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

Minutes of the meeting held on Tuesday, 10th February 2009 at Manor Hotel, Meriden.

Present

Chairman
Professor D Coggon

Members:
Dr D Bell
Professor A Boobis
Dr R Dearman
Professor C de Vries
Dr C Elcombe
Dr J Foster
Dr A Hansell
Professor D Harrison
Dr P Jackson
Dr G McNeill
Professor I Morris
Dr N Plant
Dr D Ray
Dr D Tuthill

FSA Secretariat:
Dr D Benford (Scientific Secretary)
Mrs J Shroff (Administrative Secretary)
Ms B Gadeberg
Dr D Gott
Ms R Harrison
Mrs F Hill
Ms C Mulholland
Dr D Parker
Dr N Thatcher
Mr G Welsh

HPA Secretariat:
Ms F Pollitt Health Protection Agency (HPA)
Dr P Edwards HPA
Dr D Mason HPA

Assessors:
Mr M Hosford Environment Agency (EA)
Mr I McManus Health and Safety Executive (HSE)

Other officials in attendance:
Mr T Coleman EA
Mr M Whitworth EA
Dr H Garavini Department of Health Toxicology Unit (DH Tox Unit)
Mr K Okona-Mensah DH Tox Unit
Other officials in attendance (cont.):
- Dr K O'Leary
- Ms S Dine
- Dr G Clare

DH Tox Unit
Department for Food and Rural Affairs (DEFRA)
COM

Invited experts and contractors:
- Dr Mark Broomfield
- Dr Michael Festing

Enviros Consulting Ltd (Item 7)
University of Leicester (Item 11)

External observers:
None
# Contents

<table>
<thead>
<tr>
<th>Item</th>
<th>Paragraph</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Apologies for absence</td>
<td>4</td>
</tr>
<tr>
<td>2. Draft minutes of the meeting held on Tuesday, 25\textsuperscript{th} November 2008 – TOX/MIN/2008/07</td>
<td>5</td>
</tr>
<tr>
<td>3. Matters arising</td>
<td>6</td>
</tr>
<tr>
<td>5. Polychlorinated Naphthalenes and Chlorinated Paraffins in Food – TOX/2009/02</td>
<td>25</td>
</tr>
<tr>
<td>6. Developmental Neurotoxicity – TOX/2009/03</td>
<td>34</td>
</tr>
<tr>
<td>7. Potential exposure to substances in landfill sites – further data – TOX/2009/04 (Reserved Business)</td>
<td>38</td>
</tr>
<tr>
<td>8. Second Statement on Landfill sites (first draft) – TOX/2009/05 (Reserved Business)</td>
<td>53</td>
</tr>
<tr>
<td>11. Discussion on implications for toxicity testing of rodent strain differences</td>
<td>58</td>
</tr>
<tr>
<td>12. Paper for Information: Update on actions taken subsequent to COT advice – TOX/2009/08</td>
<td>75</td>
</tr>
<tr>
<td>13. Any other business</td>
<td>76</td>
</tr>
<tr>
<td>14. Date of next meeting – Tuesday, 7\textsuperscript{th} April 2009 at Aviation House</td>
<td>77</td>
</tr>
</tbody>
</table>
• Announcements

1. The Chairman, Professor Coggon, welcomed Members to the meeting. He informed Members that Dr Michael Festing would be attending to participate in Item 11: Discussion on implications for toxicity testing of rodent strain differences.

2. Members were informed that Dr Peter Jackson had tendered his resignation from the Committee due to other commitments, including being elected as Chair of the Clinical Committee of the British Pharmacological Society. His departure was in addition to that of Dr J Hinson who would be stepping down in March on the completion of her 2nd term, as noted at the previous meeting. The Chairman thanked Dr Jackson for his valuable contribution as a member of the Committee.

3. Members were reminded to submit details of their declarations of interest for the Annual Report and the COT website as soon as possible and that nil returns would be appreciated.

Item 1: Apologies for absence

4. Apologies for absence were received from Mrs A Williams, Dr J Hinson, Professor J Konje and Miss A Ward. In addition Mr J Battershill from the HPA Secretariat sent his apologies, as did Professor B Lake (Leatherhead Food International) who had been invited to attend this meeting prior to his joining the Committee as of 1st April 2009.

