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Design and chemical evaluation of reduced machine-yield cigarettes

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ABSTRACT

Experimental cigarettes (ECs) were made by combining technological applications that individually reduce the machine measured yields of specific toxicants or groups of toxicants in mainstream smoke (MS). Two tobacco blends, featuring a tobacco substitute sheet or a tobacco blend treatment, were combined with filters containing an amine functionalised resin (CR20L) and/or a polymer-derived, high activity carbon adsorbent to generate three ECs with the potential for generating lower smoke toxicant yields than conventional cigarettes. MS yields of smoke constituents were determined under 4 different smoking machine conditions. Health Canada Intense (HCI) machine smoking conditions gave the highest MS yields for nicotine-free dry particulate matter and for most smoke constituents measured. Toxicant yields from the ECs were compared with those from two commercial comparator cigarettes, three scientific control cigarettes measured contemporaneously and with published data on 120 commercial cigarettes. The ECs were found to generate some of the lowest machine yields of toxicants from cigarettes for which published HCI smoke chemistry data are available; these comparisons therefore confirm that ECs with reduced MS machine toxicant yields compared to commercial cigarettes can be produced. The results encourage further work examining human exposure to toxicants from these cigarettes, including human biomarker studies.

1. Introduction

Tobacco smoke is a complex, dynamic, mixture of more than 5000 identified constituents (Rodgman and Perfetti, 2009) of which approximately 150 have been documented as toxicants (Fowles and Dybing, 2003; Green et al., 2007). The toxicants are present in the mainstream smoke (MS) inhaled by a smoker and are also released between puffs as constituents of sidestream smoke (SS).

In 2001 the Institute of Medicine (IOM) reported that, since smoking related diseases were dose-related, and because epidemiologic studies show reduction in the risk of smoking related diseases following cessation, it might be possible to reduce smoking related risks by developing potential reduced-exposure products (PREPs). These they defined as (1) products that result in the substantial reduction in exposure to one or more tobacco toxicants and (2), if a risk reduction claim is made, products that can reasonably be expected to reduce the risk of one or more specific diseases or other adverse health effects (Stratton et al., 2001). To date, no combustible cigarette product has been shown to meet the general requirements outlined by the IOM.

The IOM and other groups (Life Sciences Research Office (LSRO), 2007; World Health Organization (WHO), 2007) describe a number of stages of activity which are likely to be required for a combustible tobacco product to be recognised as a PREP; however, the detailed approach and stages required to provide relevant data have yet to be agreed amongst the scientific community. For example, some groups have proposed MS yield limits for specific smoke toxicants (Burns et al., 2008) and others have suggested that biomonitoring should play a role in this assessment (Hecht et al., 2010). Recently Hatsukami et al. (2012) described a sequence of activities designed to assess modified risk tobacco products, starting with pre-human studies involving constituent yield analysis (of the kind described in this paper) prior to pre-market human studies and post-market studies. The USA FDA is also currently considering approaches for the Scientific Evaluation of modified risk tobacco product (MRTP) applications (FDA, 2011).

Abbreviations: BT, blend treatment (tobacco); CA, cellulose acetate (filter); CR20L, amine-functionalised resin; dwb, dry weight basis; ECs, experimental cigarettes; HCI, Health Canada Intense (machine smoking conditions); IOM, the Institute of Medicine of the USA; ISO, International Organization for Standardization; LOQ, limit of quantification; LSRO, the Life Sciences Research Office; MS, mainstream smoke; NAB, N'-Nitrosoanabasine; NAT, N'-Nitrosoanatabine; NFDPM, nicotine-free dry particulate matter, sometimes called "tar"; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N'-Nitrosonornicotine; PAH, polycyclic aromatic hydrocarbon; PREP, potential reduced-exposure product; RTP, reduced toxicant prototype; RMYP, reduced machine-yield prototype; SS, sidestream smoke; TSNA, tobacco-specific nitrosamines; TSS, tobacco-substitute sheet; WHO, World Health Organization; wwb, wet weight basis.

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From a cigarette design and manufacturing viewpoint, we propose the following step-wise approach to exposure assessment with modified tobacco products.

The first stage in the design of a cigarette-based PREP would involve the development of technologies which reduce the yields of smoke toxicants. Experimental cigarettes (ECs) would be assembled using these technologies and then assessed for their toxicant yields using smoking machines; comparison to relevant control and reference products would indicate the effectiveness of the cigarette design in generating reduced yields of toxicants. Those ECs that are found to reduce smoking machine measured yields of smoke toxicants, in comparison to reference products, are termed "reduced machine-yield cigarettes".

A second stage of testing is necessary to establish the ability of a reduced machine-yield cigarette to reduce smokers' exposure to toxicants, under real-world use conditions. Those that successfully demonstrate reductions in smokers' exposure to toxicants are termed "reduced toxicant prototypes". A reduced toxicant prototype designation is insufficient to satisfy the IOM's definition of a PREP and further assessment would be required to demonstrate that these cigarettes can reasonably be expected to reduce the risk of one or more specific diseases or other adverse health effects.

Over many years there have been numerous attempts to develop cigarettes with reduced machine yields of toxicants. These have been reviewed in depth on a number of occasions (e.g. NCI, 1968; Wnyder and Hoffmann, 1979; Gori and Bock, 1980; Gori, 2000; Hoffmann et al., 2001; Proctor et al., 2003; Baker, 2006a,b; Rees and Connolly, 2008; O'Connor and Hurley, 2008).

