have limited use as a food ingredient. The COC last considered the carcinogenicity of areca nut in 1994 but since then, a number of relevant new papers had been

published. Consequently, the FSA sought the view of the COC as to whether the new information was sufficient to warrant updating its previous advice, and whether any conclusions could be drawn about the use of areca nut as a food ingredient.

3.6 In the previous COC assessment, the Committee had considered a range of data and had concluded the following:

- There was evidence of mutagenic and carcinogenic activity of areca nut extracts and derived compounds in experimental systems. In particular, the potent carcinogenic activity of the areca-derived nitrosamine, 3-(methylnitrosoamino)-propionitrile (MNPN), had been confirmed and methyl and cyanoethyl adducts had been detected in the DNA of the target tissues in which the tumours developed. There was evidence that endogenous nitrosation of areca nut alkaloids can occur in animals and humans; and areca nut derived nitrosamines, including MNPN, have been detected in the saliva of betel quid chewers.

- There were very limited data from epidemiological studies on the effect of betel or areca nut products without tobacco, which did not allow any conclusion to be drawn. There was, however, sufficient epidemiological evidence of a link between the chewing of betel quid containing tobacco and cancer in humans.

- Use of these products without tobacco was possibly carcinogenic in humans.

3.7 In 2003, the International Agency for Research on Cancer (IARC) assessed the use of betel quid, and concluded that both the chewing of betel quid and areca nut should be categorised as Group 1 carcinogens (carcinogenic to humans). In its conclusions, IARC stated that there is sufficient evidence in humans to conclude that betel quid chewed without tobacco causes oral cancer. IARC also concluded that there was sufficient evidence in animals to confirm the carcinogenicity of betel quid and areca nut without tobacco. This followed a previous IARC review in 1985, which concluded that there was inadequate evidence for the carcinogenicity of betel quid chewed without tobacco.

3.8 The COC reviewed a number of epidemiology studies published since 1993 and new animal studies which attempted to identify mechanisms for the carcinogenic potential of areca nut extracts and agreed with the IARC (2003) conclusions. The committee considered that most of the evidence suggested that areca nut is a site of contact carcinogen acting by a genotoxic mechanism, and noted that there was some evidence that a non-genotoxic oxidation mechanism could also be involved. The carcinogenic mechanism was likely to involve the nitrosation of precursor chemicals present in areca nut. It was not clear why there appeared to be an increased site-of-contact cancer risk for those who swallowed the juice. The COC also considered that there was no clear evidence that the use of areca nut without tobacco caused primary liver cancer or other cancers at distant sites.

3.9 The COC concluded that there was now sufficient evidence for a causal link between the chewing of areca nut without tobacco and oral cancer and that, overall, areca nut should be regarded as carcinogenic to humans. Members also agreed that the use of areca nut as a food ingredient was potentially carcinogenic.

Folic acid fortification and carcinogenesis

3.10 In 2005, the Scientific Advisory Committee on Nutrition (SACN) asked for advice from the COC on whether dietary folic acid intake is associated with increased cancer risk. After reviewing the available data, the COC recommended a precautionary approach in considering mandatory fortification of flour with folic acid. Subsequently, SACN had asked for further clarification of this advice and the Chairman had agreed that aiming to increase low level intakes whilst ensuring that high level intakes do not increase is consistent with a precautionary approach. As a result, the SACN concluded that mandatory fortification should only be introduced if it is accompanied by action to reduce folic acid intakes from voluntarily fortified foods to ensure that the numbers of people with intakes above the EVM's upper intake level do not exceed current levels and there is no substantial increase in mean intakes or in the folate status of the UK population.

3.11 At its July 2007 meeting, the COC discussed further recent publications on folic acid, including two editorials from the British Medical Journal. An abstract of a randomised clinical trial with folic acid supplements for the prevention of colorectal adenomas had been seen by the committee when it considered this topic last year; the full paper had now been published. This had failed to show any evidence that folic acid supplementation protects against colorectal cancer but that there was an excess of prostate cancer cases in the folic acid group compared to controls. It was suggested that this might be due to promotion of pre-neoplastic lesions to neoplasms. Members were reminded that the study had not been designed to investigate the effect of folic acid supplementation on prostate cancer risk and that the authors of the study had themselves commented that this could be a spurious association. It was also noted that there may have been a differential rate of PSA testing between the two groups, and that mandatory fortification of food with folic acid had been introduced in the US while this study was in progress and total intake of folic acid would have exceeded the guidance level for upper intake of 1 mg/day recommended in the UK by the Expert Group on Vitamins and Minerals (EVM). According to the SACN report, there was no clear change in the incidence of prostate cancer following mandatory fortification in the US. A second paper reported a study on dietary intake of folate and co-factors in folate metabolism.

