

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Discussion Paper on the Carcinogenicity Testing of Tobacco Products

Introduction

1. Tobacco smoke, generated as a result of smoking tobacco products, is a complex matrix of chemicals, some of which are classified as carcinogens ^[1].
2. Unlike other products which are harmful to health, there is currently little or no regulation of tobacco products and there are no internationally agreed approaches to toxicological testing of tobacco products (in burnt or unburnt forms) which can generate appropriate data to compare tobacco products. ^[2] New products with claims of reduced toxicity based on existing methodologies are being presented (in different forms) as healthy alternatives to conventional smoking. It is not possible to draw meaningful conclusions about the effects of added ingredients (which are chemicals, compounds, flavours, etc added to tobacco products during manufacture) or other constituents on the overall toxicity of tobacco products (in burnt or unburnt form). There is thus a need to establish protocols to assess the toxicity of tobacco mixtures or the contribution of added ingredients in alternative products.
3. Assays for testing the carcinogenicity of tobacco products, which fall under the umbrella of 'toxicity testing', are reviewed in this discussion paper, albeit with the main focus on the Mouse Dermal Promotion Assay (MDPA). The scientific evidence underpinning the applicability of this assay to tobacco products, and improvements made to the assay regarding standardisation to measure the tumourigenic potential of cigarette smoke ^[3], have been examined. Suggestions in the published literature regarding the use of the standardised assay for the comparison of tobacco products have also been considered.
4. A discussion of the use of the proposed cancer risk indices by Fowles and Dybing ^[4] for the prioritisation of carcinogens in cigarette smoke has been included.

Background Information

5. The tobacco products directive (2001/37/EC) stipulates that one of the major areas that require special attention is "methodologies for realistically assessing and regulating exposure and harm attributable to tobacco products" ^[5]. The Secretariat presented a discussion paper to the COT, COM and COC in 2004 which sought advice on the toxicological approaches used for the evaluation of tobacco products and the results obtained from them, with a view to responding to the European Commission's request for advice on the toxicological testing of tobacco products ^[6].
6. The Committees considered the evidence in the paper (the COM on the 7th of October, the COT on the 26th of October and the COC on the 18th of November 2004) and issued a statement, which included the following ^[7] (Annex 1):-

- *"that there were considerable difficulties in designing a toxicological testing strategy for the reassessment of tobacco products and that it was not possible to design a valid strategy given current understanding of the diseases associated with smoking tobacco"*

- *“that there is no strategy which could be used to compare Potentially Reduced Exposure Products (PREPS) for carcinogenic potency and that the approaches used are not informative on the risk of tobacco induced carcinogenicity”*
- *“that analysis of tobacco smoke constituents was not useful in comparing tobacco-based PREPS or predicting risks associated with tobacco smoking”*
- *“that future progress on the proposed approach to reduce tobacco smoke-induced disease by modification of tobacco products could only be made when detailed mechanistic information on tobacco-induced diseases were available”*

7. In February 2007, COT members revisited the issue of toxicological testing of tobacco products as part of the horizon scanning exercise and raised concerns about an appropriate testing strategy for the toxicological evaluation of tobacco and its products ^[8]. Members noted that studies were being published by the tobacco industry which reported that additives have no impact on the toxicity or carcinogenicity of tobacco and that there might be a potential role for the COT in considering toxicological methods used to assess tobacco products.

8. At the February 2008 meeting ^[9] (Annex 2) of the COT, the Secretariat presented a scoping paper on the toxicology of tobacco products which provided an overview of the toxicological evaluation of tobacco products. This paper covered different aspects of toxicological testing of tobacco products. Paragraphs 54 – 59 of this paper specifically dealt with carcinogenicity testing with specific emphasis on dermal carcinogenicity and the Secretariat indicated that these sections would be referred to the COC at its November 2009 meeting for advice.

Regulatory considerations surrounding the Toxicological Testing of Tobacco Products

9. Existing toxicity tests are not considered suitable for assessing the toxicological relevance of additives/smoke emissions to humans or for comparison of the toxicological profiles of products ^[10]. It has also been pointed out that there are no international agreed approaches to toxicological testing of tobacco products ^[9], which could form the basis for effective regulation of these products.

10. The WHO noted in one of its monographs on tobacco product regulation that existing toxicological tests (in-vitro cytotoxicity, in-vitro mutagenicity, subchronic toxicity in rodents, dermal initiation/promotion assay in mice) are inadequate to evaluate the total toxicity of tobacco products, as “they were not intended to measure the biological or the epidemiological activity of these products” ^[2]. Recommendations from this report include calls for the development of new methods to evaluate the total toxicity and health impact of tobacco products based on a range of biological activities, a call for 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. It was also noted in this report that the potential of a product to induce different types of toxicological or mutagenic damage to humans needed further investigation ^[2].

11. Another area of concern is the marketing of novel products, most of which purport to reduce harm to users based on reduced exposure to toxicants when compared with conventional cigarettes ^[11]. However, the available tests may not be the most suitable for the toxicological testing of these products or give enough information to draw meaningful conclusions on the toxic potential of additives used in existing or novel products and resulting emissions. The Department

of Health has therefore requested advice on the toxicological assessment of tobacco products in order to derive appropriate policies.

12. The toxicological investigation of tobacco products often uses exposure to smoke generated using smoking machines. However, smoking machines are known to produce exposures that do not correlate well with human smoking behaviours [12, 13]. There is thus a movement for existing methods to be replaced by realistic machine smoking methods that would match more closely with human behaviours and provide predictions of human exposure [14]. Whilst it is currently recognised that no current machine smoking method can truly mimic human smoking, these methods are well suited for product characterisation under standardised conditions [15].

13. Annual reports submitted by tobacco product manufacturers to the Department of Health on added tobacco products ingredients indicate that these ingredients have little or no effect on the overall toxicity of the cigarette. However, there is an element of uncertainty as to the real effects of these ingredients, since there are no reliable models for assessing the individual contribution of these added ingredients to the toxicity of tobacco smoke. In the European Union, the European Commission requires Member States to submit ingredient and emission information on tobacco products (including any available toxicological data), submitted by manufacturers or importers of these products in their respective countries, although the specific tests to be used to generate such data are not specified.

14. Although it was the opinion of members in their 2004 joint statement that there were no realistic methods for assessing toxic exposure due to tobacco smoke, the rapid pace of tobacco industry product development and marketing since 2004, coupled with new scientific evidence, highlights the importance of an update of the statement. Further, scientific advice from an independent committee such as the COC, COT or the COM would inform policy on the areas of concern, which are discussed in the following sections.