Technological developments for reduction in yields of smoke toxicants have included modified agricultural and curing practices (O'Connor and Hurley, 2008), selective removal of tobacco constituents (Gori and Bock, 1980), the substitution of tobacco with alternative, diluent materials (Sittig, 1976), addition of chemical species to the tobacco blend (Hatsukami et al., 2004) and selective reduction of cigarette smoke toxicants through use of filter materials such as cellulose acetate (NCI, 1968), resins (Horsewell, 1975), and activated carbon (Kensler and Battista, 1963; Tokida et al., 1985; Norman, 1999; Rouquerol et al., 1999; Laugesen and Fowles, 2006; Rees et al., 2007; Polzin et al., 2008; Hearn et al., 2010; Branton et al., 2009; Branton and Bradley, 2010).

A number of these technological approaches have been employed in commercial or test marketed cigarettes such as ADVANCE (Breland et al., 2003,2006; Advance, 2001; Counts, 2002), OMNI (Hatsukami et al., 2004; Counts, 2002), and Marlboro Ultra-Smooth (Laugesen and Fowles, 2006; Rees et al., 2007).

Alternative approaches to conventional cigarettes have included devices that heat but do not burn tobacco, such as PREMIER (RJ Reynolds, 1988), ECLIPSE (Eclipse Expert Panel, 2000), ACCORD (Holzman, 1999; Patskin and Reininghaus, 2003) and HEATBAR (Rees and Connolly, 2008). Further descriptive details of these products were found at the website Tobaccoproducts.org (Tobaccoproducts, 2011).

However, despite the range of approaches described above, to date none of these attempts have led to a commercially successful PREP.

In recent papers, in an extension to previous published studies, we have described four different individual technological approaches to the reduction of toxicants in cigarette smoke, two of which modified the tobacco blend (McAdam et al., 2011; Liu et al., 2011), and two of which modified the cigarette filter (Branton et al., 2011a,b). The two tobacco blend technologies, a tobacco-substitute sheet material (TSS) and a tobacco blend treatment (BT), acted to reduce the generation of toxicants at source within the burning cigarette. The two filter technologies, an amine functionalised resin material (CR20L) and a high activity, polymer-derived, carbon adsorbent, acted to remove volatile species from the smoke stream after formation. The technologies described in those reports are summarised in Section 2.1 below.

This current paper describes the design of three ECs made using combinations of these blend and filter technologies. The goal of the current work was to assess whether the technologies could be combined into ECs which reduce machine yields of toxicants in comparison to commercial products, and have the potential to reduce exposure of smokers to toxicants as a consequence of human smoking. Four considerations shaped the approach taken in the development of these ECs: first, a lack of consensus in the scientific community over which toxicants in smoke are priorities for reduction; second, uncertainty over the extent of reductions necessary for a biologically substantial effect; third, a desire to avoid inadvertent and substantial increases in yields of any toxicants when changing cigarette design to make ECs; and fourth, the need to maintain consumer acceptability when reducing overall yields of smoke constituents – a principle recognised by Wnyder and Hoffmann (1979).

In terms of priorities for reduction, a major unresolved challenge in understanding the causes of smoking-related diseases is identification of the key smoke toxicants mechanistically involved. Without this detailed knowledge, modifications to cigarette design cannot precisely target the smoke constituents involved in driving disease processes. However, even if this knowledge were available, with few exceptions, it is unlikely that specific smoke constituents or chemical classes could be entirely eliminated from MS, and a more pragmatic approach is to develop cigarettes with substantially reduced overall smoke toxicant yields.

Testing the ECs under a variety of smoking machine conditions and analysing the yields of smoke constituents on a per cigarette basis and as a ratio per milligram of nicotine yield, permits comparisons with relevant commercial comparator cigarettes, and also to a wide range of products reported in the literature. The results presented in this work demonstrate that the development of combustible reduced machine-yield cigarettes is feasible. Further studies on these ECs to assess their ability to reduce exposure to toxicants in smokers have been conducted and will be reported separately.

2. Materials and methods

2.1. Design of experimental, control and comparator cigarettes

The approach taken was to develop ECs that gave reductions in a wide range of machine smoked yields of toxicants, without overall increases in MS emissions. This was considered the most appropriate strategy for the initial stages in combustible PREP development, bearing in mind the constraints discussed above. Consequently, the ECs described here were constructed from combinations of blend and filter technologies that were developed to reduce specific chemical classes of smoke toxicants or their precursors in tobacco (Table 1). For each EC individual tobacco grades with low tobacco-specific nitrosamine (TSNA) and metal contents were selected and blended to provide a low toxicant starting point for the design of experimental cigarettes.

The BT process was described in detail by Liu et al. (2011). Briefly, the tobacco blend is subjected to an aqueous extraction step and the extract is subsequently passed through two stages of filtration to remove polyphenols and proteins. The residual tobacco solids are treated with protease to remove insoluble proteins. After washing and enzyme deactivation, the tobacco solids and filtered aqueous extract are re-combined. The BT process results in reduced smoke yields of phenolics, aromatic amines, HCN, and a number of other nitrogenous smoke constituents; however, there are also increases in the yields of formaldehyde and isoprene (Liu et al., 2011).

Table 1
Technologies used in the construction of experimental cigarettes.