Item 2: Minutes of the meeting held on Tuesday, 25th November 2008 – TOX/MIN/2008/07

5. The minutes of the 25th November 2008 meeting were agreed subject to the following amendments:

   • Item 3, section 2 title: Matters arising: “Pyrrrolizidine Alkaloids in Food” and “Risk to Consumers of Eating Foods Derived from Animals that have eaten Bracken”
   • Para 14, line 6: “hypothesis, that early”
   • Para 17, line 4: “expert (Professor Holgate), who was”
   • Para 20, line 4: “Lack et al. (2003)² could”
   • Para 27, line 7: “metals and other elements”
   • Para 28, line 2: “(TDI).”

Item 3: Matters arising

Second Draft Statement on the review of the 1998 COT recommendations on peanut avoidance – TOX/2008/36

6. This statement had now been published.

Second Draft Statement on the 2006 Total Diet Study of Metals and other Elements – TOX/2008/37

7. This statement had now been published.
Members’ self assessments
9. Before these could be passed to the Chair, those Members who had not yet completed them would need to submit them to the Secretariat.

Item 4: Horizon scanning – potential future discussion items – TOX/2009/01

10. Paper TOX/2009/01 identified ongoing topics and future agenda items of which the Secretariat was currently aware, and potential discussion topics. The Chairman reminded Members that topics for discussion can be suggested at any point throughout the year to either himself or the Secretariat. He also asked Members to consider whether additional expertise would be required on the Committee.

11. Items already scheduled for future discussion included:
   • papers on para-occupational exposure to pesticides and on possible toxicological mechanisms of Multiple Chemical Sensitivity (both following up on the Royal Commission on Environmental Pollution (RCEP) review of bystander exposure to pesticides),
   • possible health effects of organophosphates,
   • toxicological testing of tobacco products,
   • advances in toxicogenomics,
   • the toxicological aspects from a review on iron to be issued for consultation by the Scientific Advisory Committee on Nutrition (SACN).

Ototoxicity of solvents such as toluene and ethylbenzene

12. It was anticipated that a COT view would be requested, and possible additional expertise was discussed.

Vitamin E and pregnancy

13. Vitamin E is a generic description for a group of eight lipid soluble chemicals. There are two classes, tocopherols and tocotrienols, which exhibit a biological antioxidant effect. Vitamin E is synthesised in plants, the highest levels being found in plant oils, with lower levels in the leaves and other green parts. The cellular mechanism of action of Vitamin E is to prevent lipid oxidation and maintain membrane integrity.

14. In 2003, the Expert Group on Vitamins and Minerals (EVM) established a Safe Upper Level (SUL) of 540 mg (800 IU) for supplemental vitamin E, equivalent to 9 mg/kg bw/day in a 60 kg adult (EVM, 2003¹). This was based on two studies (Gillilan

et al; 1977², Meydani et al, 1996³) in which supplemental vitamin E was given to human volunteers and no adverse effects were apparent in a range of biochemical parameters. Two papers had since been identified which suggested that high doses of vitamin E taken during pregnancy could be associated with reduced birth weights. These were Boskovic et al. (2005)⁴ and Poston et al. (2006)⁵.

**Boskovic et al. (2005)**

15. This was a prospective observational study. Two groups of women were enrolled after calling the Motherisk program, a Canadian counselling service which advises pregnant women and their health professionals on evidence-based risk assessments of drug, chemical and other exposures during pregnancy. The study group included healthy women who took high doses of vitamin E during the first trimester as part of a healthy lifestyle. Women with conditions which could adversely affect pregnancy, such as diabetes, were excluded. The women were interviewed on enrolment and 4-6 months after delivery, with letters being sent to the women’s physicians to verify the findings.

16. Birth weights were significantly reduced in the study group (3173 ± 467 g) compared to the control group (3417 ± 56 g). In the study group there was one major malformation reported (omphalocele). There were no statistically significant differences in the rates of live births, pre-term deliveries, miscarriages or stillbirths.

