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TOX/2008/01

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

TOXICOLOGY OF TOBACCO PRODUCTS – SCOPING PAPER

(Previous discussion paper TOX/2007/03)

Introduction

1. This draft discussion paper presents a preliminary review of published literature on the toxicological evaluation of tobacco, ingredients and additives used in the manufacture of tobacco and its emission products. The proposed review will focus mainly on the validity of the toxicological tests used to evaluate tobacco and tobacco product toxicity but the use of biomarkers is considered briefly too.

2. In 2004, the Committee released a joint statement with the Committees on Carcinogenicity (COC) and Mutagenicity (COM) on the re-assessment of the toxicological testing of tobacco products (Annex 1). The Committees reviewed the *in-vitro* tests and *in-vivo* approaches used to evaluate the toxicity of tobacco products and tobacco-based potentially reduced exposure products (PREPs) and concluded that they are uninformative on the risk of diseases induced by tobacco smoke. The Committees also noted that comparative assessments between PREPs can be undertaken for some *in-vitro* mutagenicity data, but that the data cannot be extrapolated to *in-vivo* mutagenicity. The Committees agreed that there were considerable difficulties in designing a toxicological testing strategy for the re-assessment of tobacco products and that it was not possible to design a valid strategy given the current understanding of the diseases associated with smoking tobacco based products. During the 2004 review, the Committees considered a limited number of references.

3. At the February 2007 meeting, the Committee revisited the 2004 joint COT/COC/COM statement on toxicological testing of tobacco products as part of the horizon scanning exercise. The Committee was informed that the toxicological testing strategy for tobacco and tobacco products had been reported in published reviews as an emerging area for concern,¹⁻⁴ and that the Committee might have a role with regard to considering the toxicological methods used to assess tobacco products. Further, the Department of Health, through the COT Secretariat, has agreed that it is an area with increasing level of concern and thus needs to be reviewed.

4. The Secretariat has undertaken a preliminary overview of the scientific evidence underpinning tobacco product research and developments within the tobacco field. Four key areas of research have been identified:

1. Validity of toxicological tests used for the evaluation of tobacco and its products

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2. Potential effects of ingredients, additives and flavours on tobacco product emissions
 3. Whether there are validated biomarkers of effect for tobacco and its products which can be used to predict risk, harm, injury or disease
 4. Whether PREPS have an associated reduced level of harm, risk or injury.
5. Of these areas, the validity of toxicological tests used for the evaluation of tobacco and its products was considered appropriate for further investigation as it has relevance to tobacco product regulation and relates to the COT 2007 horizon scanning exercise undertaken at the February 2007 meeting. In this paper, the term 'validity of toxicological tests' does not mean being valid to extrapolate to human risk but rather, refers to the ability of tests to produce accurate reliable data that could be used in tobacco and tobacco product comparison.
6. This paper presents preliminary findings from a systematic approach to literature searching regarding the validity of toxicological tests used for the evaluation of tobacco and its products and forms part of the horizon scanning exercise, supporting and extending the 2004 joint COT/COC/COM statement. It considerably increases the number of papers reviewed to evaluate the validity of toxicological tests used in the assessment of tobacco and its products.

Background

Tobacco Products

7. The WHO report (2006) on tobacco product regulation recognises that there is a wide array of tobacco products on sale worldwide ranging from smokeless to combustible tobacco products.¹ The cigarette remains the most popular of all the products,⁵ but other products/devices include cigars, pipe tobacco, smokeless tobacco, kreteks, leaf tobacco, water pipe tobacco or shisha products, bidis, electronically heated devices and liquid nicotine delivery systems that are not intended for nicotine replacement therapy. Although most of these products contain tobacco as the main ingredient, they vary considerably in their composition such that mainstream and sidestream smoke from the combustible products and emissions from the smokeless products can all differ.
8. Recent public health activities suggest that since the joint COT/COC/COM statement there has been the introduction of new tobacco products onto the UK market and the development of other tobacco products and devices worldwide.