3.12 The committee concluded that, on balance, it was content with the proposals by the FSA Board to recommend to UK health ministers that there should be mandatory fortification of a food with folic acid, with controls on voluntary fortification and guidance on use of supplements, monitoring of the folic acid intakes and status of the UK population and postulated risks – including cancer incidence- and a review of the data on the benefits and possible risks 5 years after introduction of mandatory fortification. Members asked to be informed of the outcome of the 5 year review.

3.13 Following the meeting, Members were informed that the Chief Medical Officer had decided to convene a special subgroup of the Scientific Advisory Committee on Nutrition (SACN) to examine further two papers on the potential adverse effects of folic acid on the risk of colorectal cancer and that the Chairman had been invited to participate in this subgroup.

Formaldehyde

3.14 At the 2006 horizon scanning exercise, the committee was informed that the International Agency for Research on Cancer (IARC) had concluded that there is "strong but not sufficient evidence for a causal association between leukaemia and occupational exposure to formaldehyde". This conclusion was tempered because it was not possible to identify a mechanism for leukaemia induction. A literature paper had argued against there being a causal relationship for formaldehyde and leukaemia risk on the grounds that there is no evidence to suggest that formaldehyde reaches any target organ beyond the site of contact, no indication that formaldehyde is toxic to the bone marrow, and no credible evidence that formaldehyde induces leukaemia in experimental animals. In 2007, it considered the conclusions of a recent COM review of formaldehyde and the extract from the recent IARC monograph on formaldehyde which summarises the epidemiological findings on leukaemia.

3.15 The COM advice is given in paragraph 2.17 of this document. The COC noted its conclusions and considered a key point to be that inhalation exposure to exogenous formaldehyde would have no effect on the endogenous concentration of formaldehyde. Members therefore questioned how IARC had concluded that there was a causal association between leukaemia and occupational exposure to formaldehyde, particularly in view of the metabolic data in the IARC summary. The Committee decided that it did not wish to review the epidemiological evidence in detail.

Quantitative structure-activity relationships (QSAR)

3.16 The COC considered two recently published papers on the use of QSAR to predict carcinogenicity. One paper, from the US Food and Drug Administration (FDA) described an evaluation of the performance of a particular software, MDL-QSAR predictive discrimant analysis modelling of rodent carcinogenicity, to estimate the carcinogenic potential of small, organic, naturally occurring chemicals found in the human diet. The predictive performance for this group of chemicals and a control group of 19 known syntheic dietary constitutents was 97% sensitivity and 53% specificity. The second paper, by Benigni *et al* (2007), described and discussed preliminary findings from a project on (Q)SARs for mutagenicity and carcinogenicity initiated by the European Chemicals Bureau and carried out by the Istituto Superiore di Sanita', in the context of the forthcoming REACH legislation.

3.17 Members considered that it would be useful to review the final results of the REACH project when this had been published and then to decide whether to undertake a full review of QSAR for prediction of carcinogenicity.

Risk communication

3.18 In 2005, the COC and COM decided that they should collaborate to improve the way in which the advice on the potential carcinogenicity and mutagenicity of chemicals is presented to the general public. In 2006 and 2007, the COC discussed the ways in which the carcinogenic risk of chemicals might be better communicated and put into context alongside other risks. Members noted that the primary role of the COC and COM was to provide advice to government departments and regulatory

agencies which were responsible for risk management and risk communication. However, it was agreed that a wider understanding of the low risks associated with exposure to environmental carcinogens was desirable.

3.19 One suggestion was that the risks of environmental carcinogenesis could be compared with other involuntary risks, using established risk scales such as the Calman risk scale or the "Risk It" game developed by the HPA for radiation. However, the committee considered that it would not be possible to develop such an approach for chemicals as selection of exposure scenarios would be very difficult. It suggested that a more suitable approach might be to compare closely connected risks e.g. those of natural toxins and synthetic pesticide residues in or on the same foodstuff. The COC also agreed that current approaches to drafting statements needed to be improved, with more information on the reasons for undertaking reviews and non executive summaries developed for all statements. It asked the secretariat to consider carefully the audience in any further paper for the COC.

3.20 As part of this item, the FSA asked the COC to comment on the potential usefulness of the Margin of Exposure (MOE) approach being developed by the European Food Safety Agency (EFSA), the World Health Organisation (WHO) and the International Life Sciences Institute (ILSI) as a way of prioritising the risks associated with unavoidable exposure to genotoxic chemical carcinogens in food. The MOE is the numerical value obtained by dividing a reference point on the dose response curve for carcinogenicity in experimental animals by estimated human exposure to the chemical. The preferred reference point of the above organisations is the lower limit of the confidence interval on the benchmark dose (BMD), although they have noted that the T25 could be used in cases where the dose-response data were not adequate to define the BMD or the lower limit of its confidence interval of the (BMDL).