**Poston et al. (2006)**

17. In a randomised placebo-controlled trial, 2410 pregnant women at risk of pre-eclampsia were given 1000 mg vitamin C and 400 IU natural source vitamin E (RRR α-tocopherol) or placebo from the second trimester of pregnancy until delivery. Significantly more low birth weight (<2.5 kg) babies were born to women in the treated group than in the control group but the prevalence of small size for gestational age (<5th centile) did not differ did not differ significantly. Treatment was also associated with the occurrence of an umbilical artery pH of less than 7.0. The authors considered that this might be due to an earlier onset of pre-eclampsia in the treated group. There was no difference in the incidence of severe or early onset pre-eclampsia between the groups, but more women in the treatment group developed gestational hypertension for which they needed treatment. A post-hoc analysis of the data suggested that the women in the placebo group who took pregnancy-specific multi-vitamins, independent of the study, had a lower risk of low birth weight, though it was noted that this finding should be treated with caution since it was not a pre-specified analysis.

**Other data**

18. Preliminary searches have identified a number of small clinical trials predating that of Poston et al. (2006) where anti-oxidants (usually vitamins C and E) have had

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beneficial or neutral effects on pre-eclampsia but have not affected birth weights. A prospective study by Spinnato et al. (2008)\(^6\) suggested that supplementation with vitamins C and E could increase the risk of premature rupture of the membranes.

19. Some mechanistic data suggesting that vitamins E and C have detrimental effects on placental cells \textit{in vitro} have also been identified (Aris et al., 2008\(^7\)). There have been few studies of vitamin E in laboratory animals, but those that are available do not indicate any adverse effects. There are a number of studies in laboratory animals demonstrating beneficial effects of vitamin E, when given with known chemical toxicants.

20. The Committee agreed that the study of Boskovic \textit{et al.} (2005) was small with major potential for selection bias, and was not worth considering further. The study of Poston \textit{et al.} (2006) generated suspicion although the findings may have resulted from chance. The studies employed high doses that were not achievable in the diet. Members requested additional information, including the EVM report, a check on whether there were other pregnancy cohort studies, toxicological data and whether the Cochrane review of 2005 had been updated.

\textit{Methyl glyoxal}

21. Methyl glyoxal is an \(\alpha\)-dicarbonyl compound that has been reported to be formed in foods and beverages during processing, cooking and prolonged storage. The highest concentrations have been reported in Manuka honey, which is derived from the Manuka tree (\textit{Leptospermum scoparium}) in New Zealand. Manuka honey exhibits pronounced antibacterial activity, which has recently been attributed to the methyl glyoxal content. It has been suggested to be an intermediate in acrylamide formation via the Maillard reaction. Methyl glyoxal is also formed endogenously in the human body. It has been proposed that the products of ketosis, \(\beta\)-hydroxyacetone, acetoacetate, acetone and acetol, are potential precursors of methyl glyoxal. Methyl glyoxal levels are elevated in patients with type 1 and type 2 diabetes mellitus, and it is one of a number of reactive intermediates implicated in formation of advanced glycation endproducts (AGEs), which are involved in the aetiology of diseases such as the vascular complications of diabetes, atherosclerosis, hypertension and Alzheimer’s disease. There is currently little information on uptake from dietary exposure to methyl glyoxal, relative to endogenous formation.

22. Members were asked to advise on whether a full review of methyl glyoxal would be worthwhile, taking into account its relation to acrylamide, and the possibility that individuals with diabetes could be a population at increased risk. It was agreed that this topic should be considered further, starting with a more thorough literature search, a review of the chemistry, including enzymic and non-enzymic pathways, and consideration of genotoxicity. If possible, a comparison should be made between dietary exposure to methyl glyoxal and endogenous production.


23. Members’ views were requested on emerging toxicological issues which should be included in the programme of work for 2009. A Member alerted the Committee to over-the-counter use of antacids by pregnant mothers and evidence of increased asthma in their children. Another Member noted that there were some animal data on this association.

24. Finally the balance of expertise on the Committee was discussed. It was agreed that ‘dietary exposure assessment’ and ‘systems biology’ should be added to the list of specialist expertise required by the Committee. The clinical pharmacologist expertise would need to be restored in future recruitment.

