

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Minutes of the meeting held on Monday 2nd February 2004 in the Dylan Thomas Suite, Marriott Hotel, Cardiff.

Present

Chairman:	Professor Hughes	
Members:	Professor Boobis Dr Carthew Professor Chipman Dr Hinson Dr Jackson Dr Joffe Dr Piersma Prof Ray Prof Rowland Dr Rushton Ms Salfield Dr Smith Dr Stanley Dr Tucker Miss Ward	
FSA Secretariat:	Dr Benford Mr Butler Dr Gott Dr Tahourdin Ms Ngarize Dr Sivapathasundaram Mr Maycock	(Scientific Secretary) (Administrative Secretary)
DH Secretariat:	Mr Battershill	Items 1-5
Also in attendance:	Dr Barlow Dr Burgess Ms A Ashelford Dr R Fielder Dr O'Sullivan Mr R Alexander Dr R Sharpe	FSA, CST - Item 6 FSA, CST – Items 6 FSA, COMS DH Food Safety Promotion Board, Ireland Chief Environmental Health Adviser to the Welsh Assembly Medical Research Council, University of Edinburgh- item 9

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ANNOUNCEMENTS

1. The Chairman welcomed all those who were attending the meeting. He announced that Professor Chipman, Drs Joffe, Smith and Tucker and Ms Salfield would be completing their current terms of office at the end of March and were not eligible for reappointment under the OCPA guidance. He also informed members that, due to increased work commitments, Dr Piersma was unable to continue as a member of the Committee after the end of his current term of office (March 2004). The Chairman thanked them for the valuable contributions to the work of the Committee.
2. He informed Members that interviews for new appointments were being held on 9 February and Members would be informed of the outcome following the decision of the Chairman of the Food Standards Agency and the Chief Medical Officer.
3. The Chairman welcomed Ms Sekai Ngarize as a new member of the Secretariat and announced that this would be Dr Gott's last meeting before he departed on secondment to the European Food Safety Authority. On behalf of the Committee, the Chairman thanked him for his work in the Secretariat and wished him well.

ITEM 1: APOLOGIES FOR ABSENCE

4. Apologies for absence were received from Professors Lunec and Strobel.

ITEM 2: MINUTES OF THE MEETING HELD ON 9 DECEMBER 2003: TOX/MIN/2003/06

5. The minutes of the 9 December meeting were agreed subject to the following amendments (in italics):
 - Para 6,3rd sentence: The fermentate had been analysed for mycotoxins *for up to 400 cycles* without anything being detected. Although the half-life of the column had not been accurately established, it was thought that the resin would *not* last for longer than 300-400 cycles.

ITEM 3: MATTERS ARISING

6. The Chairman informed members that discussions on tryptophan and phosphorus would continue at the next COT meeting in April. Matters arising from other agenda items were described in information paper TOX/2004/04.

ITEM 4: HORIZON SCANNING (TOX/2004/01)

7. In 2001 Members agreed that it would be useful to have an annual agenda item to discuss potential future topics. The Committee was informed that future agenda items would include:

- Adverse effects of acid sweets
- Disinfection by-products in drinking water and adverse birth outcomes, following on from a COT discussion in 2001.
- The chemical incapacitant spray PAVA (nonivamide), following on from COM and COT discussions in 2001-2002
- Receipt of the report from the Lowermore Subgroup

8. Members were asked for suggestions for specific issues to be discussed, whether as routine agenda items, topics for one-day open meetings, or generic issues requiring the setting up of a new working group. Issues suggested included:

- interactions between diet and pharmaceutical agents,
- effects of diet regimes,
- the relation of plant sterols and stanols to vascular disease,
- potential risks associated with the increasing use of nanomaterials,
- safety of disinfection by-products that may be left as residues on food.

9. It was noted that some of these topics would require cross-committee views in order to deal adequately with the relevant issues. Members were reminded that they may draw additional issues to the attention of the Secretariat at any time.