Approach to literature review

15 Following the request by the Department of Health to update the joint 2004 statement on tobacco products, an extensive search of the scientific literature, steered by discussions by DH officials, was undertaken by the Secretariat (1998 – August 2009). The initial searches focused on the approach to toxicological testing of tobacco products. Additional searches were undertaken on the marketing of novel products with implied reduced harm, and modifications of existing products with claims of reduced toxicity in comparison with conventional cigarettes.

16. Scientific journals, online scientific engines (such as PubMed) and toxicological databases (such as toxline) were employed for the review of available evidence. Search terms such as mouse dermal promotion assay, biomarkers of effect, harm reduction, were used to search for relevant studies, although studies were narrowed down by using more specific terms, such as tobacco, smoking machines and cigarettes. The studies identified were subject to further selection according to five areas identified for consideration by the Committee as follows:-

- i.) The carcinogenic testing of tobacco products (Area 1);
- ii.) Implied harm reduction through comparison of products (Area 2);
- iii.) The effect of ingredients or additives on the toxicology of emissions (Area 3);
- iv.) Evaluation of biomarkers of harm, risk, injury or disease for tobacco products (Area 4);
- v.) The use of a cancer risk index for prioritisation of toxicants in tobacco smoke (Area 5)

17. The abstracts of the key studies for Area 1 (which had the most number of publications), are presented in tabular form in Annex 3 for Members' information. Abstracts of the key studies for areas 2, 3 and 4 are presented in Annexes 4 – 6, while short paragraphs of all key studies reviewed for the identified areas (i.e. 1 – 5) are presented in the following sub-sections. In addition, copies of selected references have been appended and the key paper for area 5 is presented in Annex 7. Annex 8 presents a 3-stage model proposed for the evaluation of PREPs, while Annex 9 presents an extract from the Scientific Committee on Emerging and Newly Identified Health Risk (SCENIHR) report on smokeless products which is a summary of studies on carcinogenic effects in experimental animals after snuff application,. Annex 10 presents an extract from the a WHO Technical Series Report, while Annex 11 presents examples of publications relevant the draft discussion paper. A further review of papers will be undertaken in accordance with any requests made by the COC.

SECTION I – CARCINOGENICITY TESTING OF TOBACCO PRODUCTS (ANNEX 1)

18. Tobacco smoke is a complex mixture consisting of thousands of chemicals in the form of particulate phase suspended in a mixture of gases and semi-volatile compounds. Data from smoking machines indicates that the composition of tobacco smoke may differ considerably between different tobacco products. Thus, it has been suggested that different responses may be elicited by tobacco products in carcinogenic assays based on the fractions (mainstream – inhaled by smoker or sidestream – from the lit end of the cigarette) of the smoke analysed.

19. Tobacco smoke has been causally associated with lung cancer and cancer of a wide range of other organs in humans (e.g. bladder, nasal) ^[16, 17]. However, many animal inhalation studies of tobacco smoke have yielded negative results for cancer. ^[14]. Many of the approaches (described below) which are used to assess the carcinogenic potential of tobacco products, despite being extensively described in literature for testing tobacco carcinogenesis, are not validated for cigarette smoke and the relative contribution of many of the potential carcinogens is unknown ^[18]. The main objective of this section is to consider the human relevance of the current approaches used for the evaluation of the carcinogenic potency of tobacco products and smoke components, with the main focus on animal models.

Use of Animal Models for Tobacco Carcinogenesis

20. Many carcinogenicity studies using tobacco smoke undertaken in experimental animals (including primates, hamsters, dogs, rabbits, rats, mice and ferrets) have yielded either negative or weakly positive findings. ^[19, 20, 21]. Despite this, Hetch (2005) in an extensive review of animal models explained the rationale for their use to assess cigarette smoke exposure, ^[21] and emphasised their importance for exploring the mechanisms involved in cigarette smoke-related cancers/cigarette smoke induced carcinogenesis and other degenerative diseases ^[21, 22]. There have been a number of publications reporting improvements in the design of animal models for carcinogenicity testing of tobacco products in recent years ^[22], although their relevance to humans is still unclear.

21. In mouse models, several exposure approaches, such as skin-painting, nose-only inhalation and whole-body smoke have been used to demonstrate the carcinogenicity of tobacco smoke condensate ^[21], each with its limitations. A number of mice strains, including A/J, Swiss Albino (which are next in sensitivity to A/J mice in terms of lung carcinogenesis), Balb/c, B6C3F1, SENCAR and DBA/2, have been employed for studying tobacco carcinogenesis but

most studies have used the A/J mouse model developed by Witschi (1997) [23]. An overview of the mouse models has been given below.

Inhalation Carcinogenesis of Tobacco Smoke

22. Mice are obligate nose breathers and thus inhalation studies in mice result in different pattern of particle deposition and lower doses compared to smoke inhalation in humans [21]. This may have contributed to the relatively reduced carcinogenic potency seen in mice in inhalation studies.

23. Reviews of chronic inhalation studies with mainstream cigarette smoke, conducted in 2001 and 2002, found no statistically significant increase in the incidence of malignant tumours of the respiratory tract in mice, rats, hamsters, dogs, and nonhuman primates, following prolonged exposures (from 12 months to more than 24 months) to high doses of smoke [24, 25].

24. The most prominent medium-term bioassay for cigarette smoke carcinogenesis was developed by Witschi et al. (1998) and involves the whole-body exposure of A/J mice or other mouse strains to cigarette smoke for 5 months followed by recovery in filtered air for 4 months [26]. The model evaluates the tumourigenic potential of tobacco smoke mainly by measuring tumour incidence (fraction of exposed animals with any tumour) and tumour multiplicity (average tumours per lung inclusive of non-tumour bearing animals). The A/J mouse model has been widely used to show statistical significant increases in the yield of surface lung tumours in many studies following exposure to tobacco smoke from various sources [27, 28, 29, 30, 31, 32, 33].