Technological application	Cigarette component	Description	Potential toxicant reduction	Reference
Tobacco substitute sheet (TSS)	Blend	Tobacco-substitute sheet reducing tobacco combustibles and giving glycerol dilution of smoke	Whole smoke	McAdam et al. (2011)
Tobacco blend treatment (BT)	Blend	Protease treated tobacco, reducing protein nitrogen and polyphenols in the blend	Phenolics and nitrogen-based constituents: aromatic amines, NAB, NAT, NNK, NNN	Liu et al. (2011)
Amine-functionalised Resin Beads (CR20L)	Filter	Amine group functionalised resin included in filter stage	HCN, HCHO, acetaldehyde and other carbonyls	Branton et al. (2011b)
High activity carbon	Filter	Polymer-derived, spherical carbon beads included in filter stage	Vapour phase constituents	Branton et al. (2011a)

The TSS used in the current study was made from calcium carbonate, bound with sodium alginate, loaded with glycerol (approximately 12.5%) and coloured with caramel E150a, as described in detail by McAdam et al. (2011). Incorporation of the TSS into a tobacco blend reduces the quantity of tobacco in a cigarette, thereby diminishing the overall potential for the cigarette to generate toxicants. The TSS also contains glycerol and, when heated, the TSS releases glycerol into the smoke stream contributing to the total amount of particulate smoke, measured as nicotine-free dry particulate matter (NFDPM, also known as "tar"). As most cigarettes are designed to meet a specific NFDPM yield value, incorporation of glycerol into the smoke stream effectively results in a reduced contribution of the tobacco combustion products to the overall NFDPM value: this process is termed "dilution." The incorporation of TSS into cigarettes results in reductions in a wide range of smoke constituents, including both particulate and vapour phase toxicants (McAdam et al., 2011).

The polymer-derived, high activity carbon granules used in the dual and triple stage filters was obtained from Blucher GmbH, Germany. It possesses a pore structure different from the carbon commonly used in commercial cigarettes, which is typically derived from coconut shells. As a result it has superior adsorption characteristics for a range of volatile smoke toxicants, as described in detail by Branton et al. (2011a).

CR20L is a specific grade of a commercial ion-exchange resin (CR20, Diaion, Mitsubishi Chemical Corporation, Tokyo). It is an amine functionalised resin bead material which can also be incorporated into cigarette filters. In comparison to filters containing conventional carbon, CR20L offers superior reductions for HCN, formaldehyde and acetaldehyde. However, carbon is more efficient than CR20L in removing other volatile constituents from a smoke stream. The characterisation and use of CR20L in ECs was described in detail by Branton et al. (2011b).

Cigarettes were constructed from these technologies with ISO NFDPM target yields of 1 and 6 mg.

Three scientific control cigarettes were also manufactured to allow an evaluation to be made of the contribution of the filter technologies to toxicant reductions from ECs. Two commercial comparator cigarettes, a 1 mg ISO design and a 6 mg ISO design, were also used in these studies. Comparisons with commercial brands were conducted because realistic control cigarettes are required to assess the success with which the different toxicant reduction technologies can be brought together into a coherent and consumer acceptable cigarette design. Also, the use of commercial cigarettes allows examination of the extent with which toxicant reductions can be realised against real-world cigarettes, rather than scientific controls. Finally, use of commercial reference products allows relevant comparisons to be made of sensory acceptability and human exposure under real-world use. The commercial comparator products were of similar ISO machine smoked toxicant yields to the market leading brands at 1 mg and 6 mg (ISO) from Germany in 2007–8. BAT group comparator cigarettes were chosen, rather than the actual market leading brands, in order that full information was available on blend and cigarette design characteristics, and to allow product masking to be conducted for human sensory and exposure evaluations. Samples of both commercial cigarettes were therefore manufactured specially for these studies, without brand marking or other identification, in order to support human smoking studies (described elsewhere).

2.2. Specifications for experimental, comparator and control cigarettes

Common features were used in the design of the ECs: all were constructed to the same basic dimensions, of 84 mm cigarette length (a 57 mm tobacco rod plus a 27 mm filter), 24.6 mm circumference and the filters were all based on cellulose acetate (CA) fibres plasticised with triethyl citrate. Tobacco grades with low TSNA and metal contents were identified and combined for the tobacco blends used in these prototypes. Three different experimental cigarettes were prepared, and the design features of the three ECs are summarised and compared with control cigarettes and commercial comparators in Table 2 and described below.

The experimental cigarette BT1, combined a Virginia style tobacco blend containing BT treated tobacco (75.4% treated Virginia tobacco, with 4.3% Oriental tobacco and 20.3% untreated Virginia tobacco) with a filter containing a CR20L stage (to reduce formaldehyde, acetaldehyde and HCN yields) and a polymer-derived, high activity carbon filter containing stage (to reduce yields of isoprene and other volatile toxicants). The target NFDPM yield from this cigarette was 1 mg under ISO machine smoking conditions. The experimental cigarette TSS1 was also designed to yield 1 mg of NFDPM under ISO smoking machine conditions and was based on a US-blended style containing TSS (a blend of Virginia, Burley and Oriental tobaccos, with the inclusion of approximately 20% TSS) and the same filter used in experimental cigarette BT1. The experimental cigarette TSS6 also used 20%TSS in a different US style blend, and was designed to give an NFDPM yield of 6 mg under ISO machine smoking conditions. A different filter construction was used with this cigarette: a dual segment filter containing 80 mg of the high activity carbon interspersed amongst CA fibres adjacent to the tobacco rod with a CA stage at the mouth end.

The commercial comparator cigarette CC1 contained a US-blended style of tobacco, including some Maryland tobacco. The commercial comparator cigarette, CC6, was also a typical US-blended cigarette but with a different blend to CC1. The design features of the three ECs are summarised and compared with control cigarettes and commercial comparators in Table 2. Both commercial comparator cigarettes used single stage cellulose acetate filters. The three "scientific control" (SC) cigarettes had identical construction to the relevant experimental cigarettes BT1, TSS1 and TSS6, with the exception that the filter used in each control cigarette was a single stage 27 mm CA filter without additional filter adsorbent media.

Table 2 shows that the cigarette constructions of BT1 and CC1 were very similar, with well matched filter ventilation and paper permeability. There were differences in tobacco density (BT1,

Table 2
Cigarette construction details.