ITEM 5: TOXICOGENOMICS/PROTEOMICS/METABONOMICS: UPDATE ON LITERATURE RETRIEVED DURING 2003 (TOX/2004/02)

10. A joint symposium was held by the COT, COC and COM in October 2001 on the use of genomics and proteomics in toxicology, and a statement outlining the conclusions of the committees was produced. Since then a number of papers had been published and preliminary information from a trial by the ILSI Health and Environmental Sciences Institute (HESI) had become available. The committee was asked to review this new information and to determine whether there was any basis to change the conclusions of COT/COC/COM.

11. Members noted that there had been a large number of published studies using transcriptomics in the last 2 years. There had been improvements in the design and reproducibility of studies, and the approaches to analysis of raw data and statistical approaches to evaluation and identification of toxicologically relevant patterns for gene changes. There was still a long way to go in terms of dose-response analysis and, in particular the distinction of adverse from non-adverse effects at low doses, and tissue analysis. The lack of published studies which included a reversal phase was commented on. Such data would be important in validating the application of transcriptomic data to risk assessment.

12. A Member noted that only a tiny proportion of significant (as defined by study investigators) changes in gene expression were readily identifiable by cluster analyses as being toxicologically relevant. The majority of changes were likely to be

unrelated to toxicity and had been termed adaptive changes. Such changes in gene expression represented a considerable “background noise” which hindered the evaluation of critical gene changes in transcriptomic studies. The significance of changes in the expression of many genes was unknown as the functions of the genes were unknown. However, specific patterns of changes may be predictive of toxicity, and thus pattern recognition methods such as hierarchical clustering were important.

13. It was noted that the cost of transcriptomics had limited the number of arrays that could be run in some studies and there was a tendency to pool samples to save money. Members felt this reduced the quality of experiments, a conclusion which had also been reached by the ILSI/HESI working group on toxicogenomics.

14. A Member considered that the overall conclusion of the previous statement, that genomics and proteomics had not sufficiently advanced to be used in risk assessment, was still valid. Genomics and proteomics data should be considered as part of the overall data package, but could not be used in the absence of prior knowledge about the toxicity of the chemical.

15. Members noted that high density microarrays were complex and costly to establish but could, given appropriate methods of analysis, provide information on new hypotheses for toxicological risk assessment. Low density microarrays were cheaper and easier to evaluate but could potentially miss relevant gene changes. Low density arrays could be targeted at specific toxicological mechanisms and could serve as a useful adjunct to conventional toxicological methods. The COT confirmed it was important to quantify key gene changes, such as by PCR analysis of mRNA. Members were aware of the need to avoid focusing on gene changes which were not part of toxicologically relevant pathways. The importance of experimental design was emphasised. Members agreed a need for additional information on the reproducibility of replicate analyses, the approach to assessment of background changes, use of housekeeping genes, variation between laboratories regarding analysis of mRNAs, in particular the use of different platforms, and validation of the genes incorporated into microarrays. These were examples of the potential sources of variation in transcriptomic analyses.

16. Members requested more information on the statistical methods used in the analysis and interpretation of toxicogenomic studies. It was suggested that an expert in this area be asked to either attend a future COT meeting or to write a paper for the committee. Members noted that normal statistical methods could not be used due to the huge number of multiple comparisons being made. It was noted that there were actually two levels to the statistical analysis employed in toxicogenomic studies: processing the raw data to identify gene changes and then analysing for clusters of gene changes. Application of Principal Component Analysis (PCA) should be part of any contracted review of statistics. Development of bioinformatic approaches (such as database set-up and use) should also be considered.

17. It was agreed that transcript changes in isolation would not be suitable as biomarkers, but that they could be useful in identifying potential biomarkers.

18. Members considered that proteomics had shown the greatest advance in the past 2 years, with the introduction of solid-phase separation techniques. It was considered to have great potential value but more information was needed on the function of proteins identified by these methods. Both 2D gel and solid-phase-based techniques were of value and should be considered as complimentary techniques. A Member noted that it was important that transcriptomics and proteomics were not considered in isolation.

19. Metabonomics had not been considered in the joint COT/COC/COM meeting. An overview of published papers was presented. Members heard that several pharmaceutical companies were developing techniques in metabonomics and it was likely that more data would be published during the next year. Members noted that metabonomics had advanced rapidly and that patterns in tissues (using magic angle NMR spectroscopy), and not just biological fluids, were being studied. Metabonomics was truly quantitative and allowed whole systems to be studied, not just specific tissues. It was noted that metabonomics had the potential to identify biomarkers of effect. Members also noted the useful approach of deriving trajectomes which provided a visual perspective on the development and recovery from toxicity. It was noted that techniques other than NMR were being developed, such as HPLC-electrochemical detection systems.