Item 5: Polychlorinated Naphthalenes and Chlorinated Paraffins in Food – TOX/2009/02

25. Dr Elcombe declared a personal, specific interest as he had carried out work on chlorinated paraffins (CPs) paid for by Industry, both at the University of Dundee and with CXR Biosciences of which he is a shareholder. In addition, he had acted as a Scientific Expert, providing data on the compounds to the European Chemicals Bureau. It was agreed that he could answer technical questions but should not take part in decision-making.

26. Drs Foster and Bell also declared personal, specific interests as they had both previously worked on CPs at the Central Toxicology Laboratory. These interests were deemed to have lapsed as neither had worked on the compounds during the last 8 years at least.

27. The two different groups of compounds were discussed separately, and therefore, the declarations of interest were only related to the CPs discussion.

28. The Food Standards Agency had recently completed an investigation of PCNs and CPs in food. Members were provided with an unpublished draft of the Food Survey Information Sheet, and were asked to advise on approaches to risk assessment for dietary exposure to these substances.

Polychlorinated naphthalenes (PCNs)

29. Members considered that a toxic equivalency factor (TEF) approach is not appropriate for PCNs as they do not fulfil the criteria set out by van den Berg et al. in 2006 due to a lack of information on persistence and the absence of repeat dose toxicity studies using 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as a positive control. The in vitro data could not be used to establish TEFs as they do not take into account biological half-life, although they do give some indication of potency in vitro relative to TCDD. As it would be reasonable to assume PCNs are less persistent than TCDD, summing concentrations in food on the basis of these data would provide a highly conservative approach to cumulative risk assessment. On this basis the results of the FSA investigation did not suggest a concern for human health.

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30. Members noted that it could not be assumed that all toxic effects of PCNs would be mediated via the aryl hydrocarbon receptor (AhR). There may be differences depending on the degree of chlorination, with higher chlorinated congeners exhibiting dioxin-like effects. However a contribution of potent dioxins occurring as impurities could not be excluded. The data on toxic effects other than AhR mediated dioxin-like activity would need further analysis before the Committee could comment on them.

Chlorinated Paraffins (CPs)

31. The Committee agreed with an HSE report that the liver and thyroid tumours from rodent carcinogenicity studies are unlikely to be relevant to humans. There was some discussion regarding the possible mechanism of induction of kidney tumours in male rats.

32. The data available on these compounds were considered to be robust and of good quality, and considering the negative genotoxicity data, it was determined that the lowest relevant NOAEL could be used to derive a health-based guidance value, with conventional uncertainty factors. For short chain CPs, the lowest NOAEL was determined to be 10 mg/kg b.w./day as reported by the WHO (1996)\(^9\). For medium chain compounds, it was decided that Members with relevant expertise should assess the study quoted by the HSE identifying a NOAEL of 0.4 mg/kg b.w./day, and also the studies showing reproductive effects, with a view to identifying a NOAEL on which to base the risk assessment.

33. While the Committee wished to review the studies, there was no immediate concern for human health from the levels determined in the FSA investigation. Further consideration should include some form of dietary exposure assessment, whilst recognising the limitations in the currently available data. A future possibility might be to measure these substances in a Total Diet Study in order to provide more robust information on dietary exposure.

Item 6: Developmental Neurotoxicity – TOX/2009/03

34. Professor Ray indicated that he had been a member of the ILSI group that had produced the papers on which the Committee was invited to comment. This was not considered to be a conflict of interest.

35. Members considered that the ILSI papers identified important issues in the interpretation of developmental neurotoxicity studies, and proposed a suitable strategy for assessing such studies. It was noted that substantial information was required on a study for its thorough evaluation. This level of detail might be available in reports of regulatory developmental neurotoxicity studies (and could be required by regulatory authorities and in guidelines) but was unlikely to be available for published studies due to editorial constraints on space. This would be reflected in the discussion of uncertainties and might influence the weight given to findings.

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36. One member noted that developmental neurotoxicity studies for pesticides carried out for the US-EPA had not found more sensitive endpoints than conventional studies. In response to a question regarding the extent to which well established neurotoxicants produced effects in developmental neurotoxicity studies, it was stated that although they had not necessarily been tested under the precise conditions used in regulatory studies, it was likely that they would produce positive results under such conditions. Professor Ray offered to provide some unpublished case studies on established neurotoxicants which had been prepared for the ILSI discussions but were not incorporated in the published paper. Members welcomed this offer.