Cigarette Code	CC1	SC-TSS1	TSS1	SC-BT1	BT1	CC6	SC-TSS6	TSS6
Tobacco								
Blend technology	Conventional	TSS	TSS	BT	BT	Conventional	TSS	TSS
Blend addition	–	20% TSS	20% TSS	75% BT	75% BT	–	20% TSS	20% TSS
Density[a] (mg/ml)	217	216	216	247	247	226	235	235
Blend weight (mg)	570	572	572	654	654	605	622	622
Moisture (%)	13.5	11.4	11.4	13.5	13.5	14.1	11.4	11.4
Filter								
Format	Mono	Mono	Triple	Mono	Triple	Mono	Mono	Dual
Total Length (mm)	27	27	27	27	27	27	27	27
Mouth end stage	CA	CA	CA 7 mm	CA	CA 7 mm	CA	CA	CA 15 mm
Middle stage	–	–	CA 10 mm + 20 mg CR20L	–	CA 10 mm + 20 mg CR20L	–	–	–
Tobacco end stage	–	–	CA 10 mm + 60 mg C	–	CA 10 mm + 60 mg C	–	–	CA 12 mm + 80 mg C
Total filter weight (mg)	244	234	310	234	310	197	207	292
Filter PD (mmWG)	86	97	97	91	91	85	109	109
Filter ventilation (%)	78	81	81	79	79	52	46	46
Cigarette								
Total weight (mg)	856	842	918	924	1000	844	865	950
Paper permeability (CU)[b]	50	50	50	50	50	50	50	50
ISO NFDPM target (mg)	1	1	1	1	1	6	6	6

BT, blend treatment; C, high activity, polymer-derived carbon; CA, cellulose acetate; CU, CORESTA units; NFDPM, nicotine-free dry particulate matter ("tar"); PD, pressure drop; TSS, tobacco-substitute sheet.

[a] Density calculated at 13% moisture.

[b] CU = volume of air (cm^3) passing through 1 cm^2 paper min^{-1} at constant pressure difference of 1.0 kilopascal.

247 mg/ml and CC1, 217 mg/ml) and filter pressure drop (BT1, 91mmWG and CC1 86mmWG), with BT1 higher than CC1 for both parameters. The cigarette constructions of TSS1 and CC1 were also very similar. The filter pressure drop was higher from TSS1 than the commercial comparator (97 and 86 mm WG respectively). For TSS6 and CC6 less filter ventilation was used than with the 1 mg (ISO) products. Comparing the two 6 mg (ISO) products gave higher tobacco densities (TSS6 235 mg/ml; CC6 226 mg/ml), pressure drop values (TSS6 109mmWG; CC6 85mmWG) and lower filter ventilation (TSS6 46%; CC6 52%) from TSS6 than from CC6.

2.3. Tobacco blend analysis

A 100 g sample of each tobacco blend was split into five separate aliquots and each aliquot was processed separately. All samples were ground using a centrifugal mill with 0.25 mm mesh and titanium accessories. For metals content, samples of 0.25 g ground tobacco were digested with 6 ml nitric acid (Fluka Analytical, 'trace grade') in a pressurised vessel with microwave heating. A reference tobacco blend was digested as a separate control with each sample run. Metal content was determined by inductively coupled plasma – mass spectrometry, using reagent blanks and reference calibrations for each metal.

For TSNAs, 0.5 g of ground tobacco was extracted with 20 ml methanol (HPLC grade, Rathburn Chemicals, Wakerburn, UK) and sonication for 30 min and then centrifuged for 5 min at 4600*g*. An internal standard of deuteriated mixed TSNAs (Kinesis, Cambs., UK) was included with each extraction. From the supernatant, approximately 1 ml was transferred to an autosampler vial for analysis by liquid chromatography using a C18 column (Phenomenex, Macclesfield, UK), a mobile phase of 5 mM ammonium acetate (ReagentPlus grade, Sigma–Aldrich, St. Louis, USA) with a gradient of acetonitrile (HPLC grade, Rathburn Chemicals, Wakerburn, UK) and tandem mass spectrometry detection. A reference tobacco blend was extracted with each set of samples.

2.4. Smoke chemistry analysis

Prior to smoke chemistry analysis, cigarettes were conditioned according to the specifications of ISO 3402 (1999). Routine chemical analyses were performed according to the smoking conditions specified in ISO 4387 (2000) (i.e., a 35 ml puff of 2 s duration taken every 60 s, abbreviated as 35/2/60) and ISO 3308 (2000) which was developed for NFDPM and nicotine analysis.

Approximately 150 smoke constituents have been described as toxicants (Fowles and Dybing, 2003; Green et al., 2007) and some regulatory authorities have requested yield data on a subset (approximately 40) of them. Yield restrictions for some of these toxicants have been proposed (Burns et al., 2008) along with an approach to their biomonitoring (Hecht et al., 2010). For these reasons and in order to characterise the ECs more precisely, the MS yields of an extended range (47 analytes) of smoke constituents were measured. The other, approximately 100, toxicants not examined in this work were not measured due to the lack of available validated analytical methods. However, a wider screen of smoke constituents from cigarettes containing the tobacco substitute sheet is reported by McAdam et al. (2011). Values for benzo(a)pyrene yields were obtained twice, through a direct measure and also as part of a suite of polycyclic aromatic hydrocarbons (PAHs).

Slight modification to the ISO smoking parameters was required for the measurement of some analytes, as described by Gregg et al. (2004) and the current methods are available from British American Tobacco, on the Internet (British American Tobacco, 2011). Measuring the yield of smoke constituents from a smoking machine does not mimic human smoking yields and so all cigarettes were tested under a range of different smoking machine settings in order to allow machine yield performance to be assessed over a wide range of possible smoking conditions. These modified smoking conditions are described in Table 3.

Sidestream smoke (SS) yields were also measured as described by Health Canada (1999) but only under ISO smoke generation parameters and for a wider range of smoke constituents. The SS testing was conducted by Labstat International ULC.