20. Members considered that proteomics, transcriptomics and metabonomics needed to be all considered as part of an integrated approach. Thus transcriptomics provided no useful information on post-translational changes in proteins, whereas a proteomic approach might provide appropriate information. It was suggested that a paper be prepared for the committee on 'systems biology', an integrated approach to evaluating chemical mediated physiological and pathological changes.

21. The Committee discussed a number of general themes associated with all of the Annexes of TOX/2004/02. There was some discussion of whether transcriptomics, proteomics and metabonomics could lead to a reduction in animal testing. It was considered that they could eventually lead to a refinement of the risk assessment process. These techniques might potentially help to refine and reduce animal testing, but it was too early to draw any conclusions.

22. Members noted that there were no epidemiological studies available which had used a toxicogenomic approach and asked that any relevant studies should be considered. It was speculated that potentially, in the long term, the techniques may permit more human studies if end points could be identified below doses that caused adverse effects.

23. Members heard that the WHO International Programme on Chemical Safety (IPCS) had recently held a meeting to consider methods of use and evaluation of toxicogenomic studies in risk assessment. It was hoped that a draft report could be made available to the COT in the near future. One of the key questions considered by the IPCS working group was whether a single gene change could be indicative of a toxic effect. The consensus view was that any single gene change needed to be part of a toxicologically relevant pathway for such a change to be indicative of a toxic effect. The term "phenotypic anchorage" had been used to describe the need to keep gene changes in perspective of toxicologically relevant pathways. The IPCS group

had also identified the approach to determination and validation of NOAELs using toxicogenomics as a key area for further consideration.

24. A Member queried the co-ordination between UK Government departments with regard to developing expertise in assessing microarray experiments.

25. Overall, Members confirmed that toxicogenomic approaches could potentially be used as a screening process for toxicological mechanisms and for the investigation of mechanisms. It was agreed that it was currently too early to use such data in risk assessment.

ITEM 6: STUDY TO INVESTIGATE UNUSUAL RESPONSES TO THE LIPOPHILIC SHELLFISH TOXIN MOUSE BIOASSAY (TOX/2004/05)

26. In the UK, the mouse bioassay (MBA) is used for statutory monitoring of shellfish for lipophilic toxins of algal origin. The MBA involves intraperitoneal injection of a shellfish extract and the assay is considered positive for the presence of lipophilic toxins if severe symptoms or death are observed in the animals. Since June 2001, an atypical response has been observed in some MBAs after testing shellfish extracts, primarily cockle extracts, in the monitoring programmes in England, Wales and Northern Ireland. A figure was tabled showing the percentage of atypical responses observed during cockle testing carried out each month since 2001 in England, Wales and Northern Ireland.

27. The Agency commissioned a toxicity study to investigate the nature of the atypical response and to provide information for an assessment of the possible public health implications of ingestion of cockles that have shown the atypical response in the MBA. The Committee was provided with the initial results from the preliminary stages of the study and asked to comment on its design and whether it would provide sufficient information to allow a risk assessment given that only a limited amount of cockle extract was available.

28. Members asked whether the atypical response could be an artefact of the testing method because it had only been observed after the monitoring programme for England and Wales had moved from a Scottish to an English laboratory. The Committee was informed that the atypical response had also been observed during monitoring at a Northern Irish laboratory over the same period of time. In addition, studies instigated by the Agency, such as investigations of the residual solvent levels in the extracts, had suggested that the cause of the atypical response was not methodological in nature. A limited study had shown that injection of at least 5 μ L of one of the extraction solvents, diethyl ether, was required to produce a clinical response in mice but that the response had been considered different to that seen when cockle extracts were tested. In addition, the concentrations of the extraction solvents, diethyl ether and acetone, in the cockle extracts used in the toxicology study were very low compared with their LD₅₀ values. In response to a question about whether water-soluble agents could be carried over into the cockle extracts, Members were informed that the extraction procedure included aqueous backwash steps to remove such components.