Tobacco Product Regulation

9. Tobacco product regulation is in its infancy.¹ The WHO Framework Convention on Tobacco Control has established the base for the regulation of

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the contents, emissions, disclosure of additives and ingredients, packaging and labeling of tobacco products.⁶ However, there are no internationally accepted guidelines or standards governing contents, emissions and designs, and tobacco products continue to be unregulated or under-regulated in many WHO Member States.¹ Currently, limits on emissions from tobacco products have only been implemented for machine-measured yields of tar, nicotine and carbon monoxide.^{1;3} As yet, there are no internationally agreed approaches to hazard assessment of tobacco products.

10. The major academic/risk assessment groups interested in the toxicity of tobacco, its additives and ingredients are listed in Annex 2. With the advent of the development of a diverse range of tobacco products and the increase in the use of technology within the tobacco industry, regulators and the public health community have a clear need for the assessment of the validity of toxicological methods used in the evaluation of tobacco product ingredients, additives and emissions.

Tobacco Product Testing

11. Manufacturers often undertake comparative toxicological assessments of their tobacco and tobacco products to demonstrate reduced toxicity against standardised and conventional tobacco and tobacco products respectively. Marketing strategies may result in manufacturers claiming a reduction in exposure to or harm from tobacco products.^{3;7}

12. The machine-measured yields of tar, nicotine and carbon monoxide measured using the International Organisation for Standardisation/United States Federal Trade Commission (ISO/FTC), the State of Massachusetts and the Canadian Government protocols are recognised as not providing valid estimates of human exposure because some smokers may take deeper and/or more frequent puffs than specified in the ISO/FTC protocols.^{1;3} In addition, it is widely recognised that the ISO/FTC measurements of cigarette smoke tar and nicotine content are an inadequate base for the measurement, regulation and labelling of tobacco products.³

13. Many countries require the tobacco industry to submit information on ingredients, emissions and some require toxicological data based on a number of toxicological tests, with the Ames, neutral red cytotoxicity and micronucleus assays being the most commonly used. However, there have been debates amongst tobacco control experts that these tests give insufficient information on which to draw meaningful conclusions on the toxic potential of ingredients, additives and tobacco product emissions.^{2;4} The WHO have stated that as part of tobacco product regulation, there is a need for methods to assess the effects of tobacco product contents and investigation of contents that reduce tobacco product toxicity.^{1;2;4} In order to achieve this, there is a need for a systematic review of the toxicological literature. In an attempt to fill this gap, this review proposes to evaluate the validity of toxicological tests and their relevance to the assessment of tobacco products. This will be invaluable in guiding regulators in making informed

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decisions about the toxicity of tobacco product ingredients, additives and emissions.

Toxicological Test Methods

14. Tobacco smoke is a complex matrix containing around 4000 chemicals, around 60 of which are human carcinogens, and particulate matter. Although most of these chemicals are formed either during the combustion or during chewing of tobacco products, some are found naturally in tobacco. One goal of tobacco product toxicity testing has been to evaluate the potential hazards and risks associated with exposure to complex mixtures containing a wide diversity of potentially harmful chemicals and particulate matter.

15. WHO recommendations urge Governments 'to develop better measurements of the constituents and impact of tobacco products with the aim of substantially reducing their toxicity'.³

16. The most commonly used toxicological test methods employed in the assessment of the overall toxicity of tobacco and its related products are as follows⁸:-

- A bacterial test for gene mutation (e.g. Ames assay)
- A test for clastogenicity and for indications of aneugenicity
 - i. *In-vitro* metaphase analysis
 - ii. *In-vitro* micronucleus test
- Mammalian cell mutation assay (preferred choice is the mouse lymphoma assay)
- Cytotoxicity assessment using the Neutral Red uptake assay
- Sub-chronic inhalation assay

17. The WHO monograph on tobacco product regulation stated that these methods are inadequate in evaluating the total toxicity of tobacco products, as they were not intended to measure the biological or the epidemiological activity of these products.³ Recommendations from this report include the development of new methods to evaluate the total toxicity and health impact of tobacco products based on a range of biological activities, investigation into the use of biomarkers to assess the health impact of tobacco products on humans, and research to respond to claims on new products.