2.5. Statistical analysis

Statistical comparisons of tobacco blends and smoke yields between different cigarette types were conducted using a two-tailed, unpaired, Student's *t*-test, performed with Minitab v16. Any *P* value >0.05 was considered to be non-significant (NS).

Analytical uncertainty for mainstream smoke constituents was calculated by analysis of seven independent replicates of the

Table 3
Smoking machine parameters.

Smoking Regime	Abbreviation	Puff volume (ml)	Puff duration (s)	Puff interval (s)	Filter vent blocking (%)
ISO 3308/4387	ISO	35	2	60	0
Health Canada Intense	HCI	55	2	30	100
Health Canada Intense-filter vents open	HCI-VO	55	2	30	0
ISO WG 9 intense option B	WG9I	60	2	30	50

Kentucky Reference Cigarette 2R4F. An expanded uncertainty value (U) was calculated for the methods in this matrix according to EURACHEM/CITAC (2000), with a coverage factor, $k = 2$, giving an approximate 95% confidence interval. In this paper, where differences in constituent yields between products are presented, the expression of the expanded uncertainty (U) value as a percentage of the mean value for the reference cigarette facilitates the interpretation of whether differences between product yield mean values fall within or outside the expanded uncertainty for the method.

For comparisons of individual smoke constituent yields across published studies, mean values from published data sets (Health Canada, 2004; Counts et al., 2005; Australian Government, Department of Health and Ageing, 2002) were examined for normal distribution using the Anderson Darling statistic. Percentile distributions within the toxicant data were calculated using an empirical cumulative distribution analysis within Minitab v16.

3. Results and discussion

Testing of the ECs was conducted in order to examine the actual performance of the ECs from a blend and smoke chemistry perspective, by quantifying the MS constituents and specific toxicant yields under a number of machine smoking conditions.

The SS emissions from the ECs were also measured using the ISO smoking profile. The tests were conducted on a comparative basis with two commercial cigarettes and with three scientific control cigarettes. As a final step, the overall performance of the ECs was assessed both in comparison to previously published MS yield data on cigarettes from several countries and as ratios of specific toxicant yields to nicotine yields.

3.1. Mainstream smoke constituent yields

The yields of the major smoke constituents (NFDPM, nicotine and CO) and glycerol under four smoking machine conditions are shown in Table 4. Glycerol measurements are included in this table because it has been incorporated into the tobacco-substitute sheet used in the ECs TSS1 and TSS6, to dilute other smoke constituents in the smoke particulate phase.

Table 4 shows that BT1 and CC1 were well matched across the four smoking regimes for MS NFDPM and nicotine yields, but that BT1 had lower CO yields than CC1. TSS1 and CC1 were well matched across the four smoking regimes for NFDPM and nicotine yields but TSS1 had lower CO yields than CC1. The higher glycerol yield from TSS1 is consistent with the intended dilution effect due to the glycerol content of TSS. The MS NFDPM and nicotine yields from TSS6 and CC6 were well matched across the four smoking regimes, other than higher CO yields from CC6 and the expected higher glycerol yields from TSS6.

For NFDPM and these smoke constituents the yields measured followed the same rank order based on smoking machine conditions: ISO < HCI-VO < WG9I < HCI. The yield differences between the different regimes were substantially greater with the 1 mg products than with the 6 mg products, as the level of ventilation was higher and the impact of ventilation blocking for the WG9 and HCI regimes is therefore more profound for the 1 mg products.

The 47 toxicants quantified in this work were also measured under all of the smoking machine conditions shown in Table 3, except that data for the ECs TSS1 and BT1 under ISO machine smoking conditions were not collected because preliminary runs showed the yields of many constituents to be below the LOQ for the methods. The yields measured under all smoking machine conditions are available as a Supplementary table to this paper (Table S1 available online). The machine smoked yields of these toxicants generally followed the rank order noted for NFDPM, nicotine and CO shown in Table 4 and so, for the remainder of this paper, only the yields obtained under HCI conditions are described.

The use of the HCI smoking regime in this work represents the strictest test of the ECs and the commercial comparator cigarettes. Although these smoking conditions inactivate a design feature used in the ECs and commercial cigarettes (filter ventilation), they address criticism of the machine yield values obtained from ventilated cigarettes (US National Cancer Institute, 2001).

3.1.1. Metal and TSNA yields

Two groups of toxicants included on regulatory lists are the metals and the tobacco specific nitrosamines (TSNAs). Both these groups of toxicants are primarily affected by the tobacco blend used in cigarette manufacture and so careful blend selection is a major contributor to their reduction in smoke (Baker, 1999). The chemical analysis of blend metals and TSNAs are described in Table 5 and their MS yields under HCI smoking machine conditions are shown in Table 6. The yields are discussed for each EC in Sections 3.1.2.1–3.1.2.3 below.

3.1.2. Other toxicant yields

Measured smoke constituent yield comparisons between ECs and commercial cigarettes, under HCI smoking machine conditions, are shown in Table 7. The yields are discussed for each EC in Sections 3.1.2.1–3.1.2.3 below.

3.1.2.1. BT1. Measurement of blend chemistries (Table 5) showed the blend arsenic and chromium contents of BT1 were statistically significantly higher than the commercial cigarette CC1 ($P < 0.01$), lead and nickel contents of the BT1 blend were lower ($P < 0.01$), and blend mercury contents from all cigarettes were <0.05 μg/g. The MS yields for metals from BT1 were comparable to or lower than the yields from CC1, except that the arsenic and mercury yields were higher (Table 6). The higher arsenic yield may be explained by the higher blend content of this metal (Table 5). A similar comparatively high mercury yield was also found with control cigarette SC-BT1 (the same construction as BT1 except for a cellulose acetate filter). Therefore it can be concluded that the higher mercury yield from BT1 than CC1 arises from a tobacco combustion source.