29. Members suggested that a ring-trial whereby the same shellfish samples are tested at each monitoring laboratory could establish the comparability of the methodology used at the labs. The Committee was informed that a small trial had been conducted that had highlighted differences in methodology but that a standardised extraction method had since been instituted and a further ring-trial was planned.

30. The Committee was informed that there had been no reported cases of poisoning from consumption of cockles in the UK but that much of the cockle harvest was exported.

31. In response to questions about the nature of the atypical response, Members were informed that the onset of the atypical response was more rapid than that seen with known lipophilic shellfish toxins and the symptoms included convulsions and respiratory difficulty. One Member, who had viewed a video recording of the atypical response in mice, noted that the clinical signs were inconsistent with responses induced by domoic acid. It was suggested that it may be possible to determine if the cause of the repressed respiratory function was the result of direct action to induce paralysis of the diaphragm, possibly by examination of diaphragm preparations, or was via a neurotoxic mechanism. In addition, it was suggested that the transient clinical effects observed when extracts were administered to mice by oral gavage could be the result of a reversible pharmacological effect.

32. Members considered that on its own, the proposed acute study would not provide a no observable effect level that could be used to assess the human health risks. This was primarily because the potency of the test extracts used in the study was undefined and therefore, the range of potencies of future shellfish extracts could not be predicted. The test extract was considered to be more potent compared with many of the extracts tested during routine monitoring of shellfish and it was suggested that some semi-quantitative assessment of the potency could be derived by comparing the clinical signs observed during monitoring with those induced by the study extract.

33. Members also noted that each mouse was administered with extract that had been prepared from 25 g of cockle flesh and that, on a body weight basis, this was approximately 750 times higher than the dose of shellfish a human would consume. However, although this constituted an appreciable safety factor, the extraction efficiency for the causative agent was unknown. Thus, the amount administered to the animals could not be related directly to human exposure.

34. Members considered it important to identify the causal agent responsible for the atypical response so that the study extracts used in the toxicology study could be characterised and the efficiency of the extraction process could be estimated. It was suggested that a fractionation approach, with active fractions identified by MBA and analysed by LC-MS, could be used to characterise the agent. The Committee was informed that the Agency had funded a preliminary analytical study using LC-MS/MS to examine the chemical composition of shellfish extracts. However, the findings from this study were inconclusive and further work was planned.

35. In addition, the Committee indicated that the validity of basing a risk assessment on an acute toxicology study, rather than a repeat-dose study, would need to be justified. It was suggested that the results from a short-term repeat dose study could provide some justification on which to base such a risk assessment. However, there was insufficient extract available to conduct such a study at this time.

ITEM 7: TETRABISPHENOL A – A REVIEW OF TOXICOLOGICAL DATA (TOX/2004/06)

36. In 2003, the COT considered data on some brominated flame retardants (BFRs) in fish from the Skerne-Tees river system. In the course of the discussions, Members noted a need to consider other BFRs, such as tetrabromobisphenol A (TBBPA). As the Food Standards Agency is planning to conduct a survey of TBBPA in fish, shellfish and possibly free-range chicken eggs during 2004, the Committee was invited to consider the toxicological data in advance of receiving the results of the survey.

37. Paper TOX/2004/06 summarised the toxicology of TBBPA, as described in a draft risk assessment conducted under the EU Existing Substances Regulations, together with some additional *in vitro* data identified in literature searches.

38. Members noted that reported effects of TBBPA on the thyroid differed between studies. TBBPA would be expected to have thyroid hormone agonist effects and to inhibit thyroid stimulating hormone (TSH), but this did not appear to be the case. Given the lack of consistency in the data, it was considered difficult to ascertain whether TBBPA had significant thyroid effects. A reduction in T4 levels had been reported but levels had returned to baseline following a recovery period, which indicated that any effect was reversible and unlikely to be adverse. The relevance of data suggesting that TBBPA may displace T4 from transthyretin (TTR), a major T4 binding protein in the rat, was unclear in view of the limited understanding of the role of TTR.

