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.

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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

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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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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

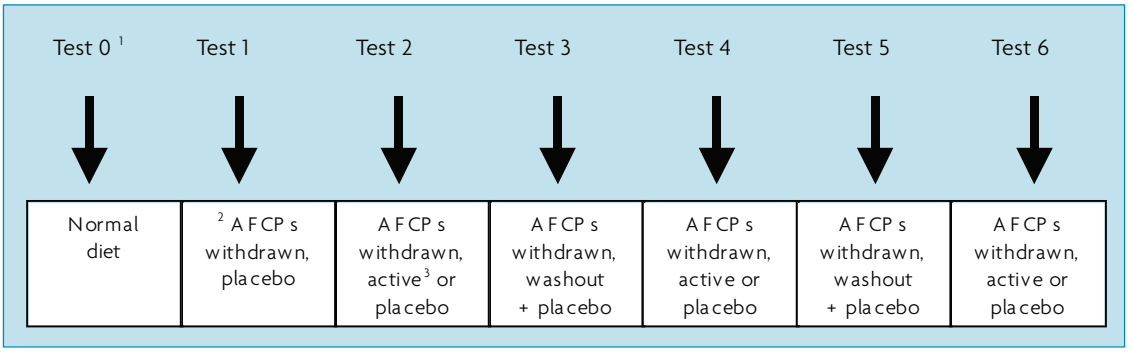
6 The primary hypothesis tested by the researchers was that mixtures of certain artificial food colours with the preservative sodium benzoate compared with a placebo increase the mean level of hyperactive behaviour of children drawn from the general population. With a minimum target sample of 80 children, 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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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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Senior pathologist, SEAC Toxicology Unit, Unilever.

Professor D Coggon OBE MA PhD DM FRCP FFOM FFPH FMedSci (from 1 April 2007)
Professor of Occupational & Environmental Medicine, University of Southampton

Professor C de Vries MA MSc PhD FRSM
Professor of Pharmacoepidemiology, University of Bath

Dr R Dearman BSc (Hons) PhD
Faculty of Life Sciences, University of Manchester

Dr J Foster BSc PhD FRCPath
Senior Principal Pathologist, AstraZeneca Pharmaceuticals
Chair of Panel of Examiners for the Toxicology Specialty, Royal College of Pathologists

Professor D Harrison BSc MB ChB FRCPath FRCPEd FRCSEd (from 1 April 2007)
Professor of Pathology, University of Edinburgh Medical School
Director, University of Edinburgh Cancer Research Centre

Dr J Hinson BSc (Hon) PhD DSc
Reader in Molecular and Cellular Endocrinology, Barts and the London,
Queen Mary School of Medicine and Dentistry, University of London

Dr P Jackson BA (Oxon) MA (Oxon) MB ChB MRCP PhD FRCP
Reader in clinical pharmacology and therapeutics, University of Sheffield

Professor J Konje MBBS MD MRCOG (from 1 April 2007)
Head of Clinical Division of Obstetrics and Gynaecology, Leicester Royal Infirmary

Professor J Lunec BSc (Hon) PhD FRC Path (upto 31 March 2007)
Director of Cranfield Health, Cranfield University

Dr G McNeill MB ChB MSc PhD RPH Nutr
Senior Lecturer in Nutrition Epidemiology, University of Aberdeen,
Head of Public Health Nutrition research group, Rowett Research Institute

Professor I Morris BSc (Hons) PhD
Professor of Pharmacology and Physiology, Associate Dean for Research, Hull York Medical School

Dr N Plant BSc PhD (from 1 April 2007)
Senior Lecturer in Molecular Toxicology, University of Surrey

Dr D Ray BSc PhD
Head of Applied Neuroscience Group, University of Nottingham Medical School

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

Dr L Rushton BA (Hons) MSc PhD CStat (upto 31 March 2007)
Head of Epidemiology, Medical Research Council, Institute for Environment and Health,
University of Leicester

Dr L Stanley BA PhD (upto 31 March 2007)
Head of Operations, CXR Biosciences

Professor S Strobel MD PhD FRCP FRCPCH (upto 31 March 2007)
Director of Clinical Education, Peninsula Postgraduate Health Institute, Peninsula Medical School, Plymouth

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

Dr D Benford BSc PhD	*Scientific Secretary*
Mrs J Shroff BA	*Administrative Secretary*
Mr J Battershill BSc MSc	*Scientific - HPA*
Dr S Creton BSc PhD	
Mr A Furmage BSc	
Ms B Gadeberg BSc MSc	
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