37. Members agreed with the Secretariat’s suggestion that a further paper should be produced summarising the key elements and issues relating to assessment and interpretation of developmental neurotoxicity in humans.

Item 7: Potential exposure to substances in landfill sites – further data – TOX/2009/04 (Reserved Business paragraphs 40 to 52 inserted @ December 2010)

38. In September 2008, the Committee considered the final draft of a report of a study by Enviros Consulting Ltd for the Environment Agency which had measured emissions of airborne chemicals, dusts and micro-organisms from two landfill sites. The Committee had asked for further information to help it to interpret the results of the report and to offer advice on the report prior to publication.

39. The requested information was provided to the Committee at this meeting. This item was discussed as reserved business as the paper contained details of unpublished draft research reports in the process of development and was therefore received by the Committee in confidence. The reserved section of the minutes would be released after publication of the reports.

40. Members were asked to look at the further data supplied to them at this meeting, to comment on the suitability of the Health Criteria Values (HCVs) used in the report, to advise on the possible health effects of the reported boundary chemical exposures, and to formulate recommendations for further work. Substances were discussed individually:

*Arsine:*

41. The Committee agreed that it did not consider it was appropriate to assume that arsine was metabolised completely to arsenic. However, the studies providing the basis for the proposed alternative HCV were short-term where the end point of interest was haemolysis, which is an acute effect. Thus, the proposed HCV did not take into account possible longer term effects such as carcinogenicity. Members asked to see any further toxicological data on arsine in animals. The Committee was informed that the detection limit for arsenic exceeded both the project-specific HCV and the CICAD guidance value. It considered that there may be a need to recommend the development of more sensitive analytical methods and that further monitoring should be carried out.

*Chromium:*
42. Members noted that the project had measured total chromium in dust, but how much of this was chromium III and how much chromium VI was unknown. Thus, further research might be desirable on this. There was a marked difference in the maximum concentration of chromium measured at each site and no clear explanation for this. It was noted that, if 10% of the chromium present in the emissions at Site B was chromium (VI), then the maximum concentration would just exceed the project-specific HCV. Members were informed that the average concentrations for all the chemicals analysed had been calculated using the assumption that, when the chemical was below the limit of detection, its concentration was zero. They asked that these values be recalculated using the assumption that a non-detected chemical was present at the limit of detection. Members also asked for data on background concentrations of all of the chemicals and background intakes from food and drinking water, so that the concentrations and likely intakes from the two landfill sites could be put into context.

1,2-dichloroethane:

43. Members had doubts about the HCV as it was derived from a study using the inhalation route. The use of an uncertainty factor of 50,000 was also questioned. Members commented that the average exposures were below the HCV, but asked to see a copy of the inhalation study before commenting on the appropriateness of the HCV.

Dimethyl sulphide (DMS) and dimethyl disulphide (DMDS):

44. The HCV for these substances was set as a combined value as the two substances are similar chemically and biologically. It was noted that there are only limited toxicity data on these chemicals, in particular on DMDS. The derivation of the HCV appeared to be conservative and it was probably as accurate as the data allow. However, it should be adjusted for continuous exposure. The secretariat was asked to try to obtain the report of the 6-month inhalation study from which the LOAEL for DMS was derived.

Formaldehyde:

45. Members noted that humans were less sensitive to the nasal cytotoxicity observed in rodents because of differences in respiratory physiology. They were content to adopt the approach used by the WHO whereby it was assumed that, if the respiratory tract in humans is not repeatedly damaged, exposure to low, non-cytotoxic concentrations of formaldehyde would be associated with a negligible cancer risk. Members were therefore content to use the WHO Air Quality Guideline for Europe of 100 mcg m$^{-3}$ as a 30 minute average. They asked for information on background indoor air concentrations of formaldehyde to assist in putting exceedances of the HCV into context.

Methylmercaptan:
46. The Committee considered that the data on which the HCV was based were poor but that the derivation of the HCV appeared to be conservative. Members requested sight of the paper by Tansy et al on which the HCV was based.