18. There are no reliable models for assessing the individual contribution of ingredients and additives to the toxicity of tobacco smoke. It would be valuable to establish a standard for the evaluation of the toxicity of inhaled tobacco and its emissions. This is in line with some of the discussions and debates among tobacco control expert bodies, such as WHO advisory networks (Tobacco Regulation (TobReg), Tobacco Laboratory Network (TobLabNet), Working Group to the Conference of Parties to the WHO Framework Convention on Tobacco Control (WHO FCTC)), the Health and Consumer Products of the European Commission (DG SANCO) and government research centres.

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Biomarkers

19. WHO has recommended that better measures, including biomarkers, be developed to assess the health impact and measure composite toxicity of various tobacco products.³

20. The use of biomarkers to predict harm or disease has been discussed extensively among public health experts. However, it is uncertain whether there are valid biomarkers for tobacco related diseases, for example cardiovascular disease, therefore this possibility will have to be further explored. Although this review focuses mainly on the validity of toxicological tests, peer-reviewed publications on biomarkers will be reviewed at a later stage.

Approach Taken and Evidence Reviewed

21. The Secretariat undertook a literature search for approximately 10 years prior to the COT/COC/COM joint statement to provide a more substantive review of the literature and the development of toxicological approaches to the evaluation of tobacco products. The search criteria used for this review are listed in Table 1 together with the number of peer-reviewed papers listed in PubMed for each of the search terms:

Table 1 Search Criteria

Search Term	No of Papers
Tobacco Ames assay	96
Tobacco carcinogenicity	258
Tobacco cardiovascular toxicity	87
Tobacco dermal	58
Tobacco developmental toxicity	39
Tobacco inhalation rat studies	60
Tobacco inhalation toxicity	150
Tobacco micronucleus assay	87
Tobacco neutral red cytotoxicity assay	18
Tobacco reproductive toxicity	168
Testing tobacco	1469
Testing strategy tobacco	39
Toxicity testing tobacco	481
Toxicity testing cigarettes	134
Toxicity testing smokeless	37
Comparative risk assessment tobacco	173
Risk assessment tobacco products	62
Toxicological tests assessment tobacco	6
Toxicological tests tobacco	28
Total no of papers	3450
No of duplicated papers	672
No of citations for period 1995 – 10/12/07	1952
Final paper selection based on abstract/paper content	72

22. After removal of the duplicated citations, the number was reduced to only include those published in the period 1995 to date to increase the data

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reviewed by the Committees. This was judged as a reasonable timeframe based on scientific data available. The remaining papers were screened according to their relevance to toxicological testing of tobacco and its products. The final selection of papers for this discussion paper were chosen to provide information on toxicological assays employed in the assessment of tobacco and tobacco products, testing strategies and the risk assessment of tobacco and tobacco products. Thus, there is evidence to show that tobacco manufacturers are using a relatively common testing strategy. It is proposed to review these papers in detail to further evaluate the rationale for the testing strategy. The objective is to ask the Committee to help focus further review work into discrete areas or topics which would be taken forward at meetings during 2008.

Testing Strategy for Tobacco Products

23. Testing generally follows a tiered strategy: mainstream cigarette smoke chemistry studies; *in-vitro* studies include genotoxicity (Ames and sister chromatid exchange (SCE) and cytotoxicity studies (neutral red); *in-vivo* studies include a 13-week inhalation study in Sprague-Dawley rats and a 30-week dermal tumour promotion study in SENCAR mice.⁹ A scientific consensus on how to measure and characterise the risk associated with tobacco smoke is lacking.⁸

24. *In-vitro* bioassays assessing the toxicological properties of mainstream cigarette smoke utilise smoke generated from cigarettes machine-smoked under either FTC or ISO conditions. The composition of mainstream cigarette smoke varies with smoking conditions set on smoking machines and the methods of collection operated under FTC/ISO and Health Canada Intensive protocols.¹⁰ The FTC/ISO protocol conditions can affect the relative amounts of pyrolysis products produced and consequently the outcome of *in-vitro* assays for cigarette smoke condensate can vary.¹⁰