25. Witschi (2002) in studies with the A13 mice strain (male and female) exposed (6h/day, 5days/week for 5 months, followed by a 4-month recovery period in air) to environmental tobacco smoke from Kentucky IR4F reference cigarettes, at concentrations of total suspended matter of 50 to 90 mg/m³, showed significant lung tumour multiplicity than in concomitant controls. The author concluded that the strain A13 lung tumour model might be suitable to study tobacco smoke toxicity and carcinogenicity. Further, in analysis of several independent studies using the protocol described above, Witschi found a significant increase in lung tumour multiplicities in tobacco smoke exposed mice relative to air exposed mice, and a good correlation between exposure (50 - 150mg/m³ tobacco smoke) and lung tumour multiplicities, whereas lung tumour incidences varied. He concluded that the lung tumour model might be suitable for future evaluation of chemo-preventive agents or modified tobacco products [34]. Despite this, comparative analysis of 18 individual studies reported by four different laboratories (concentrations of 50 – 170 mg/m³ total suspended smoke particulates) indicated that cigarette smoke is a weak animal carcinogen [35] due to the comparatively low tumour multiplicities (average of 1.1 – 2.8 tumours per lung). Further, a study by Witschi in 2004 which used the same protocol confirmed cigarette smoke as a weak mouse lung carcinogen, partly due to the flat shape of the dose-response curve [36].

26. A 2005 study by Hutt et al. demonstrated that lifetime whole-body exposure (6hrs/day, 5days/week for 30 months starting at age 6 weeks) of female B6C3F1 mice to high doses of mainstream cigarette smoke at a concentration of 250 mg/m³ significantly increased lung cancer incidence (when compared with sham-exposed animals) [37]. Adenocarcinoma in cigarette smoke treated mice was 20.3% (67 out of 330) compared to 2.8% in controls (9 out of 326) and lung adenoma was 28.2% in treated mice and 6.7% in sham-exposed mice. This is considered to represent a relatively strong carcinogenic response [21, 37].

27. Balansky et al. (2007), in evaluating whether whole body exposure of mice to cigarette smoke (CS) early in life enhances carcinogenic response, used Swiss albino mice to provide evidence that when these species are exposed 12hrs after birth, mainstream CS behaves as a potent carcinogen [22]. Cigarette smoke in this study induced a high incidence and multiplicity of lung tumours (mostly microadenomas and adenomas), as well as malignant lung tumours, tumours of the liver and urinary tract, and bronchial and epithelial hyperplasias [22]. The susceptibility of mice to the carcinogenicity of mainstream cigarette smoke, when exposure starts a few hours after birth, was ascribed by the authors to a variety of mechanisms, such as the induction of oxidative DNA damage and formation of bulky DNA adducts, increased proliferative rate in neonatal organs, and alterations of xenobiotic metabolism. The authors concluded that this bioassay is suitable for studying cigarette smoke-related carcinogenesis, the mechanisms involved in the process and chemoprevention studies.

28. Although there are a number of studies that have used the Balb/c mice to model lung carcinogenesis [38], Santiago (2009), in a study conducted to investigate the effect of different doses of CS in urethane-induced Balb/c mice using the same model, found no statistically significant difference in the mean number of nodules and hyperplasias between the different exposure groups [38]. Seven to 13 week-old Balb/c male mice were treated with two intraperitoneal injections of urethane (1.5g/kg) at 48hour intervals and randomly divided into four groups for whole body sidestream CS exposure (control, Group 1, Group 2 and Group 3) once or twice a day, 5days a week for 16 weeks after which they were sacrificed. Exposures were performed in a closed chamber. The control group (n=18) received urethane only; Group 1 (n=17) was exposed to the smoke of three cigarettes for 10 minutes once every day, 5 days a week for 16 weeks; Group 2 (n=28) was exposed to the smoke of three cigarettes for 10 minutes twice a day; and Group 3 (n=18) was exposed to six cigarettes for 10 minutes twice a day. The authors suggested that the Balb/c mice model is unsuitable for exploring tobacco pathogenesis of smoking induced tumour progression in the lungs.

Dermal Carcinogenesis of Tobacco Smoke

29. The mouse dermal promotion bioassay involves the exposure of the skin of mice to 7, 12-dimethylbenz[a]anthracene – DMBA (initiation - usually for a week), followed by the repeated administration of cigarette smoke condensate (CSC) or its constituents (promotion - usually for 29 weeks) [3, 30]. Whilst this assay has been used for years to study cigarette smoke, the methodologies employed were highly varied, until a standardised protocol using female SENCAR mice was described [39] by Meckley in 2004. This standardised protocol, based on combined data from four independent studies, was described by the author as an effective model that may be used to evaluate changes in cigarette design, new materials used in cigarettes, changes in tobacco processing or the development of new technologies. The assay meets the criteria (previously identified from a review of earlier studies using the MDPA) of a standardised CSC collection method, a clear quantitative dose-response, short-term results, ready availability/sensitivity of test species and strain and ability to verify responsiveness at regular intervals with a reference cigarette. Thus, it is claimed that the standardised protocol provides a highly reproducible, quantitative study design to assess the tumour promotion activity of CSC, can be used to detect changes in the biological activity of CSCs [39] and is considered the preferred method for studying carcinogenic activities of cigarette smoke [40, 41].

30. Comparisons by Hayes (2007) of the dermal tumour promoting potential of CSCs (conc. of 9, 18, or 36 mg) from flue cured (Heat Exchange Process) tobacco cigarettes with flue cured (Traditional Direct Fire Process) tobacco cigarettes, using the standardised 30-week MDPA, found no statistically significant difference between the tumour promotion potential of the two

types of cigarettes ^[42]. The assay was able to discriminate between controls and each of the types of flue cured tobacco cigarettes, as evidenced by statistically significant differences between the number of tumour bearing mice and numbers of dermal tumours for the smoke condensates of the three. However, the assay did not show any statistically significant difference between the tumour promotion potential of the heat exchange process cured tobacco and the traditional direct fire cured tobacco.

31. A number of studies have demonstrated that CSCs which show significantly different tumourigenic potentials in the standardised 30-week MDPA could be discriminated by employing short-term indices of sustained hyperplasia and/or inflammation ^[43, 44]. However, Smith et al. (2006) asserted that, since the MDPA can only detect a subset (not described in paper) of the IARC carcinogens in smoke, it is plausible that it would not give a picture of the overall toxicity of carcinogens in tobacco smoke, unless used in a sequential weight of evidence approach to include smoke chemistry and the results of other toxicity studies. The author considered this method as the only validated method capable of producing tumours in experimental animals from cigarette smoke condensate ^[3].