**Polycyclic Aromatic Hydrocarbons (PAHs):**

47. Members were generally happy with using the EPAQS standard as the basis for the HCV although they considered that a recalculation would be necessary for site B, where the relative contribution of BaP to the carcinogenicity of total PAHs measured was much lower than in the occupational study used by EPAQS. However, they asked to see the most recent advice on PAHs from the Committee on Carcinogenicity (COC) before making a final decision, including its advice on dibenzo[a,l]pyrene (DBalP), which is more potent that BaP and which is not taken into account in the EPAQS approach.

**Stibine:**

48. Members considered that the data available were not sufficient to set an HCV. The proposed HCV was based on very limited data and the maximum exceedance found near the landfill sites sampled in the project was 40 times this HCV. Members therefore considered that reducing the uncertainties around stibine was a fairly high priority for further work.

**Styrene:**

49. No questions were raised about the proposed HCV for styrene and the Committee considered that there were unlikely to be any health concerns arising from the styrene emissions recorded at the two sites.

**Toluene:**

50. The Committee agreed that use of the US EPA reference concentration was appropriate as a basis for the HCV, as it was based on a more complete data package. Members were reassured that the levels of toluene measured at the test sites were within this HCV.

**Vinyl Chloride:**

51. It was noted that average concentrations at both sites were well below the HCV, which was based on a WHO risk estimate for cancer. However, the maximum concentration at Site B exceeded this level. Members noted that, as vinyl chloride is a genotoxic carcinogen, it was not possible to set a level of no concern. They asked for information on the frequency of samples testing positive for vinyl chloride, the detection limit, and range of results.

52. Members concluded by stating that suitable caveats should be added to those HCVs that were set using limited safety data.
Item 8: Second Statement on Landfill sites (first draft) – TOX/2009/05
(Reserved Business)

53. A first COT statement on landfill sites, published in 2001, discussed a large study by the Small Area Statistics Unit (SAHSU) on birth outcomes near landfill sites in Great Britain. The preliminary draft statement in this paper summarised new data on landfill sites seen by the COT in the last 2 years. It covered the emission study and the two SAHSU studies considered in 2007. Although the emission study was still under discussion, it was considered appropriate to begin work on the statement so that both the report and statement could be published as soon as possible. The two SAHSU studies, which were discussed in July 2007, have both now been published, as have the minutes of their discussion by the COT.

54. Due to lack of time it was decided that editorial comments on the draft statement could be taken by correspondence. A Member was identified to review the conclusions, particularly with respect to the epidemiological evidence. In addition, it was noted that more emphasis should be placed on the lack of data on possible developmental effects.

Item 9: Glucosamine and hepatotoxicity – TOX/2009/06

55. Glucosamine is a popular food supplement taken alone or in combination with chondroitin sulphate, usually by sufferers of osteoarthritis. In view of a small number of case reports linking glucosamine and hepatitis, including one that became the subject of a Scottish Fatal Accident Inquiry, the COT were asked to consider whether a causal link was plausible.

56. At the November COT meeting, Members had made a number of comments on the structure and text of a first draft statement, which had subsequently been incorporated into a revised draft. Due to lack of time, Members were asked to send in any comments on the revised draft to the Secretariat in writing. It was agreed that the statement could be finalised by Chairman’s action.


57. Members agreed the general content of the report. It was decided that editorial comments could be submitted by correspondence.

Item 11: Discussion on implications for toxicity testing of rodent strain differences

58. The Chair welcomed Dr Michael Festing, acknowledged correspondence between Dr Festing and the COT, which provided a summary of the topic for discussion, and invited Dr Festing to give an introduction. Dr Festing had previously submitted evidence to the COT Working Group on Variability and Uncertainty in Toxicology about strain differences in xenobiotic metabolism. His proposal was to use multiple genetically well-characterised inbred strains of laboratory animals in toxicity testing (a multi-strain assay; MSA) in place of an equal number of animals from one or two outbred strains. He had also replied in writing before the meeting to
a number of questions, which members of the COT had asked, concerning the proposed use of a multi-strain assay

59. The following possible reasons for using MSAs were given to the Committee:
• To minimise false negative results associated with strain resistance to toxicities,
• MSAs would not require more animals than equivalent studies on outbred animals,
• Minimisation of within-strain variability allowing more powerful experiments,
• To compare genes and xenobiotic metabolism between strains to identify susceptibilities that could be compared to human metabolism and genes,
• Analysis of inter-strain differences in xenobiotic metabolism could identify mechanisms of toxicity.