Blend nitrosamine content of BT1 was lower than US-blended commercial comparator CC1, as has been seen previously in comparison of Virginia and US-blended cigarettes (Gregg et al., 2004; Counts et al., 2005). The MS yields of nitrogenous constituents were expected to be lower from BT1 than from CC1 for two reasons: first, the tobacco treatment reduces precursors of nitrogenous smoke constituents; and, second, Virginia style tobaccos typically generate lower yields of nitrogenous smoke constituents than US-blended cigarettes (Gregg et al., 2004). Measurement of the yields of nitrogenous compounds showed the anticipated differences: yields of the TSNAs were statistically significantly

Table 4
Mainstream smoke yields using different smoking machine conditions.

Cigarette	Design NFDPM Yield (ISO)	ISO (mg/cigarette)					HCI-VO (mg/cigarette)					WG9I (mg/cigarette)					HCI (mg/cigarette)				
		NFDPM	Nicotine	CO	Glycerol	% Glycerol	NFDPM	Nicotine	CO	Glycerol	% Glycerol	NFDPM	Nicotine	CO	Glycerol	% Glycerol	NFDPM	Nicotine	CO	Glycerol	% Glycerol
CC1	1	1.2	0.1	2.3	0.2	18	5.5	0.6	8.7	0.6	10	9.8	0.9	15.2	1.1	11	18.9	1.3	23.8	1.4	7
TSS1	1	1.0	0.1	1.0	0.2	18	5.3	0.5	6.1	0.7	13	9.8	0.9	11.1	1.4	14	17.3	1.2	18.2	2.0	11
BT1	1	1.2	0.1	1.0	0.1	9	5.5	0.5	4.7	0.6	10	10.2	1.1	9.9	0.7	7	17.8	1.5	18.1	1.0	6
CC6	6	5.0	0.5	5.8	0.4	9	15.2	1.2	16.4	1.1	7	21.2	1.5	21.9	1.3	6	24.4	1.6	24.6	1.4	6
TSS6	6	5.3	0.4	4.9	0.9	18	15.1	1.2	13.8	2.4	16	16.8	1.3	15.4	2.8	17	20.7	1.4	18.5	3.0	15

Yield values given to 1 decimal place;%values rounded to the nearest whole number.

(83–96%) lower from BT1 than from CC1 (Table 6); aromatic amine yields from BT1 were 26–57% lower than from CC1 (Table 7); and the yields of other nitrogenous compounds from BT1 were also substantially lower (HCN by 82%, NO by 79%, ammonia by 75%, pyridine by 97%, quinoline by 67% and acrylonitrile by 69%) than the respective yields from CC1 (Table 7). These data confirm that the blend selection, use of the BT process (and incorporation of CR20L in the filter in the case of HCN yields) produced the expected lower yields of toxicants from the ECs.

The BT process also reduces blend polyphenol levels and so reductions in MS phenols yields would be expected; however, higher yields of phenolics are generally expected from Virginia style products than from US-blended products (Gregg et al., 2004) and this tobacco type difference could mitigate any reductions from the BT process. Comparison between phenolic compound yields from CC1 and from BT1 showed higher catechol and hydroquinone yields from BT1 (Table 7).

The BT process does not influence benzo(a)pyrene yields (Liu et al., 2011) and analysis of PAHs in the current study showed comparable yields from BT1 and CC1 for fluorene, phenanthrene, pyrene and benzo(a)pyrene.

Lower carbonyl yields (26–74% lower) were obtained from cigarette BT1, apart from formaldehyde, which showed a 41% higher yield from BT1. The volatile hydrocarbon yields from BT1 were lower, with a range from 66 to 94% for benzene, toluene, styrene and naphthalene, when compared to the respective constituent yields from CC1; however, the 1,3-butadiene yield was 35% higher from BT1 compared to CC1. Most of the observed differences in volatile constituent yields are consistent with the use of a high activity adsorbent in the filter of BT1. Formaldehyde yields are driven in part by sugar levels (Baker et al., 2006), which are normally higher in Virginia blends than in US blends (Baker, 2006a,b). Formaldehyde yields are also increased by the blend treatment process (Liu et al., 2011). Hence the higher formaldehyde yields from BT1 are understandable on the basis of knowledge of formaldehyde generation in cigarettes. The higher yield of 1,3-butadiene from BT1 was unexpected from the anticipated effect of the high efficiency filter and lack of reported sensitivity of 1,3-butadiene yields to the tobacco treatment process (Liu et al., 2011). However, the increased 1,3-butadiene mainstream yields from BT1 were confirmed by subsequent repeated analysis, and as described in Section 3.5 sidestream 1,3-butadiene yields were also found to be higher (24%) from BT1 than from CC1.

3.1.2.2. TSS1. The overall blend metal content was higher in TSS1 than in CC1 for some metals: arsenic ($P < 0.01$), chromium ($P < 0.01$) and nickel ($P < 0.05$); lower for cadmium content ($P < 0.01$) and not different for other metals (Table 5). The TSS contains a high proportion of calcium carbonate from non-synthetic sources, which would contribute to the blend metal content. Analysis of the TSS alone showed a higher level of chromium and comparable or lower levels of the other measured metals than the TSS1 blend (data not shown). Hence, the higher chromium content of TSS1 than of CC1 reflects the inclusion of TSS material in the blend; whereas, the higher arsenic and nickel levels were due to the different tobacco types used across these blends. It should be noted that the transfer of metals from the TSS would not necessarily occur with the same efficiency as from tobacco, due to possible differences in the chemical form (and therefore volatility) of trace metals in calcium carbonate and in tobacco. Overall, the metal yields in MS under HCI smoking machine conditions were either lower or not statistically significantly different when TSS1 was compared to CC1 (Table 6). The blend nitrosamine content of TSS1 was lower (23–72%) than that of CC1 (Table 5) and the MS yields of the TSNAs under HCI

Table 5
Blend metal and tobacco-specific nitrosamine contents.