3.21 The COC agreed that the use of the MOE to prioritise the risk of exposure to genotoxic carcinogens was consistent with the most recent COC guidelines and would also contribute to international harmonisation in this area. It discussed the relative merits of using the BMDL10 or T25 and noted that the BMDL10 takes the quality of the data into consideration, due to the use of confidence intervals, whilst the T25 does not. The T25 was usually derived from poorer data sets. Overall, it would be necessary to decide which to use on a case-by-case basis depending on the quality of the available data.

3.22 A system for banding MOE values was agreed and is given below in Table 1. This expands on proposals for the interpretation of the magnitude of the MOE that had been made by JECFA and EFSA, where there was a consensus that an MOE greater than 10,000 indicated low concern. It was hoped that the banding system might improve the communication of advice on genotoxic carcinogens to wider audiences.

Table 1: Banding of MOE values for risk communication

MOE Band	Interpretation
<10,000	May be a concern
10,000-1,000,000	Unlikely to be a concern
>1,000,000	Highly unlikely to be a concern

3.23 Having agreed the MOE approach in principle for the communication and prioritisation of risks, the committee considered in detail the methodology used to derive the BMDL10. It reviewed an illustrative exercise for 3 genotoxic soil contaminants (hexavalent chromium, benzo(a)pyrene and 1,2-dichloroethane) and advised on several aspects of the methodology which should be used. Members involved in the international fora recommended that the COC should review ongoing work on model averaging when published.

Technical guidance for derivation of DNELs and risk characterisation of non-threshold effects in the context of REACH

3.24 At the March 2007 meeting, the HSE made a short presentation to inform members about progress with the development of technical guidance for the risk assessment of substances under REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals - a new European Regulation). This focused on the proposed guidance for dealing with "non-threshold" genotoxic carcinogens. Two approaches were proposed: one is based on linear extrapolation from animal bioassay data to a predetermined low level of risk and the other is based on application of a large uncertainty factor (UF) to a suitable reference point on the dose-response for carcinogenicity. The latter approach was included because the UK, unlike most other EU member states, does not use the linear extrapolation approach. Both approaches generate a Derived Minimum Effect Level (DMEL) which is defined as the level of exposure above which humans should not be exposed. Members were informed that both the predetermined low level of risk and the uncertainty factors were decided at policy level and were non-negotiable.

3.25 The COC considered that the UF and DMEL methods are essentially equivalent but differ with respect to risk communication. Members noted that the UF approach differed from the MOE approach in that, in the REACH methodology, a predetermined UF of 10,000 was to be used. Members were concerned about the derivation of an UF of 10 for 'the nature of the carcinogenic process' given the limitations of current knowledge about DNA repair.

3.26 There was discussion of the units chosen for the DMEL values with some members favouring the use of moles/micromoles rather than dose/weight/day, because the biological response is based on the interaction of molecules of a chemical, not the weight administered. However, it was pointed out that this could be difficult since the purity of the active ingredient is not always known and it would be difficult to assess substances, as opposed to single chemicals, on a molar basis. The committee also discussed the proposal that "biological criteria" should be used to assess the relevance of a carcinogenic response, rather than statistics alone. It was pointed out that all tumours are biologically significant but it is important to know whether they are compound related and this is, in part, based on statistical analysis.

3.27 The proposals included using the Threshold of Toxicological Concern (TTC) approach to setting DMELs for somatic cell mutagens with no cancer data. However, the COM considers it premature at present to set levels such as TTCs for mutagens with no cancer data, as no agreement had been reached about how to rank such mutagens.

3.28 HSE commented that the committee's comments would be reported to the EU working group drawing up this guidance.

Horizon scanning

3.29 The COC undertakes "horizon scanning" exercises at regular intervals to identify new and emerging issues which have the potential to impact on public health. After an extensive literature search, a number of topics were identified by the secretariat for consideration by the committee at the 2007 exercise. From these, and committee members' own proposals, the COC considered that the following topics should be taken forward, together with those outstanding from 2006:

- the carcinogenicity of mixtures
- further consideration of the Human relevance framework/Mode of action
- a further review of proteomics
- mutational spectra (jointly with the COM).

Ongoing topics

Chlorinated drinking water and cancer

3.30 During 2007, the COC reviewed a number of new epidemiological studies on chlorinated drinking water and cancer. A statement will be published in 2008.

Non-Hodgkin's lymphoma (NHL)

3.31 Incidence rates for NHL have increased in all age groups in Great Britain in recent years, particularly during the early 1980s and 1990s. A number of epidemiological studies have been carried out on the association between chemicals and NHL and the committee reviewed these during 2007 to see if there is any evidence that exposure to environmental chemicals could have accounted for the increase in incidence of NHL. A statement will be published in 2008.