25. An *in-vitro* toxicology test battery has been used to evaluate and compare the toxicities of processed tobaccos,⁹ machine-generated mainstream cigarette smoke condensates from tobacco-heated and tobacco-burning cigarettes,¹¹⁻¹³ mainstream whole cigarette smoke,^{14;15} and particulate matter from cigarettes with and without additives.¹⁶⁻¹⁸ Further, *in-vitro* tests have been used to evaluate the toxicities of particulate phase combustion products prepared from individual pure tobacco constituents absorbed onto a cellulose matrix, and it is claimed that this experimental approach could be used to identify toxic chemicals in cigarette total particulate matter and as a guide in the modification of tobaccos to reduce the levels of constituents that contribute to the toxicity of cigarette smoke condensate.¹⁹ The test battery used commonly includes two or more of the following: the Ames assay using various Salmonella strains, SCE, chromosome aberration, neutral red cytotoxicity assay in Chinese hamster ovary (CHO) cells (WBL strain) or mouse embryo BALB/c 3T3 cells.¹⁸

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26. An example of the use of a test strategy for toxicity assessment is a study undertaken to assess the influence of 482 tobacco ingredients on *in-vitro* genotoxicity and cytotoxicity of smoke particulate matter and inhalation toxicity of cigarette smoke.¹⁷ Various mixtures of the ingredients were added to blended tobacco and the tobacco then made into cigarettes to assess the effects of ingredients on smoke chemical and biological properties. Assays used were the Ames, micronucleus and neutral red cytotoxicity assays combined with a rodent sub-chronic inhalation study. The specific smoke particulate matter was unchanged by the addition of the ingredients to the cigarette. There was no difference in the histopathological and histomorphometric assessments of rats in 90-day sub-chronic inhalation studies exposed to smoke from control cigarettes and cigarettes containing various ingredient mixtures. This study demonstrates that strategies have been developed where the objective is to compare different tobacco products but the adequacy of this approach has not been considered.

In-Vitro Mutagenicity Evaluation

27. The COM has previously considered the use of the Ames test in monitoring tobacco smoke condensate (see joint COT/COC/COM statement appended as Annex 1) and advised on the limitations of the SCE and Syrian hamster embryo (SHE) assays. The COM have also recently completed a review of approaches to fractionation and monitoring of mixtures using mutagenicity tests. It is noted that there have been a number of developments in testing approach for tobacco products as summarised below. It is proposed that the COM should review these data.

Ames assay

28. Differences in smoking conditions and methods of collection on smoking machines appear to affect the mutagenicity (Ames assay) and cytotoxicity (Neutral Red cytotoxicity assay) of cigarette mainstream smoke.^{10;20}

29. The mutagenicity of total particulate matter collected from tobacco-burning and extracts from smokeless tobacco products has been assessed using the Ames assay.²¹ The authors stated that it was possible to compare the mutagenic potency of mainstream smoke condensate from tobacco-burning products with that of smokeless products if the comparisons of mutagenic potency were expressed on a per mg nicotine basis. The Ames assay has also been used to assess the mutagenicity of tobacco smoke condensates generated at different pyrolysis temperatures.²⁰

30. The Ames assay is used for assessing mutagenicity of cigarette main- and sidestream smoke using condensates or extracts.^{11;13;16;18;19} A modification of the Ames assay has been proposed to detect the mutagenic activity of cigarette smoke whereby the bacteria are exposed to atmospheres containing whole smoke and gas vapour phase of cigarettes.²²

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31. Another modification of the Ames assay employing a microsuspension modification of the Ames Salmonella/microsome assay with strains TA98 and YG1024 in the presence of 5% S9 metabolic activation has been used to assess the mutagenicity of particulate matter concentrated from environmental tobacco smoke.¹²

Micronucleus induction in V79 cells

32. A novel exposure system has been designed which allows direct exposure of V79 cells to whole cigarette smoke without an intervening layer of medium between the cells and smoke and the presence of S9.²³ Using the system, the induction of micronuclei in response to the smoke exposure was measured without a layer of medium between the cells and the smoke.²³

Mouse lymphoma assay

33. This assay has been used to assess and compare the mutagenicity of cigarette smoke particulate matter derived from cigarettes machine-smoked under different conditions, and from single tobacco type and conventional cigarettes.²⁴

SHE assay

34. An initiation-promotion cell transformation assay system using SHE cells at pH6.7 has been developed and applied to the determination of the carcinogenic potential of cigarette smoke total particulate matter.²⁵ It is considered by the authors that chemicals found to be positive in the traditional SHE cell transformation assay could be further classified as initiators or promoters using this alternative method.