	Units	CoV (%)[a]	CC1		TSS1		BT1		CC6		TSS6	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Metals (dwb)												
Arsenic	μg/g	8.1	0.2	0.0	0.3	0.0	0.3	0.0	0.2	0.0	0.2	0.0
Cadmium	μg/g	6.1	1.3	0.1	0.9	0.0	1.2	0.1	0.7	0.0	1.2	0.0
Chromium	μg/g	5.9	0.5	0.0	1.7	0.1	0.9	0.2	0.9	0.1	1.8	0.2
Lead	μg/g	9.3	0.8	0.1	0.8	0.0	0.5	0.0	0.6	0.0	0.6	0.0
Mercury	μg/g	-[b]	<0.05[c,d]	–	<0.05[c]	–	<0.05[c]	–	<0.05[c]	–	<0.05[c]	–
Nickel	μg/g	24.3	1.3	0.1	1.4	0.1	1.1	0.1	1.6	0.1	1.6	0.2
Selenium	μg/g	15.3	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0
Nitrosamines (dwb)												
NAB	μg/g	78.0	0.1	0.0	0.03	0.0	0.01	0.0	0.04[e]	0.0	<0.01[c]	0.0
NAT	μg/g	29.7	1.4	0.0	0.6	0.0	0.1	0.0	0.8[e]	0.0	0.5	0.0
NNK	μg/g	42.8	0.3	0.0	0.2	0.0	0.1	0.0	0.4[e]	0.1	0.2	0.0
NNN	μg/g	23.8	3.2	0.1	0.9	0.0	0.1	0.0	1.3[e]	0.1	0.6	0.0

Mean and standard deviations of five replicates for each blend are shown, except as noted. All values rounded to 1 DP except NAB and mercury values.
dwb, dry weight basis.
[a] The CoV for the reference blend from Kentucky reference cigarettes 3R4F measured contemporaneously with the blend samples is shown.
[b] The reference cigarette blend always ran below the limit of quantification for the assay and so no CoV was calculated.
[c] For values with a "<" symbol, the limit of quantification for the assay is shown.
[d] Retested due to inconsistent data – value from retest is shown.
[e] Six replicates.

Table 6
Mainstream smoke yields of metals and nitrosamines measured under Health Canada Intense smoking machine conditions.

	Units	Uncertainty (%)	CC1	TSS1		BT1		CC6	TSS6	
			Yield	Yield	Δ (%)	Yield	Δ (%)	Yield	Yield	Δ (%)
Metals										
Arsenic	ng/cig	29	2.9	1.3	**−55**	4.4	**52**	3.7	4.3	16
Cadmium	ng/cig	42	38.7	6.2	**−84**	11.3	**−71**	36.5	11.7	**−68**
Chromium	ng/cig	67	<1.2[a]	<1.2	–	<1.2	–	<1.2	<1.2	–
Lead	ng/cig	67	14.8	16.5	11	<12.0	−19	20.1	18.8	−6
Mercury	ng/cig	120	0.3	0.3[b]	0	2.2	**633**	1.0	0.9	−10
Nickel	ng/cig	175	<2.0	<2.0	–	<2.0	–	2.4	<2.0	−17
Selenium	ng/cig	61	<4.1	<4.1	–	<4.1	–	20.1	18.8	−6
Nitrosamines										
NAB	ng/cig	27	13.6	6.6	**−51**	1.4	**−90**	12.1	7.6	**−37**
NAT	ng/cig	22	124.5	70.3	**−44**	19.1	**−85**	117.6	69.5	**−41**
NNK	ng/cig	21	57.9	48.2	−17	10.1	**−83**	80.0	44.5	**−44**
NNN	ng/cig	16	245.2	76.0	**−69**	10.2	**−96**	146.9	72.8	**−50**

The mean of five replicate measurements for each cigarette type are shown. Yield values are given to 1 decimal place and % changes rounded to the nearest whole number. Changes shown in bold type were statistically significant ($P < 0.05$) and were greater than the analytical uncertainty.
[a] For values with a "<" symbol, the limit of quantification for the assay is shown.
[b] Four replicate measurements.

machine smoking conditions were correspondingly lower (up to 69%) for TSS1 than CC1 (Table 6).

Statistically significantly lower yields were found from TSS1 than from CC1 for phenol and some cresols (50–57%), carbonyls (44–86%), some PAHs (36–71%) and miscellaneous volatile and organic constituents (27–94%); although for the dihydroxybenzenes, quinoline, pyrene and benzo(a)pyrene, these differences did not achieve statistical significance (Table 7). These data demonstrate lower toxicant yields from TSS1 across all of the analyte classes examined and, therefore, support the expectation that the TSS and the three stage filter should function to give overall MS toxicant yield reductions in an EC.

3.1.2.3. TSS6. The blend metal contents of TSS6 and CC6 were similar, other than statistically significantly higher chromium and cadmium blend levels in TSS6 ($P < 0.01$). As noted above, the higher chromium level was due to the TSS; whereas, the higher cadmium content reflects a difference in the tobacco types used between the two blends. The MS yields of metals determined under HCI smoking machine conditions, were not elevated in TSS6 compared to CC6 (Table 6). However, MS cadmium yields were significantly reduced.

The blend nitrosamine contents were lower (38–54%) from TSS6 than those measured for the CC6 blend (Table 5). Again, this lower blend nitrosamine content translated to 37–50% lower MS yields for these TSNAs under HCI smoking machine conditions (Table 6).