32. Curtin, in 2006, employed a modified initiation-promotion protocol to further evaluate CSC-induced hyperplasia with respect to time of induction (ToI), existence of a threshold (T) and suitable dynamic range (DR) for detectable responses, and reversibility ^[44]. CSC exposure (9 - 36 mg tar), 3 times a week for 3 – 15 weeks, was found to induce treatment-related increases in epidermal thickness, proliferative index (assessed by 5-bromo-2-deoxyuridine (BrdU) labelling) and ornithine decarboxylase (ODC) expression. Increases in interfollicular BrdU labeling and ODC expression, which were partially reversed after promotion had ceased, remained elevated within the perifollicular epidermis at a similar level to that observed during CSC application. The author noted that perifollicular ODC expression assessments appear to provide an opportunity to discriminate between test articles with reference to a rapid ToI, low T and expanded DR of responses, and the potential to account for irreversible changes. These findings were considered by the author to suggest that ODC expression may be necessary for tumour promotion and that mouse skin tumours stem primarily from the perifollicular epidermis.

33. Members are asked to comment on the utility of mouse carcinogenicity studies with tobacco smoke and CSC.

SECTION II – TOBACCO “HARM REDUCTION” - COMPARISON OF PRODUCTS (ANNEX 4)

34. The purpose of this section is to ask members’ advice on the available information on the toxicological basis of claims of reduced exposure, harm or risk posed by existing and novel tobacco products. A number of studies have been published to support such claims since the 2004 joint statement on the toxicological evaluation of tobacco products. Many of these studies have shown statistically significant reductions in several toxic emissions ^[45, 46, 47, 48] but it is not known whether these reductions translate to reduced risk, harm or injury in humans ^[49].

35. Harm reduction has been defined as an attempt to reduce exposure in persisting tobacco users, either by reducing amounts of toxins/carcinogens in smoke or reducing amounts of smoke inhaled ^[50], as a way of “minimizing harms and decreasing total morbidity and mortality without completely eliminating tobacco and nicotine use” ^[51].

36. Members may be aware that “light cigarettes”, were promoted as harm reduction products even though epidemiological studies suggest that there is no difference in lung cancer risk among people who smoke light or ultralight cigarettes compared with regular cigarettes ^[52]. Instead of reducing harm to smokers, “light cigarettes” enabled smokers to inhale nicotine dosages required to sustain their addiction (i.e. compensatory smoking), while yielding lower values when tested by smoking machines, such as the Federal Trade Commission (FTC) or International Organisation for Standardisation (ISO) methods, which are known to underestimate yields of smoke toxicants ^[53].

37. Gray and Henningfield (2004) demonstrated the technical and commercial feasibility of making cigarettes with substantially lower levels of benz-*a*-pyrene (BaP) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), compared with typical commercially available cigarettes, and this approach could potentially be adopted to lower the levels of carcinogenic substances in cigarette smoke, with a view to setting regulatory standards ^[50]. Although substantiated reduced exposure is probably indicative of reduced risk, Haussmann (2007) suggested that due to the changes of aerosol constituents in novel and conventional products and the lack of etiological understanding, there is no single smoke constituent, nor any combination thereof, that could be used to determine that reduced exposure would be linearly related to reduced risk ^[54].

38. Whilst it is possible that harm reduction principles could potentially be beneficial by reducing exposure of smokers who are addicted or unwilling to quit ^[55], it should not detract from cessation and prevention of initiation, which are the primary approaches to reducing tobacco related mortality and morbidity. This view was also clearly stated in the 2004 joint COT/COC/COM statement on tobacco toxicology. Members are asked to review the information that follows on selected studies.

Comparison/Modification of Products

39. There is a wide array of tobacco products on sale all over the world ranging from smokeless to combustible tobacco products, which employ different technologies. Most of these products contain tobacco as the main ingredient, while some do not. These products are so varied in their composition that they yield different types of mainstream and sidestream smoke for combustible products and different emissions for the smokeless products. There is evidence to suggest that there are a number of newly developed novel tobacco products on the market since the COT statement in 2004, A number of these products have been subjected to carcinogenicity assessment.

40. Biomarker studies in humans suggest that there is a spectrum of potential exposure across different types of tobacco or nicotine-containing products, with the greatest exposure associated with the conventional combustible tobacco products and the least exposure associated with therapeutic nicotine replacement products ^[55]. In the same paper, Zeller et al. (2009) suggested that, based on current knowledge, novel combustible products are unlikely to substantially reduce risk for disease because of the number of toxic combustion constituents associated with cigarette smoke, coupled with the fact that human studies on modified, reduced-toxicant cigarette products have shown only modest reductions of some toxicants, no significant reductions of other toxicants and increases in some other toxicants ^[55].

41. Currently, most of the novel products are not controlled within a proper regulatory framework and there is concern from a public health standpoint that the claims of reduced risk or exposure are not substantiated. These claims may pose a threat to tobacco control efforts

(cessation and prevention) and may lead to the recruitment of fresh smokers or re-initiation of ex-smokers who think that these products pose lower risks to health. There is thus a clear need for advice as to whether these products (new or existing products) actually reduce exposure and risk [55].

42. A number of investigators have published results indicating different tumour promoting potential between tobacco products. These studies have been reviewed below.

Potentially Reduced Exposure Products (PREPS)

43. Potentially Reduced Exposure Products (PREPS) are products designed or modified to potentially reduce exposure to toxic substances in tobacco smoke and are marketed as potential aids to reduce harm, risk or injury relative to conventional cigarettes. By changing or modifying the properties of the tobacco products, their smoking characteristics/performances can be controlled when machine or human smoked [56]. Currently, there is a wide range of these products. These include modified tobacco products that contain low levels of one or more toxins; cigarette-like devices, such as those that heat rather than burn tobacco; and oral non-combustible products, such as smokeless tobacco, that are modified to reduce exposure to specific toxicants [49].

44. There are a few clinical studies involving third generation Electrically Heated Cigarette Smoking Systems (EHCSS) and conventional cigarettes, using biomarkers of exposure, in adult smokers who were switched from conventional cigarettes to EHCSS [46, 57] for 8 days. These studies all demonstrated significant reductions (up to 97%) in exposure to several particulate and gas phase smoke constituents in controlled, clinical settings [46, 47, 57].