In-Vivo Mutagenicity Evaluation

DNA adduct formation

35. The smoke condensate from machine-smoked cigarettes was painted on the skin of female SENCAR mice for 29 weeks. After sacrifice the entire lung, heart and a portion of the treated skin were removed and analysed for DNA adduct formation using ³²P-postlabelling.²⁶ DNA adducts were found in all tissues examined. In another study, B6C3/F1 mice were exposed by inhalation to mainstream smoke from tobacco-burning and tobacco-heating cigarettes and DNA adduct measurement by ³²P-postlabelling was used for comparative genotoxicity assessment.²⁷

Micronucleus induction

36. The assessment of micronuclei formation in bone marrow and peripheral blood polychromatic erythrocytes has been used in studies where animals were exposed to tobacco smoke to support comparative cigarette type genotoxicity evaluation.²⁷

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Cytotoxicity Evaluation

37. In the 2004 joint COT/COC/COM statement, the COT concluded that *in-vitro* cytotoxicity testing could be used as part of an overall approach for comparing PREPs but the data could not be extrapolated to the *in-vivo* situation and the outcome measured in such tests had no predictive value with regard to disease associated to tobacco smoke (Annex 1). There have been a number of developments in the assessment of cytotoxicity since this statement and it will be important to evaluate the new proposals given the weight of evidence that has been attributed to the assay in some publications.

38. To test the hypothesis that cigarette constituents that inhibited chicken chorioallantoic membrane (CAM) growth would also inhibit mammalian cell growth, the effects of 6 cigarette toxicants on human umbilical vein endothelial cells (HUVECs), human microvascular endothelial cells (HMVECs) and NIH 3T3 cells was determined using a cell proliferation/survival assay.²⁸ Overall, HUVECs were shown to be more responsive to treatment than HMVECs.

39. A 'physiological' *in-vitro* model has been proposed for standardised studies of the cellular and histological effects of cigarette smoke.²⁹ The cigarettes were machine-smoked and the smoke bubbled through cell culture medium without additives to collect the water-soluble constituents. The smoke constituents were then added to cultured HUVECs and cell death was quantified cell cultures *in-vitro*. The smoke extracts were analysed using liquid chromatography/mass spectrometry.

40. Cultured HUVECs have been used to study the ability of aqueous extracts of chewing tobacco, dry and moist snuff to stimulate the accumulation of inflammatory leukocytes at the site of placement.³⁰

Glutathione (GSH) measurement

41. Dose-dependent intracellular ATP/ADP and glutathione levels have been analysed using cultured human bronchial epithelial cells, human lung fibroblasts and human alveolar cell line A549 exposed to machine-generated cigarette smoke from different cigarettes in a novel test atmosphere system based on the cell cultivation system CULTEX.³¹⁻³⁵ The *in-vitro* test procedure involved a strategy to mimick the human smoking process as far as possible.³³ The authors considered that the detectable change in glutathione content confirms that the exposure strategy induces representative effects in the exposed cells.³⁴ Further, that the testing strategy could facilitate *in-vitro/in-vivo* comparisons, the analysis of complex mixtures,³⁴ evaluate the effects of smoke particulate and vapour phases independently,³¹ and detect differences in the toxicological action of smoke from different types of cigarettes³³ but the biological basis for these claims was not explained.

Neutral Red cytotoxicity assay

42. A manufacturer evaluated eight *in-vitro* assays: Neutral Red, lactate dehydrogenase (LDH) release, kenacid blue binding, MTT (3-(4,5-

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dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) formation, XTT (23-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) formation, acid phosphatase activity, sulforhodamine B binding and resazurin binding for their sensitivity towards assessing cytotoxicity in CHO cells following exposure to cigarette smoke condensate.³⁶ For short exposure periods, the LDH assay was considered the most sensitive, measuring membrane integrity, and neutral red and kenacid blue assays, measuring total cell number, were considered the most sensitive for longer exposure periods.³⁶ Based on their results, Putnam et al. (2002)³⁶ recommended the LDH assay for exposure periods of 1 hour and the neutral red assay for exposure times greater than 3 hours.