39. One Member reported on a 1-generation study on behavioural effects, which is currently in progress. An effect on T4 was observed at a dose of 25 mg/kg bw/day TBBPA, but not on T3 and TSH. The effect was small, not associated with histological changes or toxic effects, and not clearly dose-related.

40. Members considered it unfortunate that the relevance of the thyroid effects was not investigated in the reported studies. It was possible that enzyme induction could account for the findings, but that needed to be demonstrated. UDP-glucuronosyltransferase levels had not been measured and the extent of possible liver enlargement was unclear. TBBPA had been shown to have weak oestrogenic activity in some *in vitro* assays, but Members considered that this was unlikely to contribute to the effects observed *in vivo*.

41. Members noted that there were sporadic findings with the spleen and platelets, which indicated that blood may have been affected. It was noted that many chemicals cause disturbances in haem metabolism, but that TBBPA did not affect δ -aminolevulinic acid synthase, which would be expected if there was an effect on haem.

42. Members discussed the results of neurotoxicity studies in rats. The unpublished study by Hass *et al.* reported an effect on habituation at a dose of 250 mg/kg bw/day. The reported decreased motor activity could have many possible causes and was not necessarily related to CNS effects. However, the changes in exploring activity (i.e. the rate at which the animal becomes bored with its environment and seeks to explore) appeared more convincing. A total of 96 separate neurobehavioural tests were used in the Hass study, with 11 producing statistically significant results. These included sporadic findings of effects in the Morris water maze test. A Member explained that a Morris maze was used for the learning and re-learning trials, with the first trial being a real indication of effect, and subsequent trials a measure of severity. Therefore significant results would not be expected in all trials. However there was no consistent pattern of effects in males versus females across the dose groups which raised concerns about the biological plausibility of the effect.

43. An unpublished study by MPI research reported no consistent pattern in habituation data, casting doubt on the Hass data. A decrease in parietal cortical thickness was observed at 1000 mg/kg bw/day at day 11. Members were informed that new data, not described in the EU risk assessment, showed that this effect was not present at day 60.

44. In comparing the data, Members noted that the Haas study indicated a NOAEL of 50 mg/kg bw/day, with an effect on habituation at 250 mg/kg bw/day, but the MPI study raised the question of whether the effect was real. The issue of chance statistical findings for multiple endpoints is discussed in the OECD guideline on neurotoxicity testing. Another factor to be considered is the question of number of animals per litter to be tested. Testing of one pup of each sex is feasible but can make interpretation of results difficult. It was agreed that if the MPI study was comparable to the Hass study then the apparent effects on habituation in the Hass study could be discounted. A Member offered to examine the unpublished study reports in detail.

45. The EU draft risk assessment report concluded that its risk characterisation for oral exposure should be based on a repeat dose study in which rats were administered TBBPA in corn oil. No significant toxicological effects were reported in this study at the highest dose of 1000 mg/kg bodyweight/day. Based on the limited solubility of TBBPA in the dosing vehicle (corn oil) the EU report assumed that only 50% of the TBBPA was bioavailable. However the Committee considered that assumptions on absorption should not be based on solubility.

46. Members considered that, if it was agreed that the Haas findings did not represent a real effect, it was possible to identify a no observed effect level (NOEL) of 1000 mg/kg bw/day from the repeat dose study. A total uncertainty factor of 1000 would be required. This comprises the default uncertainty factor of 100 to allow for inter- and intra-species variation and an additional factor of 10 for the absence of chronic toxicity studies and because of the ambiguity over some of the minor effects reported. It was noted that no long-term carcinogenicity study was available. However, the results of mutagenicity studies were negative and the subchronic studies provided no indication of a mechanism to suggest that TBBPA could lead to carcinogenesis following life-time exposure.

47. It was agreed that a draft statement would be prepared for discussion at a future meeting. This would provide additional information on the rationale for the planned survey.

ITEM 8: PROPOSAL FOR A STUDY ON ERYTHRITOL IN CHILDREN (TOX/2004/07)

48. Erythritol is manufactured by Cerestar. Dr Carthew declared a personal non-specific interest and left the meeting while this agenda item was discussed. Professor Rowland declared a non-personal non-specific interest and remained for the discussion.