45. A similar study evaluated biomarkers of tobacco smoke exposure in adult smokers of conventional cigarettes using carboxyhemoglobin (COHb) as the biomarker for nicotine and carbon monoxide (CO) exposure, total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) for NNK exposure, and total 1-hydroxypyrene (1-OHP) for pyrene exposure and as a surrogate for polycyclic aromatic hydrocarbons, urinary 3-hydroxypropylmercapturic acid (3-HPMA) for acrolein exposure and S-phenylmercapturic acid (S-PMA) for benzene exposure. Nicotine and its five main metabolites in urine were calculated as nicotine equivalents (NE) [57]. Smokers were randomised to smoke similar tar EHCSS or conventional cigarettes over a 12-week period of unrestricted smoking. Nicotine equivalents were reduced by 33%, total NNAL was reduced by 63%, 1-OHP by 38%, carboxyhemoglobin by 23%, 3-HPMA by 25% and S-PMA by 49%. This study demonstrated significant reductions of studied biomarkers in EHCSS smokers relative to conventional cigarette smokers, in a normal life setting [45].

46. A review of published literature with charcoal-filtered cigarettes by Coggins and Gaworsky (2008) demonstrated reductions in the concentrations of gas-vapour phase constituents of mainstream smoke with corresponding reduced toxicological activity. However, this did not correlate with reduced smoking related disease. Charcoal filters provided no evidence for the reduction of particulate phase smoke components [58].

47. A study evaluated the potential of mainstream CSC of Eclipse (EC; a new cigarette which primarily burns rather than heats tobacco) to produce DNA adducts in lung, heart and skin tissue of dermally-exposed mice relative to the CSC of 1R4F (RF) Kentucky reference cigarette [59]. SENCAR mice were exposed to 30, 60 or 120 mg CSC from either EC or RF three times a week for 30 weeks and tissues were collected after 1, 4, 14 and 29 weeks for analysis of DNA adducts in lung, heart and skin tissues employing 32P-postlabeling (P1 nuclease modification). No radioactive zones were observed at any dose from the DNA of mice treated with CSC from EC or

control, whereas time and dose-dependent radioactive zones were seen in corresponding tissues of mice treated with RF condensate. The relative adduct labelling (RAL) values of lung, heart and skin DNA from RF CSC-treated animals were significantly greater ($p < 0.05$) than those of the control animals or EC CSC. The authors concluded that the results provide evidence that the smoke condensate from EC is significantly less genotoxic than the smoke condensate of the reference cigarette evaluated in this study.

48. Short-term *in vivo* analyses employing 5 – 7 weeks old SENCAR mice (2 weeks quarantine period) treated with 9, 18, 27, or 36 mg CSC from reference or prototype cigarettes, 3 times a week for 4, 8, or 12 weeks, demonstrated the utility of this short-term assay for the discrimination of CSCs with distinct tumour-promotion potentials [43]. The total tumours for the reference condensate were 11, 184 and 242 at concentrations of 9, 18, and 36 mg after 29 weeks, while the total tumours for the prototype (EC) condensate were 1, 11 and 31 at the same concentrations, respectively. RF CSC showed statistical and dose-dependent increases for all indices examined (tumours), whereas prototype CSCs showed significant reductions in the potential for inducing changes which the authors regarded as consistent with sustained epidermal hyperplasia and/or inflammation. The authors concluded that selected short-term analyses associated with sustained hyperplasia and/or inflammation are able to discriminate between CSCs with distinct tumour-promotion potentials.

49. Comparisons of the dermal tumour promoting potential of the CSC of EC and of RF using the two-stage MDPA (initiated with DMBA and promoted with CSC of either EC or RF), showed significant reductions in the endpoints assessed [39]. The standard Federal Trade Commission smoking conditions were used to generate smoke condensates and 5 – 7 week old (with a 15-day quarantine period) female SENCAR mice were exposed to 10, 20, or 40 mg EC CSC or RF CSC 3 times a week for 29 weeks. Significant increases in the number of microscopically confirmed tumour-bearing animals and the total number of microscopically confirmed dermal tumours were reported at all the doses of the RF CSC and the highest dose of EC CSC in DMBA initiated mice. Reductions of 83%, 93% and 67% in the number of tumour-bearing animals at the low-, mid- and high-doses were achieved for the EC CSC relative to the RF CSC. Reductions of 91%, 94% and 87% at the low-, mid- and high-dose, respectively, were also achieved for induction of total tumours for the EC CSC compared to the RF CSC, with a dose-dependent increase in total tumours at all doses of the RF CSC and the mid and high dose of EC CSC. The author considers the EC to have significantly lower biological activity than the RF as demonstrated by the significant reductions in dermal tumour promotion potential of EC compared to the RF CSC.

50. Although a few studies (as above) have either used the MDPA or other approaches to demonstrate significant reductions of up to 90% in toxicological and biological activities of modified products such as Eclipse, relative to conventional cigarettes [57], none of these modified products have been extensively analysed to assess their potential health impact. Hetch noted in 2005 that objective evaluation of the health effects of PREPs is critical [21], thus it would be worth evaluating if reported significant reductions in these studies translate to reduced risk of developing smoking-related diseases, as PREPs could potentially be beneficial to public health [49].

51. In 2008, comparative studies were undertaken, using the MDPA, to assess the tumorigenic potential of smoke generated from EHCSS relative to both commercially available cigarettes and the 2R4F Reference cigarette [60]. These products were smoked under multiple smoking regimens (including ISO and human-based alternative puffing regimens) and mice were divided into 5 groups for the dermal assay (Control, EHCSS - EC, Merit Ultima - CC1, Marlboro Ultra Lights - CC2, 2R4F - RF). CSC concentrations of 30, 60 and 90 mg/week (over a 26-week

exposure period) were employed in the exposed groups (EC, CC1, CC2 and RC). Normal body weight gains were recorded for mice during treatment with minor drops in mean weights in the exposed groups when compared with the DMBA/acetone control group (C). Survival at the end of the study was 92 - 98% in each of the groups, except for CC1-90 and the RF-90 groups, which had 80% and 86% survival, respectively. No tumours were observed in the control group, latency for tumour onset in CSC-exposed groups was 11 - 12 weeks, with the exception of the EC-30 and EC-90 mg/week groups in which the first tumours were observed in weeks 16 and 14, respectively. There were small differences between the tumour responses of the 30, 60, and 90mg/week CSC-exposed groups, which is indicative of no dose-response relationship, except for the EC-30 response, which had fewer tumours and shallower dose-response curve compared to the EC-60 and 90 responses. Data for tumour bearing animals and tumour multiplicities are presented in Table 1 below. The incidences in the EC-90 and CC1 and CC2 groups did not differ significantly from similar 2R4F exposure groups, while the incidences in the EC-30 and 60 groups were 22 and 72% lower than the incidences in similar 2R4F groups.