Toxicogenomics

3.32 The COC, COM and COT are updating their review of toxicogenomics. The COC has focussed on two areas in particular: whether toxicogenomics is useful in distinguishing between genotoxic and non-genotoxic chemicals, and whether it is useful in testing the hypothesis for a mode of action or of help in proposing a mode of action for a chemical. A joint statement will be published in 2008.

Statements of the COC

Statement on Prostate Cancer and Pesticide Exposure

Introduction

1 In 2004 the committee published a statement on prostate cancer, in which we discussed the known and potential risk factors associated with this disease. The statement included an evaluation of the epidemiological and other data on occupational groups and chemical exposures for which an association with prostate cancer has been proposed. Two occupational groups were considered: rubber workers, for whom we concluded that there was no convincing evidence of an increased risk of prostate cancer, and farmers/farm workers/pesticide applicators. In the case of this latter group, we concluded:

> "...that there was some limited evidence to suggest an association between farmers/farm workers, exposure to pesticides and increased risk of prostate cancer. The possibility of such an association being causal could not be discounted and the published literature should continue to be monitored for further studies. The committee commented on the need for improved measures of exposure to pesticides and in particular herbicides. It was considered that the potential association between herbicide use by farmers and farm workers should be kept under review" (COC, 2004)

2 Recently, we were asked by the Pesticides Safety Directorate of the Department of the Environment, Food and Rural Affairs (Defra) to consider a report commissioned from the Institute of Occupational Medicine (IOM) entitled "Desk Study on Prostate Cancer and Pesticide Exposure"(IOM, 2006) and to advise on whether the report alters the conclusion of our 2004 statement. At the same time, we considered a newly published review and meta-analysis of cohort studies of prostate cancer risk in pesticide manufacturing workers (Van Maele-Fabry *et al*, 2006). We report here on our conclusions.

3 The IOM report is a narrative review of the epidemiology of occupational exposure to pesticides and the risk of prostate cancer, and of the potential mechanisms that might underlie any association. The report concludes that epidemiological studies of manufacturing workers exposed to pesticides have not reported an excess risk of prostate cancer. It also concludes that there are a large number of studies of agricultural workers exposed to pesticides but that, nevertheless, the results have been inconsistent: many have been negative or inconclusive, with a few showing positive results. We note that the report proposes that pesticides might cause prostate cancer through a potential endocrine-disrupting mechanism based on androgen imbalance. This proposal may be derived from data that suggest that low dose exposure to certain chemicals may lead to an increase in prostate gland weight in young mice (vom Saal *et al*, 1997) but these data are disputed by others (Ashby J *et al*, 1999) who were unable to reproduce these observations. Moreover, pesticides are not a generic group of chemicals but comprise compounds of different structures and biological activities. It is unlikely, therefore, that a single mechanism of action would apply to all pesticides.

4 The meta-analysis by Van Maele-Fabry *et al* (2006) was conducted on 16 studies of workers ever employed in pesticide manufacturing and with potential exposure to pesticides. The analysis combined relative risk estimates from both incidence and mortality studies to derive an overall meta-rate ratio of 1.28 (95% CI 1.05 -1.58). After grouping the data into specific chemical classes of pesticide, increased pooled rate ratios were reported for each class but statistically significant results were only reported for phenoxy herbicides contaminated with polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans. We note that there are some unresolved methodological issues in this analysis, for example, adjustment for confounding might have been unsatisfactory, as little is known about the risk factors for prostate cancer.

5 We consider that the individual studies to date of exposure to pesticides in farmers/farmworkers and in pesticide manufacturing workers provide no consistent support for an association with prostate cancer. A recent meta-analysis by Van Maele-Fabry *et al* (2006) provides some limited evidence of a weak association between pesticide exposure in manufacturing workers and prostate cancer. It is not uncommon that the more formal approach of meta-analysis differs in its conclusions from a narrative review based on individual papers. Causality cannot be inferred from the available data. We recommend that the literature on this topic is kept under review.