49. Erythritol was discussed by the Committee in October 2003. It is a polyol which has potential uses as a sweetener and as a binding agent, thickener, bulking agent, sequestrant, flavour enhancer and freezing point depressant in a variety of foods and/or beverages. Excessive bolus ingestion of erythritol can cause laxation, which the committee considered to be a local physiological effect. Children may be more susceptible to laxation caused by erythritol due to the shorter length of the GI tract. The Committee considered that in order to determine a NOEL for erythritol information would be required on the relative sensitivity of young children to adults. It suggested that it may be possible to obtain this information from post-market surveillance of foods and beverages (where authorised) containing erythritol or other polyols.

50. The manufacturers had submitted a protocol for a study of erythritol in young children for comment. Whilst it would not be appropriate to approve a specific protocol, it was considered useful for the Committee to discuss some of the generic issues raised.

51. The proposed study would not help to clarify whether erythritol would have a lesser laxative effect than other polyols in young children, and a comparative study would be needed. However, it was considered that studies in young children would only gain ethical approval if any potential distress was minimal and if there was a potential benefit to children. A study in a small group of healthy individuals may not be representative of all potential users. The study may provide reassurance that erythritol was safe at its proposed use level in healthy children, but concerns would remain that some children would be more susceptible. If the dose tested was the proposed use level, it would not be possible to apply an uncertainty factor to account for inter-individual variation.

52. A Member noted that differences between intestinal microflora may lead to variability between different children and between children and adults. It was suggested that studies of fermentability in faecal samples might support some correlation between children and adults. However, this would not allow for differences in gastric emptying and transit time within the gut.

53. It was agreed that the general issue of how to assess safety of substances in young children if it is not possible to conduct studies in children should be referred to

the COT working group on Variation and Uncertainty in Toxicology (VUT). Members agreed that their reservations regarding the study protocol should be expressed to the manufacturers and re-iterated their previous advice that information on the relative sensitivity of young children might be obtained from post-market surveillance of erythritol in countries where it is currently authorised.

ITEM 9: UPDATE ON THE COT DISCUSSIONS ON ADVERSE TRENDS IN THE DEVELOPMENT OF THE MALE REPRODUCTIVE SYSTEM AND POTENTIAL CAUSES (TOX/2004/08)

54. In February 2003 the Committee was invited to consider whether it was an appropriate time to review the available evidence for adverse trends in development of the male reproductive system and possible contribution of chemical exposure to these trends. The Committee noted that the subject had been reviewed extensively, including a comprehensive recent (2002) review by the International Programme on Chemical Safety (IPCS), and discussed a draft statement summarising the Committee's views in the area. A second draft statement was discussed by the Committee in May 2003, and subsequently revised.

55. The draft statement had not been finalised as the Chairman wished to consider some additional evidence. Members were asked to consider a paper that provided a brief overview of some of the key activities and publications since the previous COT discussion. The paper outlined the continued support for some of the adverse trends mentioned previously, and alternative hypotheses relating to possible causes.

56. Dr Sharpe, from the Medical Research Council at the University of Edinburgh, attended the meeting for this item to advise the committee. He noted that the paper was a balanced update with a fair reflection of the current state of play. He also noted that contrary to the information regarding the degeneration of the genes on the Y chromosome, there is now evidence which suggests that the Y chromosome has developed a novel mechanism for dealing with its unique situation, in which mutations in single copy genes might arise. This involves a process termed gene conversion whereby multiple palindromic copies of genes exist within the Y chromosome between which recombination may occur.

57. Dr Sharpe considered that there was a lack of evidence of any chemicals playing a significant role in endocrine disruption in humans. However, there was evidence that wildlife had been affected. Such cases included intersex in fish due to exposure to estrogens in river effluents, and imposex as a result of exposure to tributyltin. It was considered that the nature of the effects reported in wildlife studies had similarities to developmental disorders in humans. Furthermore, there is increasing evidence that these disorders are of fetal origin.

58. Studies in experimental animals have produced effects similar to those seen in human testicular dysgenesis syndrome (TDS), e.g. with certain phthalates. A potential link between chemicals and TDS could not be dismissed on the basis of the currently available evidence. There is a need to obtain good data to resolve this issue, initially targeting chemicals with mechanistical plausibility and high exposures.