43. Confluent monolayers of NCI-H292 human lung epithelial cells on semipermeable membranes were placed in a purpose-designed Perspex chamber at an air-liquid interface and exposed to dilute whole mainstream cigarette smoke. Cytotoxicity was assessed using the Neutral Red assay.³⁷

44. Cultured mammalian CHO cells have been exposed to whole smoke with cytotoxicity and genotoxicity being assessed using the Neutral Red and SCE assays respectively.¹⁴

45. The cytotoxicity and gene expression profiles have been evaluated for mouse BALB/3T3 fibroblast cells exposed to whole smoke from 3 different types of cigarette.¹⁵ The authors consider that both the neutral red cytotoxicity assay and gene expression profiling can differentiate between the different types of cigarette (see paragraph 63).¹⁵

46. The Committee is asked to consider this area for a further detailed review.

In-Vivo Toxicity Studies

Subchronic inhalation studies

47. A key part in the evaluation of tobacco smoke and impact of ingredients and additives is sub-chronic inhalation studies in rodents. It will be important to evaluate the inhalation methods used for tobacco smoke assessment.³⁸

48. Rodent sub-chronic inhalation studies were performed using cigarette mainstream smoke to determine the potential biological effects of mixtures of ingredients added to blended research cigarettes.^{16;39}

49. Ninety-day sub-chronic inhalation studies have been undertaken to evaluate the effects of mixtures of additives added to blended tobacco on cigarette toxicity in terms of histopathological and histomorphometric changes.¹⁷

50. The Committee is asked to consider reviewing these studies in detail.

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Developmental toxicity

51. There are comparatively few developmental toxicity studies involving tobacco smoking.

52. Maternal exposure to high levels of mainstream cigarette smoke particulate matter during gestation and lactation caused a reduction in birth weight and growth retardation in the neonatal pups.⁴⁰ Maternal passive smoking resulted in delayed ossification *in utero* regardless of dose.^{41;42}

53. Short-term maternal exposure to tobacco smoke from different tobacco products resulted in a dose related retardation of growth but no distinctions between products were reported.⁴³

Carcinogenesis

54. Bogen and Witschi (2002)⁴⁴ have exposed strain A/J mice to environmental tobacco smoke and evaluated lung tumour formation. However, the dose-response curves in A/J mice model has been shown to be flat and its usefulness of the model in studies on product modification or chemoprevention is questioned.⁴⁵

Dermal carcinogenesis

55. The COC have previously considered approaches to carcinogenicity testing within general guidance. It is proposed the COC review the studies cited below.

56. The mouse dermal promotion assay is considered the only test that reproducibly measures the tumourigenic potential of cigarette smoke condensate when used in a weight-of-evidence approach including smoke chemistry and *in-vitro* studies using whole smoke, smoke vapour phase and particulate matter.⁴⁶ The assay can only detect a subset of the IARC carcinogens in smoke.⁴⁶

57. The SENCAR mouse skin cancer model involving mouse skin painting with mainstream cigarette smoke condensate or its constituents⁴⁷ is considered the bioassay of choice for mechanistic studies into the carcinogenic and tumour-promoting properties of cigarette smoke particulate matter.^{48;49} Selected short-term analyses associated with hyperplasia and/or inflammation are capable of discriminating between smoke condensates with dissimilar tumour promotion potentials.^{47;50}

58. A manufacturer has standardised the mouse dermal initiation/promotion assay in terms of condensate collection, duration of treatment, mouse strain, number of animals and endpoints measured to produce what is claimed as a responsive, sensitive and reproducible assay for the comparative evaluation of cigarette smoke condensates.⁵¹ The model uses female SENCAR mice and it is claimed that the model may be used to effectively evaluate changes in cigarette design, new materials used in the

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manufacture of cigarettes, alterations in tobacco processing, and the development of new technologies to increase or decrease the biological activity that results from burning tobacco.⁵¹ Comparisons of the dermal tumour-promoting potential of cigarette smoke condensates from cigarettes containing tobacco cured through different processes has been undertaken using the SENCAR mouse dermal initiation/promotion model.⁵²

59. Cancer risk indices for 158 chemical constituents found in machine-measured mainstream cigarette smoke has been proposed as a basis for discussion of the relative toxicological hazards of individual chemicals in cigarette smoke, and a possible framework for the prioritisation of carcinogens and other toxicants in cigarette smoke.⁵³ Thus, this approach differs in that risk reduction is guided by hazard assessment of individual compounds rather than measuring the toxicology of mixtures.