60. Members first considered the possible structure of a MSA and then each of the above points. A concern was raised over how different it would be to use multiple inbred strains instead of 2 outbred strains from different species. Questions were asked about the choice of inbred strains, the number of different strains needed, the selection of inbred strains and species, and implications for risk assessment. Dr. Festing pointed out that for a given species (say the rat) there was always a choice as to whether to use a single outbred stock or a MSA.

The structure of multi-strain assays and use of animals

61. Dr Festing considered that small numbers, say 4-5, of strains should be used. The use of, say, 20 strains would be unlikely to give much extra information. A multi-disciplinary approach that included toxicologists statisticians and geneticists was recommended for the implementation of MSAs and it was suggested that the COT could consider establishing such a group to look at the scientific case for using MSAs.

62. On the issue of selecting species with multiple well-characterised inbred strains, Dr Festing considered that currently only the mouse and rat were sufficiently characterised genetically to be viable choices. Of the two, an advantage of the mouse was that around 1000 such strains are now available. It was noted that some competent authorities are now concluding that the mouse is a poor model of certain forms of toxicity for humans in comparison to the rat. Members were informed that there are currently 17 genetically well-characterised rat strains and that a further 50 rat strains are being phenotyped in Japan. The EURATools consortium is also developing rat models.

63. It was stated that data are currently collected from a variety of species and strains. Dr. Festing suggested, for example that in a 28-day study with 8 animals/group a suitable subdivision of the species would be 4 strains/group and 2 animals/strain. It was acknowledged that within-strain group size of two might be a problem, but it was better than a within-group size of one when using an outbred stock where every animal is genetically different so it is not possible to get an estimate of the genetic component of response. Each group would still have 8 animals. It was noted that this would still be four groups of two animals each, and that if you had a resistant inbred strain it would reduce the statistical power to detect
effects. But should a single outbred stock prove to be resistant, this would result in even lower statistical power.

64. A second question considered was the sensitivity of a MSA in detecting low-potency carcinogens, which is a challenge under the current paradigm. Dr Festing suggested that, if an equal total number of animals was used, sensitivity would be greater with a larger number of strains but a smaller number of animals per strain. The Committee was informed that such carcinogenesis studies have not been done and Dr Festing stated that it is the total number of animals that matters, not numbers per strain. Dr Festing explained that the problem with using outbred stocks is that treated and control groups are genetically different, leading to lower statistical power.

Choice of strains and species

65. Both inbred and outbred animals are routinely used in toxicology and when selecting strains, prior knowledge of background susceptibility is taken into account. Examples of the use of such inbred strains include the F344 rat and BALB/c and B6C3F1 mice within the United States National Toxicology Program. If a MSA were to be used, strains should be as genetically different as possible through the use of computer analysis of data such as single nucleotide polymorphisms. The question of how to intelligently identify suitable strains for a particular MSA and active substance a priori could not currently be addressed, although it was proposed that genetic diversity was an experimental variable which could be better controlled by using a MSA than by using an outbred stock.

66. From experience of working with different strains, one Member felt that there was little difference in qualitative final outcomes of studies and asked how often there are significant mechanistic inter-strain differences. Although it was recognised that small quantitative differences can make the difference between detecting responses at a particular dose or not, large qualitative differences such as the expression or not of particular genes are of greater importance to decisions on hazards during risk assessment. In reply, Dr Festing questioned whether toxicologists are only interested in large differences. A MSA would be more likely to pick up both large and more subtle differences.

The utility of multi-strain assays

67. Members agreed that in the absence of data on humans, there is a need to test as wide a phenotypic range as possible that has relevance to humans, and questioned how best to achieve this. It was proposed that inter-strain differences in xenobiotic metabolism can be used to investigate mechanisms of toxicity. Whilst there was agreement that mouse models such as knockouts were useful for mechanistic investigations, it was noted that extrapolation from knock-outs to humans was not always justified at this time.