March 2007
Statement COC/07/S1

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2007 Membership of the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment

CHAIR

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Professor of Environmental Carcinogenesis, Institute of Cancer Research

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Non-specialist Member

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Mr J Battershill BSc MSc	*Scientific – Health Protection Agency*
Mrs J Cleverly MAAT	*Administrative Secretary – Health Protection Agency*
Dr L Hetherington BSc PhD	*Scientific – Health Protection Agency*
Mr S Robjohns BSc MSc	*Scientific – Health Protection Agency*

Declaration of COC members interests during the period of this report

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor D H Phillips (Chair)	Aviva Banco Santander BG Group Bradford & Bingley Centrica National Grid Servier Buller Jeffries (Solicitor)	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Honorarium Honorarium	NONE	NONE
Dr C Allen	NONE	NONE	NONE	NONE
Professor A Boobis OBE	Bank Santander Barclays Bank BG Group BT Group Centrica Halifax National Grid Transco Scottish Power Astellas Pharma	Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Shareholder Consultancy	GlaxoSmithKline ILSI HESI	Research Support Unpaid member of Board of Trustees
Dr P Carthew	Unilever	Salary	NONE	NONE
Professor P B Farmer	Santander Bradford & Bingley Foreign & Colonial Friends Provident Health Effects Institute Torotrak ILSI HESI	Shareholder Shareholder Shareholder Shareholder Research Committee Member Shareholder Committee Member	American Chemistry Council CEFIC	Research Support and Conference attendance expenses. Research support
Mrs R Glazebrook	BT Group Lloyds TSB National Grid	Shareholder Shareholder Shareholder	NONE	NONE

MEMBER	Personal Interest		Non Personal Interest	
	COMPANY	INTEREST	COMPANY	INTEREST
Professor D Harrison	The Forensic Institute University of Edinburgh Lothian NHS Response Genetics (University consultancy no fee payable.) University of Florida (University consultancy) University of Canberra (University consultancy)	Shareholder	PI Medical Research (Scotland) Chair EMMS Nazareth Member Scientific Advisory Committee, Yorkshire Cancer Research.	Non specific research funding from Cancer Research UK. Breakthrough, CSO & other grant agencies. Trustee (Healthcare Charity) Trustee
Ms D Howel	NONE	NONE	NONE	NONE
Dr B G Miller	Scottish Power	Shareholder	NONE	NONE
Professor R A Roberts	AstraZeneca HBOS P & O	Salary Shareholder Shareholder	NONE	NONE
Professor D E G Shuker	NONE	NONE	NONE	NONE
Professor P Vineis	NONE	NONE	NONE	NONE
Dr N Wallis	Pfizer	Salary Shareholder	NONE	NONE

Annex 1 - Terms of Reference

The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) is an independent body of experts that was established in 1978.

To advise at the request of:

Food Standards Agency
Health Protection Agency
Department of Health
Department for Business, Enterprise & Regulatory Reform
Department of Transport, Local Government and the Regions
Department of Trade and Industry
Health and Safety Executive
Pesticide Safety Directorate
Veterinary Medicines Directorate
Medicines and Healthcare Products Regulatory Agency
Home Office
Scottish Executive
National Assembly for Wales
Northern Ireland Assembly
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 Chair of the Food Standards Agency and 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 197);
- 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 Chair (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 Chair except where the Chair 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 Chair

The Chair has particular responsibility for providing effective leadership on the issues above. In addition, the Chair 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 included below.

(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, e.g. 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 in Discussions at Meetings and by Correspondence

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, and if items are taken by correspondence between meetings. The declaration should note whether the interest is personal or non-personal, whether it is specific to the item under discussion, or non-specific (see below) and whether it is current or lapsed. Having fully explained the nature of their interest the Chair will, 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 Chair 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 Chair 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. **Members' interests must be declared/confirmed annually on the declaration of interests form to the Committee Secretariat**.

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;
- **Membership or Affiliation**: any membership role or affiliation that you or a close family member has to clubs or organisations with an interest or involvement in the work of the Department.

Non-Personal Interests

A non-personal interest involves payment which benefits the organisation in which the member works, 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 organisation, e.g.

  i) a grant for the running of a unit or department;

  ii) a grant or fellowship or other payment to sponsor a post or a member of staff or a post graduate research programme. 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 in which they work, 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.

Specific Interests

A member must declare a personal, specific interest if they have at any time worked on a matter, product or substance under consideration and have personally received payment for that work, in any form. Declarations need not be made for generic work on test methods or research with no impact on the risk assessment of an item under consideration.

A member must declare a non-personal, specific interest if they are aware that the organisation in which they work has at any time worked on the matter, product or substance under consideration but they have not personally received payment for that work, in any form.

Non-specific Interests

A member must declare a personal non-specific interest if they have a current personal interest in a company concerned with a matter, product or substance under consideration, which does not relate specifically to the matter, product or substance under discussion.

A member must declare a non-personal non-specific interest if they are aware that the organisation in which they work is currently receiving payment from the company concerned which does not relate specifically to the matter, product or substance under discussion.

If a member is aware that a substance, product or matter under consideration is or may become a competitor of a substance, product or matter manufactured, sold or supplied by a company in which the member has a current personal interest, they should declare their interest in the company marketing the rival product, substance or matter.