52. Toxicological evaluation of the results of this study suggest that EHCSS demonstrated reduced biological activity across several biological endpoints (dermal tumour onset, incidence, multiplicity, malignant proportion etc), as well as reduced smoke exposure in *in vitro* tests and in animals when compared with commercial branded cigarettes under the same conditions. The authors noted that these results are suggestive of reduced toxicity although the mechanistic links to the human disease process and the precise knowledge of the dose-response relationship with respect to smoke exposure are currently not known. They concluded that the significant reductions in biological activity demonstrated by EHCSS relative to conventional cigarettes in this study may suggest the potential for reductions in human smokers.

Table 1 – Data for dermal tumour bearing animals (TBA) and dermal tumour multiplicity (tumours per mouse, TM) at week 26

Group	TBA Data – Exposure Dose (Mean ± SE)			Nicotine in CSC (g/L)	TM Data - Exposure Dose (mean ± SE)		
	30mg/week	60mg/week	90mg/week		30mg/week	60mg/week	90mg/week
EHCSS (EC)	14.2 ^a ± 5.0	49.5 ^a ± 7.2	60 ± 6.9	15.9	0.32a ± 0.14	2.46 ± 0.53	2.38 ± 0.43
Merit Ultima (CC1)	66.4 ± 6.7	80 ± 5.7	72.2 ± 6.9	17.2	3.66 ± 0.66	5.20 ± 0.73	3.20 ± 0.64
Marlboro Ultra Lights (CC2)	54.2 ± 7.2	67.3 ± 6.7	74.8 ± 6.3	14.7	3.10 ± 0.65	3.82 ± 0.62	3.94 ± 0.57
2R4F (RC)	64.9 ± 6.8	68.4 ± 6.8	64.4 ± 7.0	14.7	2.56 ± 0.54	4.00 ± 0.65	2.76 ± 0.49

^a Statistically different from comparable 2R4F groups, p < 0.05.

53. A three-step model, proposed by Hatsukami et al, and based on the principles for evaluating PREPs described by the Institute of Medicine ^[51] could form the basis for assessing PREPs. This model involves pre-market (preclinical evaluation and clinical evaluation of exposure reduction and health effects, and market research on labelling and advertising) and post-market evaluation (population effect, harm reduction evaluation). It is presented in Annex 8 of this paper and forms a comprehensive strategy involving testing for toxicity or hazard identification, and preclinical cell and animal testing of PREPs ^[49]. It was further proposed that the evaluation of PREP-like products must consider both individual risk (i.e. assessment of toxin exposure addictiveness and disease risk among individuals) and population effects (i.e. assessment of tobacco use behaviour, such as the rate of uptake, cessation, and relapse) resulting from the introduction of the product, and the effects of product use on morbidity and mortality ^[49].

Novel Nicotine Delivery Systems

54. There are several of these products available on the market, mainly through the internet. These products range from products that contain only nicotine to products that contain, among other things, tobacco extracts. Despite the diverse range of products and similarity to conventional cigarettes in appearance, most of the products deliver nicotine in much the same way and the contents of these devices are not burnt but electrically heated. An example of such products is E-cigarette, which is described below.

55. E-cigarettes are cigarette like, electronically heated devices, comprising three major components: a battery, an atomiser/computer aided sensor, and a nicotine cartridge/inhaler. When the user sucks on the device, the atomiser/computer aided sensor activates a heating element which vaporises a small amount of liquid contained within the cartridge ^[61]. This “vapour” is inhaled by the user and the whole process imitates the process of smoking. E-cigarettes are freely available on the market, are marketed over the internet and, with time, are bound to filter through to many countries (including the UK). Annex 9 gives a list of some of the websites freely marketing these products, with claims of harm reduction indicated on most of these sites, and Figure 1 shows an example of an e-cigarette. As these products are relatively new, and there is limited information on them, it is important for the Department of Health to get guidance on how to deal with such products.



Figure 1- Example of an e-cigarette device

56. Apart from the information obtained from internet websites and manufacturers’ promotional products, which are not supported by scientific data, there is limited information available on e-cigarette-type products. Even though these products are marketed as safer alternatives to smoked cigarettes, studies conducted by the FDA have found toxic chemicals associated with tobacco, including known human carcinogens such as tobacco specific nitrosamines (TSNAs). The WHO does not consider these products to be legitimate cessation aids for smokers trying to quit because they have not been adequately tested, nor to be proven nicotine replacement therapy (NRT) products. At present, there is no evidence to confirm safety or efficacy and there are no peer-reviewed studies on these products. However, the WHO does not discount the possibility that they could be smoking cessation aids, albeit with appropriate clinical studies and toxicity analyses ^[62].

Smokeless Products

57. Smokeless tobacco products, which are tobacco-based products either sucked or sniffed but not burned, have been recommended as alternatives for combustible tobacco products, partly as a result of studies demonstrating that these products pose lower disease risks than conventional cigarettes ^[63]. These products vary in their composition and manufacturing process, thereby leading to different levels of potentially toxic constituents, and have been found to cause some types of cancers (oral and pancreatic) ^[64, 65]. The EU has therefore proposed that future regulation of smokeless tobacco products be science-based, with product quality characteristics of principle importance, as opposed to the requirements in the current Directive, which are based on the intended use of the product.

58. A pilot study, which compared nitrosamine smokeless tobacco products to medicinal nicotine, demonstrated that exposure to tobacco carcinogens such as TSNAs can be reduced when smokers switch to smokeless tobacco products ^[66]. However, a study found smokeless tobacco to be more effective in delivering dangerous carcinogens into the body than conventional cigarettes

[67]. Human clinical studies on the effects of smokeless tobacco products on biomarkers of exposure and effect are limited [55].

59 A summary of the carcinogenic studies reviewed by the EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) is provided at Annex 10. The report noted that sample groups used in experimental animal studies are small, which makes the interpretation of data difficult. There is no consensus on the role of smokeless tobacco products as harm reduction aids although there is agreement that they could be of value compared to conventional cigarettes [55].

60. Members are asked to consider the following questions:

- if decreased exposure to the harmful substances reported in the studies of products that purport to reduce harm have been shown to be associated with decreased carcinogenic potential?
- whether it has been shown that these products are associated with a lower risk of carcinogenicity than conventional cigarettes?
- what approaches could be used to effectively assess carcinogenic potential associated with the use of novel and existing products?