68. On the topic of avoiding false negative results, it was suggested that a single outbred strain could be resistant to the toxicant under investigation. Members noted that strain susceptibility to chemicals and endpoints is taken into account when selecting species and strains. Dr Festing disputed this since most toxicity testing is simply done only on CD-1 mice and Sprague-Dawley rats as there is no way of
predicting which strains will be susceptible. Members noted the importance of historical control data in identifying low background and sensitivity to some endpoints. Members queried the implications for risk assessment when using fewer animals per strain rather than the same numbers of animals from one outbred strain. If a few insensitive strains are present in a MSA, then the overall statistical power to detect critical effects would be compromised. But Dr Festing pointed out that an outbred stock could be totally resistant, which would lead to even lower statistical power.

69. Members noted that a detailed understanding of the genetic backgrounds of MSA strains would be needed to interpret data on inter-strain variation. Dr Festing pointed out that nothing is known about the specific genetic background of outbred stocks, but on the contrary, strain differences will usually indicate polygenic inheritance. Polygenic variation is impossible to detect in a toxicity test using a single outbred stock.

Additional practicalities

70. Members expressed concern that there is a lack of historical control data for inbred strains, and noted that documented information such as a database of comparative outcomes for novel inbred strains would be required and that obtaining this would require additional animals. Dr Festing pointed out that there is already a database for inbred mice, but less work has been done on characterising rat strains. It was stated that greater genetic uniformity of inbred strains would translate to lower inter-animal variation in responses to toxicity, to which the Members responded that research on gene-environment variance has indicated that greater genetic homogeneity does not necessarily lead to less variation.

71. It was noted that if inbred strains did express narrower variability in responses then the choice of inbred strains would be critical to the accuracy and precision of MSAs. Dr Festing pointed out that strain differences are common both with inbred strains and outbred stocks, and this is the reason why more than one strain needs to be used. Dr Festing noted that a single inbred strain is used in the local lymph node assay (LLNA) where a single endpoint is investigated. This results in a highly repeatable test with a high signal/noise ratio. In such circumstance multiple strains are not necessary because any inbred strain would give a comparable result. However, it was noted that the strain used in the LLNA was not chosen for genetic reasons and that the significance of human genetic susceptibility to the sensitisation endpoint is minor. Dr Festing replied that if there was significant human variation in response to skin sensitising agents, then several strains would be needed in the LLNA to reflect the human situation.

72. If regulatory frameworks were to change to ask for multi-inbred strain assays in place of one of more inbred or outbred species, the benefits would need to be sufficiently large to outweigh the disadvantages of change. Members asked what literature studies were available to test the hypotheses that MSAs a) were as sensitive to toxicological endpoints as tests in two outbred species with the same total numbers of animals and b) were less susceptible to inter-animal variation than similar studies in outbred strains. Dr Festing stated that there were few such published parallel studies, although he was aware of one study (involving gentamicin)
in the rat in which the MSA was more sensitive than the assay using outbred animals, and he had published one on the toxicity of chloramphenicol in which the MSA was more powerful than the use of a single outbred stock.

**Conclusions**

73. It was agreed that a systematic literature review would be useful. Few studies have investigated responses in inbred and outbred animals simultaneously and there may be a bias towards publication of studies showing benefits from using inbred strains.

74. If variability between labs using the same chemicals and strains translated to a finding that repeat studies showed different results in the same inbred strains then interpretation of a systematic review would be challenging. But if a single outbred stock gives different results in different laboratories, the interpretation is equally challenging. On the subject of carcinogenesis, a question arose as to whether or not comparisons have been made between strains in terms of prevalence of or time until formation of tumours. Parallel strain comparisons with appropriate model compounds would be useful, and if large differences were identified, the COT could consider further action.

**Item 12:** Paper for Information: Update on actions taken subsequent to COT advice – TOX/2009/08

75. This paper was provided for information only.

**Item 13:** Any other business

76. No other business was raised.

**Item 14:** Date of next meeting

77. The next meeting of the Committee would take place on Tuesday, 7th April 2009 at Aviation House.