Definitions

In this Code, 'the industry' means:

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

  The Food Safety Act 1990

  The Medicines Acts 1968 and 1971

  The Food and Environmental Protection Act 1985

  The Consumer Protection Act 1987

  The Cosmetic (Safety) (Amendment) Regulations 1987

  The Notification of New Substances Regulations 1982

- Trade associations representing companies involved with such products;

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

In this Code 'the Secretariat' means the Secretariat of the COC, COM and 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 Chair 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 pages 203 and 204.

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, i.e. any necessary consultation with Members and Chair 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 (e.g. papers and minutes) confidential until an agreed opinion (e.g. statement) is available.

Issue	Proposals	Comment
Open meetings on specified topics (eg invited audience, interest groups, consumer organisations, professional societies.	Agreed. Suggestions include meeting at a 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	Publish in accordance with procedures for previous years.

* Procedures for handling confidential information is outlined overleaf.

Summary of proposals for committee openness

Procedures for handling confidential information

Background

1 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 assessment.

2 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.

3 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

4 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 (e.g. test reports, publications 333etc).

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 (e.g. where detailed information supplied in confidence such as individual patient information and full study reports of toxicological studies) will not be disclosed but that other information will be disclosed unless agreed otherwise with an individual company.

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

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

b) information on physico-chemical properties,

c) methods of rendering harmless,

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

e) methods of analysis,

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

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

Procedures 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 Chair 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 – Good Practice Agreement for Scientific Advisory Committees

Introduction

1 *Guidelines 2000: Scientific Advice and Policy Making*[10] set out the basic principles which government departments should follow in assembling and using scientific advice, thus:

- think ahead, identifying the issues where scientific advice is needed at an early stage;
- get a wide range of advice from the best sources, particularly where there is scientific uncertainty; and
- publish the scientific advice they receive and all the relevant papers.

2 The *Code of Practice for Scientific Advisory Committees*[11] (revised in December 2007) provided more detailed guidance specifically focused on the operation of scientific advisory committees (SACs). The Agency subsequently commissioned a *Report on the Review of Scientific Committees*[12] to ensure that the operation of its various advisory committees was consistent with the remit and values of the Agency, as well as the Code of Practice.

3 The Food Standards Agency's Board has adopted a **Science Checklist** to make explicit the points to be considered in the preparation of papers dealing with science-based issues which are either assembled by the Executive or which draw on advice from the Scientific Advisory Committees.

4 Scientists who serve on a scientific advisory committee which advises the Agency are expected to comply with the **Universal Ethical Code for Scientist**, launched by the Government's Chief Scientific Adviser in March 2007.

5 The Board welcomed a proposal from the Chairs of the independent SACs to draw up Good Practice Guidelines based on, and complementing, the Science Checklist.

6 These Guidelines have been developed by nine advisory committees:

Advisory Committee on Animal Feedingstuffs
Advisory Committee on Microbiological Safety of Foods
Advisory Committee on Novel Foods and Processes
Advisory Committee on Research (disbanded in 2007)
Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment[14]
Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment[15]
Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment[16]
Scientific Advisory Committee on Nutrition[17]
Spongiform Encephalopathy Advisory Committee[18]

[10] Guidelines on Scientific Analysis in Policy Making, OST, October 2005. Guidelines 2000: Scientific advice and policy-making. OST July 2000
[11] Code of Practice for Scientific Advisory Committees, OST December 2001 (www.dius.gov.uk/publications/file42780.pdf)
[12] Report on the Review of Scientific Committees, FSA, March 2002 (www.food.gov.uk/science/researchpolicy/commswork/scicomrev)
[13] Joint FSA/Defra Secretariat, FSA lead
[14] Joint FSA/HPA Secretariat, HPA lead
[15] Joint FSA/HPA Secretariat, HPA lead
[16] Joint FSA/HPA, FSA lead
[17] Joint FSA/DH Secretariat
[18] Joint Defra/FSA/DH Secretariat

7 These committees share important characteristics. They:

- are independent;
- work in an open and transparent way; and
- are concerned with risk assessment not risk management.

8 The Guidelines relate primarily to the risk assessment process since this is the committees' purpose. However, the Agency may wish on occasion to ask the independent scientific advisory committees whether a particular risk management option is consistent with their risk assessment.

Principles

9 Twenty seven principles of good practice have been developed. However, the different committees have different duties and discharge those duties in different ways. Therefore, not all of the principles set out below will be applicable to all of the committees, all of the time.

10 This list of principles will be reconsidered by each committee annually as part of the preparation of its Annual report, and will be attached as an Annex to it.

Defining the issue

1 The FSA will ensure that the issue to be addressed is clearly defined and takes account of stakeholder expectations. The committee Chair will refer back to the Agency if discussion suggests that a re-definition is necessary.