SECTION III – POTENTIAL EFFECTS OF INGREDIENTS, ADDITIVES, FLAVOURS ON TOBACCO PRODUCT EMISSIONS (ANNEX 5)

61. Due to the complexity associated with tobacco smoke, it is difficult to predict whether an added ingredient is contributing to harm to individuals or whether a product would pose an increased or reduced risk to human health when compared with conventional cigarettes. Although there are toxicological and chemical data to demonstrate that most of the additives or ingredients used in tobacco products are safe in the unburnt form, the chemical emissions of these ingredients and/or additives change when they are burned in tobacco products and when they interact with other compounds of tobacco or tobacco smoke [14]. Therefore, the most realistic way of assessing the contribution of an additive or ingredient to the overall toxicity of the tobacco product is within the matrix of the tobacco product [14].

62. A tiered testing strategy of a battery of tests (including a 30-week dermal tumour promotion assay of CSC and other tests) is usually employed to evaluate the toxicological activity of an ingredient in a tobacco product [68, 69, 70]. Many studies have evaluated the contribution of an ingredient/additive to the overall toxicity of mainstream smoke. Although these studies show minor changes in mainstream smoke composition due to the addition of an ingredient or additive, there are no significant differences in the toxicological activity of mainstream smoke, as a result of added ingredients [71, 72, 73, 74]. This is attributed to the high toxicological activity of tobacco, which swamps the responses of additives and or ingredients. A few of these studies are reviewed below.

63. Gaworsky (1999) evaluated the tumour promotion effect of 150 American cigarette flavouring ingredients, using the MDPA [75]. Female SENCAR mice (initiated with 50 mg of DMBA) were exposed 3 times a week for 26 weeks to either 10 or 20 mg of CSC from test cigarettes containing ingredient mixtures or from reference cigarettes prepared without added ingredients. The study found that tumour incidence, latency and multiplicity were related to the dose of the condensates, with a lower tumour incidence (approximately 50%), longer latency, and reduced tumour burden (4 tumours/tumour bearing animal) at the 10 mg CSC dose level.

Although the parameters measured varied between the test and reference condensates, these changes were within normal variation. Therefore, the authors concluded that the tumour promotion capacity of test CSC with added ingredients was not discernably different from reference condensates without added ingredients.

64. Comparisons of the toxicological potential of test cigarettes containing propane expanded tobacco (processed tobacco) and control cigarettes containing tobacco expanded with a traditional expansion agent (Freon-11) was made using several tests including the 30-week dermal tumour promotion study of CSC in SENCAR mice ^[68]. The study showed statistically significant differences in several smoke constituents between these two types of cigarettes. Nevertheless, the cigarettes containing propane expanded tobacco were not significantly different from Freon-11 containing cigarettes. Results of a similar study ^[69] between dry ice expanded tobacco and Freon-11 expanded tobacco also showed analogous biological activities.

65. Similarly, toxicological evaluation of cigarettes containing 9.5%, 18.5%, and 25% expanded shredded tobacco stems (ESTS) relative to control cigarettes containing no ESTS, using a range of tests including the 30-week MDPA in SENCAR mice, suggested that cigarettes with and without ESTS had a similar toxicological profiles ^[70]. In 2006, Stavanja et al. used a range of tests, including the 30 week MDPA in SENCAR mice, to investigate the possibility of using cigarettes containing 3%, 4% and 5% high fructose corn syrup (HFCS) as alternative tobacco casing materials to corn syrup/invert sugar (3%) ^[71]. The authors found no significant difference between the chemistry or biological activity of mainstream smoke or mainstream smoke condensate of the 3% corn syrup/invert sugar and the 3 or 5% HFCS with regard to the parameters investigated.

66. Another study, which compared the chemical and biological effects of cigarettes with and without banded cigarette paper technology (current marketed technologies), employing the tiered testing strategy including the 30 week MDPA, indicated that collective data on cigarettes with and without the banded technology showed similar toxicological profiles ^[76]. A similar toxicological assessment of cigarettes with and without diammonium phosphate (DAP) and urea relative to reference cigarettes showed that the addition of DAP and urea to cigarettes at the concentrations explored (up to 1% and 0.41% respectively) did not alter the biological activity compared to reference cigarettes without these ingredients ^[77].

67. Members are asked to comment on whether the approach used to test the contribution of individual or mixed ingredients or additives to the overall toxicity of tobacco products is suitable.

SECTION IV - WHETHER THERE ARE VALIDATED BIOMARKERS OF EFFECT FOR TOBACCO & ITS PRODUCTS THAT CAN BE USED TO PREDICT RISK, HARM, INJURY OR DISEASE (ANNEX 6)

68. The WHO noted in 2006 that there is insufficient understanding of how smoking causes disease to identify with confidence the rate limiting steps in the mechanistic pathways and, therefore, the changes that will reliably predict risk. It is also uncertain which changes are markers of tobacco use ^[78] (Annex 11). Although there are biomarkers which can measure the presence and extent of various systemic processes which may play a mechanistic role in disease occurrence, the diseases caused by cigarette smoking involve multiple processes and it remains unproven whether an alteration of a single process will reduce disease frequency.

69. Although biomarkers of effect need to complement biomarkers of exposure in relevant clinical studies, so far “no existing biomarkers have been demonstrated to predict tobacco-related disease [79]”. Acceptance of a given biological change as a biomarker of injury and risk requires validation that a change in the biomarker independently predicts a change in the frequency of disease occurrence [78] (Annex 11). This would suggest that this area needs to be explored further if it is to contribute to tobacco control efforts, which is consistent with WHO’s recommendation that better measures need to be developed to assess the overall toxicity of tobacco products [2].

70. There are several human clinical studies on biomarkers of exposure measured in body fluids [46, 47, 48, 57]. Urinary biomarkers of tobacco exposure, such as 1-HOP for the uptake of polyaromatic hydrocarbons, NNAL for the uptake of NNK, and cotinine for the uptake of nicotine, are all readily measured and have been applied in studies evaluating toxicant uptake by tobacco users [80]. Statistically significant reductions in biomarkers of exposure of up to 97% have been demonstrated in short-term (8 days) and long-term (12 months) clinical exposure studies in adult male and female smokers who were switched from their conventional cigarettes to electrically heated cigarettes [47, 48, 57]. The reductions in exposure in smokers of the electrically heated cigarettes are considered to be linked with statistically significant and patho-physiologically favourable changes in several cardiovascular risk factors [48]. Whilst biomarkers of exposure are useful for understanding uptake of specific smoke carcinogens and may play a crucial role in the evaluation of tobacco products, there is not much evidence of validated biomarkers of injury, harm, disease or risk [14] and no existing biomarkers have been demonstrated to be predictive of tobacco related disease [63].