59. A Member asked Dr Sharpe whether the focus should be on anti-androgenic and dioxin-like compounds rather than environmental estrogens, given that certain phthalates have an anti-androgenic mechanism. Dr Sharpe agreed that there are logical reasons for being more wary of anti-androgenic chemicals, but he was not convinced that environmental estrogens were a major issue in human reproductive disorders. However, as the data on the exposure level of the fetal testis to such chemicals are insufficient, it was not possible to be certain. Dr Sharpe noted that there were a few good studies but relatively little money had been spent on assessing exposure. He added that in the US, systems were in place to improve monitoring of certain chemicals in a representative population. If it was accepted that TDS arose during fetal development, pregnant women should be monitored, but as many women do not know they're pregnant during the early stages of fetal sexual differentiation this may be difficult. Some recent data had suggested associations but that is far from establishing cause and effect.

60. One Member questioned whether exposure to chemicals, such as DDT and phthalates, could have contributed to adverse trends that commenced 50 to 100 years ago. Dr Sharpe admitted this possibility, but added that obtaining proof one way or the other was probably impossible. He considered that the best way forward was to focus on the reproductive disorders seen at birth (cryptorchidism and hypospadias), as a starting point, and then investigate their possible causes. Denmark and Finland had carried out a comparison on the incidence of these disorders and shown that the incidence of both disorders is higher in Denmark; the next step may be to investigate any differences in lifestyle factors in the two countries. This raised the question whether contributions from factors such as maternal age and nutrition can be factored out before studying environmental factors.

61. A Member noted that genetic factors, such as familial tendencies or genetic damage from previous generations (e.g. from the grandparents' generation) may be involved, adding to the complexity of studying these disorders. However, it is difficult to separate genetic and environmental influences in familial tendencies.

62. Members considered whether existing cohorts could be used to study the disorders. The individuals from the Millenium cohort were recruited too late (at 8 months), and diagnosis of cryptorchidism and hypospadias during recruitment of the ALSPAC cohort was not considered to be sufficiently robust. Dr Sharpe informed the meeting that an impending prospective study, to compare cryptorchidism rates in Denmark and Finland, should be very informative.

63. Members considered the wording of a third working paper. It was agreed that information on prevalence for each of the disorders should be cited, and additional information incorporated from paper TOX/2004/08. New information, such as that on deletions on the Y chromosome and lifestyle factors, could be included. A sentence could be included to note a need for future review as new data emerge. In addition, results reported from wildlife studies should be emphasised, without detracting from the fact that direct evidence is still lacking for or against any chemical/s in humans, and the overall conclusions would not change.

64. It was noted that although some phthalates were anti-androgenic and caused fertility problems, these had been banned for use in consumer products and not all the phthalates had such effects.

65. The working paper will be revised and brought back to the Committee at the next meeting.

ITEM 10: 2003 ANNUAL REPORT OF THE COMMITTEES ON TOXICITY, MUTAGENICITY AND CARCINOGENICITY (TOX/2004/03)

66. Members were provided with draft text of the COT section of the 2003 Annual Report, and asked to note that the agreed statements were included and could not be altered retrospectively. Members approved the draft text and requested that the glossary be updated to include additional frequently used terms and acronyms. The increased openness of committee procedures would be mentioned in the Chairman's preface.

ITEM 11: PAPERS FOR INFORMATION

67. Members were provided with the following papers for information:

- UPDATE ON ISSUES DISCUSSED BY THE COT (TOX/2004/04)
- TOXICOGENOMICS: UPDATE ON JOINT COT/COM/COC MEETING HELD IN OCTOBER 2001 – PAPER FROM COM (MUT/04/01)
- MINUTES OF THE COM MEETING OF 29 MAY 2003 (TOX/2004/09)

ITEM 12: ANY OTHER BUSINESS

68. Members were informed of the arrangements for the open meeting of the Working Group on Variability and Uncertainty in Toxicology (VUT) that was taking place on 3 February

ITEM 13: Q&A SESSION

69. There were no observers present and therefore no questions.

ITEM 14: DATE OF NEXT MEETING

70. The next meeting of the Committee takes place on Tuesday 20th April in Aviation House.