Seeking input

2 The Secretariat will ensure that stakeholders are consulted at appropriate points in the committee's considerations and, wherever possible, SAC discussions should be held in public.

3 The scope of literature searches made on behalf of the committee will be clearly set out.

4 Steps will be taken to ensure that all available and relevant scientific evidence is rigorously considered by the committee, including consulting external/additional scientific experts who may know of relevant unpublished or pre-publication data.

5 Data from stakeholders will be considered and weighted according to quality by the committee.

6 Consideration by the secretariat and the Chair will be given to whether expertise in other disciplines will be needed.

7 Consideration will be given by the Secretariat or by the committee to whether other scientific advisory committees need to be consulted.

Validation

8 Study design, methods of measurement and the way that analysis of data has been carried out will be assessed by the committee.

9 If qualitative data have been used, they will be assessed by the committee in accordance with the principles of good practice, e.g. set out in guidance from the Government's Chief Social Researcher[19].

10 Formal statistical analyses will be included wherever possible. To support this, each committee will have access to advice on quantitative analysis and modelling as needed.

11 When considering what evidence needs to be collected for assessment, the following points will be considered:

- the potential for the need for different data for different parts of the UK or
- the relevance to the UK situation for any data originating outside the UK; and
- whether stakeholders can provide unpublished data.

12 The list of references will make it clear which references have either not been subject to peer review or where evaluation by the committee itself has conducted the peer review.

Uncertainty

13 When reporting outcomes, committees will make explicit the level and type of uncertainty (both limitations on the quality of the available data and lack of knowledge) associated with their advice.

14 Any assumptions made by the committee will be clearly spelled out, and, in reviews, previous assumptions will be challenged.

15 Data gaps will be identified and their impact on uncertainty assessed by the committee.

16 An indication will be given by the committee about whether the database is changing or static.

Drawing conclusions

17 The committee will be broad-minded, acknowledging where conflicting views exist and considering whether alternative hypotheses fit the same evidence.

18 Where both risks and benefits have been considered, the committee will address each with the same rigour.

[19] There is of guidance issued under the auspices of the Government's Social Research Unit and the Chief Social Researcher's Office (Quality in Qualitative Evaluation: A Framework for assessing research evidence. August 2003. www.strategy.gov.uk/downloads/su/qual/downloads/qqe-rep.pdf and The Magenta Book. www.gsr.gov.uk/professional_guidance/magenta_book/guidance.asp).

19 Committee decisions will include an explanation of where differences of opinion have arisen during discussions, specifically where there are unresolved issues and why conclusions have been reached.

20 The committee's interpretation of results, recommended actions or advice will be consistent with the quantitative and/or qualitative evidence and the degree of uncertainty associated with it.

21 Committees will make recommendations about general issues that may have relevance for other committees.

Communicating committees' conclusions

22 Conclusions will be expressed by the committee in clear, simple terms and use the minimum caveats consistent with accuracy.

23 It will be made clear by the committee where assessments have been based on the work of other bodies and where the committee has started afresh, and there will be a clear statement of how the current conclusions compare with previous assessments.

24 The conclusions will be supported by a statement about their robustness and the extent to which judgement has had to be used.

25 As standard practice, the committee secretariat will publish a full set of references (including the data used as the basis for risk assessment and other committee opinions) at as early a stage as possible to support openness and transparency of decision-making. Where this is not possible, reasons will be clearly set out, explained and a commitment made to future publication wherever possible.

26 The amount of material withheld by the committee or FSA as being confidential will be kept to a minimum. Where it is not possible to release material, the reasons will be clearly set out, explained and a commitment made to future publication wherever possible.

27 Where proposals or papers being considered by the Board rest on scientific evidence, the Chair of the relevant scientific advisory committee (or a nominated expert member) will be invited to the table at Open Board meetings to provide this assurance and to answer Members' questions on the science. To maintain appropriate separation of risk assessment and risk management processes, the role of the Chairs will be limited to providing an independent view on how their committee's advice has been reflected in the relevant policy proposals. The Chairs may also, where appropriate, be invited to provide factual briefing to Board members about particular issues within their committees' remits, in advance of discussion at open Board meetings.

Universal Ethical Code for Scientists

The Universal Ethical Code for Scientists, developed by the Government Chief Scientific Adviser, is a public statement of the values and responsibilities of scientists. The term 'scientists' means anyone whose work uses scientific methods, including social, natural, medical and veterinary sciences, engineering and mathematics.

Rigour, respect and responsibility: A universal ethical code for scientists

Rigour, honesty and integrity

- Act with skill and care in all scientific work. Maintain up to date skills and assist their development in others.
- Take steps to prevent corrupt practices and professional misconduct. Declare conflicts of interest.
- Be alert to the ways in which research derives from and affects the work of other people, and respect the rights and reputations of others.