71. Members are asked to comment on what needs to be undertaken to develop biomarkers of disease, harm or injury for tobacco products.

SECTION V – PROPOSED CANCER RISK INDICES (CRI) FOR THE PRIORITISATION OF CARCINOGENS IN CIGARETTE SMOKE (ANNEX 7)

72. The cancer risk index (CRI) approach to tobacco carcinogenesis is based on toxicological risk assessment of chemical analysis data and this approach has been used in literature to rank cigarette smoke carcinogens according to their potential hazards [4, 81]. However, there is a lack of adequate exposure and hazard data to evaluate carcinogenic risks in humans [14]. Thielen (2008) noted that, although there is some value in using available potency factors from acute and chronic exposure studies for the assessment of tobacco smoke as in the CRI approach, there are limitations given the special exposure conditions of human smoking [14]. There is no agreement on the approach to be taken to measure and characterise the risk associated with tobacco smoke.

73. The incremental lifetime inhalation cancer risk for specific chemicals based on a pack per day cigarette smoker has been investigated [82]. This study indicated that there are contributions from both the mainstream smoke vapour and particulate phase, and comparison of the quantity and potency of the individual carcinogenic constituents of both phases demonstrated that the potential carcinogenic contribution from the vapour phase is substantial. 1,3-butadiene was ranked the highest contributor due to its predicted high lifetime cancer risk.

74. Fowles and Dybing (2003), using multiple data sources, assessed the hazard associated with individual components of mainstream tobacco smoke measured by the ISO method and proposed cancer risk indices for some smoke constituents, including 45 known or suspected human carcinogens, based on the available data [4]. Cancer risk indices were calculated for 40

carcinogens, based on a cigarette a day, by multiplying cigarette yield data with published cancer potency factors assuming complete absorption of the chemicals in the reported yield. It was assumed that exposure would take place for an average of 60 out of a 75 year lifespan for the average person. Non-cancer risk indices were calculated for 17 compounds with known non-cancer health effects by dividing yield data with published reference exposure levels. The authors consider that this provides a means to prioritise hazards for reported chemical constituents of cigarette smoke and noted that the proposed indices could form the basis for an objective framework for prioritising carcinogens and other toxicant hazards in cigarette smoke, but not for the prediction of actual cancer risks.

75. Members are asked to comment on the utility of the CRI approach to prioritise carcinogens in tobacco smoke.

COC DISCUSSION

76. This draft discussion paper provides a review of research activities and approaches to evaluate the carcinogenic potency of existing and newly emerging products which have been employed in published literature since the 2004 joint COT/COM/COT statement. The review found, in particular, that the standardised MDPA has been used extensively for the comparison of the carcinogenic potential of different tobacco products and/or prototype products with reference cigarettes, and that the CRI approach to risk assessment has been proposed as an objective framework for the prioritisation of smoke carcinogens and other toxicants. In the light of this and the challenges associated with the toxicological testing of tobacco products, analysis of smoke constituents, lack of adequate mechanistic information on tobacco induced diseases, and limited understanding of the diseases associated with smoking tobacco and associated tobacco products, the key consideration is whether the approaches employed are adequate. An update of the statement is needed to provide the Committees' views on the new evidence and guidance to the Department of Health. This would update knowledge on the toxicological assessment of tobacco products and help to inform policy.

77. Evidence shows that tobacco manufacturers are using a relatively common testing strategy for the toxicological assessment of their products to demonstrate reduced exposure and, in some cases, reduced biological activity compared to conventional or reference cigarettes. However, to date, there has not been a critical evaluation to check the suitability of the approaches to the carcinogenic testing of tobacco products. The potential for combusting tobacco products to reduce exposure, risk, injury or harm remains unknown^[55] and there are no internationally agreed approaches to assess the hazard of tobacco products.

SPECIFIC QUESTIONS TO BE ADDRESSED BY THE COMMITTEE

78. Carcinogenic tests - Are existing approaches (e.g. mouse carcinogenicity studies with tobacco smoke and CSC) used to evaluate the carcinogenic potency of tobacco and its products suitable?

79. Harm reduction - What approaches could be used to effectively assess the carcinogenic potential of novel and existing products? Have these products been shown to have a reduced risk of cancer?

80. Added ingredients – Is the approach used to test the contribution of individual or mixed ingredients or additives to the overall toxicity of tobacco products suitable?
81. Biomarkers – What needs to be undertaken to develop biomarkers of disease, harm or injury for tobacco products, particularly in relation to cancer?
82. Cancer Risk Index – Is the CRI approach useful for the prioritisation of carcinogens in tobacco smoke?

**Secretariat/DH Toxicology Unit
November 2009**

List of Annexes

Annex 1: Committees on Toxicity, Carcinogenicity, Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COT, COC, COM). (2004). Joint Statement on the Re-assessment of the Toxicological Testing of Tobacco Products

Annex 2: Toxicology of Tobacco Products – Scoping Paper (TOX/2008/03) – Paragraphs 54 - 59

Annex 3: Tabulated Abstracts for Key Studies selected for Area 1 - Toxicity Tests

Annex 4: Tabulated Abstracts for Key Studies selected for Area 2 - Harm Reduction (Comparison of Products)

Annex 5: Tabulated Abstracts for Key Studies selected for Area 3 - Ingredients

Annex 6: Tabulated Abstracts for Key Studies selected for Area 4 - Biomarkers

Annex 7: Fowles, J. and Dybing, E. (2003). Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. *Tobacco Control* **12**, 424 -30

Annex 8: A three-step model for the evaluation of potential reduced exposure products (PREPs), with assessment recommended by an independent scientific panel or regulatory agency after each step

Annex 9: Summary of studies on carcinogenic effects in experimental animals after snuff application – an extract from the report of the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)

Annex 10: World Health Organisation (2006). The Scientific Basis of Tobacco Product Regulation. WHO Technical Report Series – No. 945.

Annex 11: Listing of selected papers submitted for the discussion paper on the Carcinogenicity Testing of Tobacco Products

- Witschi, H. (2004). A/J Mouse as A Model For Lung Tumorigenesis Caused By Tobacco Smoke: Strengths And Weaknesses. *Experimental Lung Research*. **31**(1), 3 -18.
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