Cardiovascular toxicity

60. There are comparatively few cardiovascular toxicity studies involving tobacco smoking.⁵ The effects on the cardiovascular system have been studied mainly in terms of the interaction of nicotine and carbon monoxide and the myocardium.⁵⁴

61. Sidestream cigarette smoke (SSCS) is believed to be involved in atherosclerosis and cardiovascular diseases and solutions of SSCS from different cigarettes have been shown to affect angiogenesis using the CAM assay.⁵⁵

Investigative research using genomic approaches

62. A novel exposure system employing the exposure of NCI-H292 human lung epithelial cells to cigarette smoke with measurement of mRNA levels in the secreted mucin, MUC5AC, IL-6 and IL-8 has been proposed as a useful system for further investigations into toxicological mechanisms of cigarette smoke in the lung.³⁷

63. Gene expression profiles of mouse BALB/3T3 fibroblast cells (CCL-163) exposed to whole tobacco smoke have been characterised, and the changes in cell proliferation, glutathione synthesis and consumption associated with the differentially expressed genes were proposed to have potential mechanistic involvement in the development of smoke exposure-related diseases.¹⁵ Furthermore, the differentially expressed genes were considered to have potential as biomarkers for the assessment and monitoring of the biological effects of cigarette smoke exposure.¹⁵

64. Inhalation transcriptomic markers of chronic obstructive pulmonary disease (COPD) have been investigated in lung tissue of AKR/J mice exposed to cigarette smoke and the model was considered a potential tool for studying cigarette smoke-induced COPD.⁵⁶

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65. Cigarette smoking is associated with atherosclerosis in the abdominal aorta and an integrated approach combining gene expression profiling, protein analysis, cytokine and cytotoxicity measurements has been used to research matrix-degrading and pro-inflammatory responses of cultured human aortic and coronary endothelial cells exposed to cigarette smoke condensate.⁵⁷

COT Discussion

66. The literature search was limited at this time but will be extended as appropriate to consider each area taken forward for detailed review. The Secretariat noted that much of the literature identified as being relevant to the subject of this paper was published by tobacco product manufacturers. The literature search covered the period 1995 to date which extended the search previously undertaken for the COT/COC/COM joint review of *in-vitro* tests and *in-vivo* approaches used to evaluate the toxicity of tobacco products and tobacco-based potentially reduced exposure products (PREPS), and included recent WHO reports.

67. The WHO has established a base for the regulation of the contents, emissions, disclosure of additives and ingredients, packaging and labeling of tobacco products and limits on emissions from tobacco products have only been implemented for machine-measured yields of tar, nicotine and carbon monoxide. There are no internationally accepted guidelines or standards governing tobacco product contents, emissions and designs, and no internationally agreed approaches to hazard assessment of tobacco products.

68. Manufacturers often undertake comparative toxicological assessments of their tobacco and tobacco products to demonstrate reduced toxicity against standardised and conventional tobacco and tobacco products respectively. However, there are no adequate and reliable methods for the assessment of the contribution of individual ingredients and additives to the toxicity of tobacco smoke and the evaluation of total toxicity of tobacco products. There is worldwide recognition of the need to develop better toxicological methods to evaluate tobacco and tobacco products and to contribute to the development of harm reduction products. Members will recall in the 2004 joint COT/COC/COM statement it was agreed that the ideal way forward to reduce risks and hazards of tobacco smoke was to encourage smokers to stop smoking and to discourage non-smokers from taking up smoking, and any attempt to reduce toxicity should not detract from this position.

Questions for the Committee

1. Does the Committee have any general comments on the approach?
2. Is there a need for further literature searches focusing on particular areas or tests?

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3. The Committee is asked for guidance on the areas to be taken forward for further evaluation.
4. Does the Committee agree that the areas indicated for review by the COC and COM should go to these committees?

Secretariat
January 2008

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Annexes

Annex 1: Joint COT/COC/COM statement on the re-assessment of the toxicological testing of tobacco products. Published 2004.

Annex 2: Major groups interested in tobacco toxicological assessment.