Respect for life, the law and the public good

- Ensure that your work is lawful and justified.
- Minimise and justify any adverse effect your work may have on people, animals and the natural environment.

Responsible communication: listening and informing

- Seek to discuss the issues that science raises for society. Listen to the aspirations and concerns of others.
- Do not knowingly mislead, or allow others to be misled, about scientific matters. Present and review scientific evidence, theory or interpretation honestly and accurately.

You can read the full version of the Code (launched by the UK Government's Chief Scientific Adviser in March 2007) at www.dti.gov.uk/science/science-andsociety/public_engagement/code/page28030.html

Annex 5 – 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 reference dose (ARfD): Estimate of the amount of a substance in food or drink, expressed on a body weight basis, that can be ingested in a period of 24 hours or less without appreciable health risk.

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.

Androgen: The generic term for any natural or synthetic compound that can interact with and activate the androgen receptor. In mammals, androgens (for example, androstenedione and testosterone) are synthesised by the adrenal glands and the testes and promote development and maintenance of male secondary sexual characteristics.

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.

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

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.

Enterohepatic circulation: Cyclical process involving intestinal re-absorption of a substance that has been excreted through bile followed by transfer back to the liver, making it available for biliary excretion again.

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.

Horizon Scanning: The systematic examination of potential threats, opportunities and likely future developments, which are at the margins of current thinking and planning. Horizon scanning may explore novel and unexpected issues, as well as persistent problems and trends. Overall, horizon scanning is intended to improve the robustness of policies and the evidence base

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 leukaemia's which develop from lymphoid cells and the myeloid leukaemia's 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'.

Margin of exposure (MOE) approach: A methodology that allows the comparison of the risks posed by different genotoxic and carcinogenic substances. The MOE approach uses a reference point, often taken from an animal study and corresponding to a dose that causes a low but measurable response in animals. This reference point is then compared with various dietary intake estimates in humans, taking into account differences in consumption patterns.

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)

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

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

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

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

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

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

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

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

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

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

Renal: Relating to the kidney.

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

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

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

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

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

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

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

Stakeholder: A person or organisation representing the interests and opinions of a group with an interest in the outcome of (for example) a review or policy decision.

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.

Surfactant: Also called: surface-active agent. A substance, such as a detergent, that can reduce the surface tension of a liquid and thus allow it to foam or penetrate solids; a wetting agent.

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.

Testicular Dysgenesis Syndrome (TDS): The hypothesis that maldevelopment (dysgenesis) of the fetal testis results in hormonal or other malfunctions of the testicular somatic cells which in turn predispose a male to the disorders that comprise the TDS, i.e. congenital malformations (cryptorchidism and hypospadias) in babies and testis cancer and low sperm counts in young men.

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.

Total Toxic Equivalent (TEQ): Is a method of comparing the total relative toxicological potency within a sample. It is calculated as the sum of the products of the concentration of each congener multiplied by the toxic equivalency factor (TEF).

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

Toxicogenic: producing or capable of producing a toxin.

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 ribosome's, 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 (e.g. 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.

WHO-TEQs: The system of Toxic Equivalency Factors (TEFs) used in the UK and a number of other countries to express the concentrations of the less toxic dioxin-like compounds (16 PCDDs/PCDFs and 12 PCBs) as a concentration equivalent to the most toxic dioxin 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is that set by the World Health Organisation (WHO), and the resulting overall concentrations are referred to as WHO-TEQs (Total toxic equivalents).

Xenobiotic: A chemical foreign to the biologic system.

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

Annex 6 - 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

Annex 7 – Previous Publications

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, FSA/0681/0802.++

2002 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Food Standards Agency/Department of Health, FSA/0838/0803.++

2003 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Food Standards Agency/Department of Health, FSA/0900/0504.++

2004 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Food Standards Agency/Department of Health, FSA/0992/0804.++

2005 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Food Standards Agency/Department of Health, FSA/1098/0906.++

2006 Annual Report of Committees on Toxicity, Mutagenicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment. Food Standards Agency/Department of Health, FSA/1184/0707++

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, FSA/0691/0902 (2002).++

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment: Phytoestrogens and Health, Food Standards Agency, FSA/0826/0503 (2002).++

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment: Variability and Uncertainty in Toxicology of Chemicals in Food, Consumer Products and the Environment, FSA/1150/0307 (2007).++

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

* Available on the COM website at http://advisorybodies.doh.gov.uk/com/publications.htm
** Available on the COT archive at http://archive.food.gov.uk/dept_health/archive/cot.htm
\+ Available on the COC website at http://www.advisorybodies.doh.gov.uk/coc/publications.htm
++ http://cot.food.gov.uk/cotreports/

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