MS yields from TSS6, across all of the other chemical classes measured were statistically significantly lower than the yields from CC6 (some aromatic amines (14–20%) and phenolics (17–32%), all measured carbonyls (35–85%), most PAHs (18–81%) and miscellaneous volatile toxicants (41–96%)); exceptions included 1- and 2-aminonaphthalene, 4-aminobiphenyl, cresols, quinoline and ammonia, for which the values were not significantly different (Table 7). These data again demonstrate reductions in all classes of measured toxicants, and therefore it is apparent that the TSS is functioning as expected in the EC, to give overall MS toxicant yield reductions.

3.2. Filter comparisons

From the MS yield data shown in Table 7 all the ECs gave lower yields of carbonyls and other volatile smoke constituents than the respective commercial comparator cigarettes, with the exception

Table 7
Mainstream smoke yields measured under Health Canada Intense smoking machine conditions.

	Units	Uncertainty (%)	CC1	TSS1		BT1		CC6	TSS6	
			Yield	Yield	Δ (%)	Yield	Δ (%)	Yield	Yield	Δ (%)
Aromatic amines										
1-Aminonaphthalene	ng/cig	31	20.3	17.8	−12	11.8	**−42**	22.4	22.4	0
2-Aminonaphthalene	ng/cig	43	13.1	11.5	−12	7.4	**−44**	14.6	14.8	1
3-Aminobiphenyl	ng/cig	14	3.5	3.0	−14	1.8	**−49**	4.1	3.3	**−20**
4-Aminobiphenyl	ng/cig	21	2.8	2.5	−11	1.2	**−57**	3.1	2.7	−13
o-Toluidine	ng/cig	ND	68.1	60.1	**−12**	50.6	**−26**	88.1	76.2	**−14**
Phenols and cresols										
Phenol	µg/cig	23	7.6	3.3	**−57**	6.5	−14	10.1	9.3	−8
Catechol	µg/cig	16	56.0	51.7	−8	113.8	**103**	80.5	67.0	**−17**
Resorcinol	µg/cig	30	1.7	1.2	−29	1.3	−24	2.2	1.5	**−32**
Hydroquinone	µg/cig	18	55.1	52.6	−5	78.9	**43**	86.4	67.2	**−22**
o-Cresol	µg/cig	31	1.8	0.8	**−56**	1.8	0	2.5	2.0	−20
m-Cresol	µg/cig	43	1.7	1.0	−41	2.1	24	2.4	2.2	−8
p-Cresol	µg/cig	23	5.4	2.7	**−50**	4.6	−15	6.6	6.1	−8
Carbonyls										
Formaldehyde	µg/cig	29	33.2	17.6	**−47**	46.8	**41**	60.0	31.8	**−47**
Acetaldehyde	µg/cig	16	1096.3	617.4	**−44**	811.3	**−26**	1152.2	751.4	**−35**
Acetone	µg/cig	17	563.3	224.6	**−60**	311.9	**−45**	570.0	213.5	**−63**
Acrolein	µg/cig	24	130.5	52.5	**−60**	75.0	**−43**	139.4	62.3	**−55**
Propionaldehyde	µg/cig	19	94.6	43.9	**−54**	62.3	**−34**	98.4	44.9	**−54**
Crotonaldehyde	µg/cig	37	41.6	6.0	**−86**	10.9	**−74**	45.2	7.0	**−85**
Methyl ethyl ketone	µg/cig	19	133.0	30.5	**−77**	48.8	**−63**	140.7	33.3	**−76**
Butyraldehyde	µg/cig	23	76.2	22.4	**−71**	24.1	**−68**	80.2	24.8	**−69**
Miscellaneous volatile constituents										
Hydrogen cyanide	µg/cig	18	333.4	125.5	**−62**	59.2	**−82**	307.4	179.3	**−42**
Ammonia	µg/cig	23	16.2	11.9	**−27**	4.1	**−75**	14.9	16.9	13
1,3-Butadiene	µg/cig	32	39.6	27.2	**−31**	53.4	**35**	63.6	36.8	**−42**
Acrylonitrile	µg/cig	32	21.2	6.0	**−72**	6.6	**−69**	24.1	7.2	**−70**
Isoprene	µg/cig	31	419.8	126.1	**−70**	331.9	−21	412.2	156.3	**−62**
Benzene	µg/cig	27	70.4	11.9	**−83**	22.8	**−68**	77.9	13.6	**−83**
Toluene	µg/cig	43	136.5	<31.4[a]	**−77**	<46.9	**−66**	122.9	<38.1	**−74**
NO	µg/cig	ND	324.7	191.5	**−41**	69.1	**−79**	272.5	160.7	**−41**
Miscellaneous organic constituents										
Pyridine	µg/cig	30	31.6	2.0	**−94**	1.1	**−97**	31.1	1.8	**−94**
Quinoline	µg/cig	56	0.3	0.2	−33	0.1	**−67**	0.4	0.2	−50
Styrene	µg/cig	31	25.2	2.1	**−92**	1.4	**−94**	26.6	1.1	**−96**
PAHs										
Naphthalene	ng/cig	ND	2182.5	643.8	**−71**	484.9	**−78**	2952.3	565.6	**−81**
Fluorene	ng/cig	ND	230.5	148.3	**−36**	247.3	**7**	315.7	240.9	**−24**
Phenanthrene	ng/cig	ND	524.4	191.4	**−64**	541.5	3	739.8	589.7	**−20**
Pyrene	ng/cig	ND	70.4	64.6	−8	75.3	7	108.1	80.3	−26
Benzo(a)pyrene	ng/cig	ND	11.9	11.1	−7	11.5	−3	16.8	13.7	**−18**
Benzo(a)pyrene[b]	ng/cig	37	11.3	9.6	−15	10.6	−6	17.8	12.2	−31
NFDPM	mg/cig	10	18.9	17.3	−8	17.8	−6	24.4	20.7	**−15**
Nicotine	mg/cig	10	1.3	1.2	−8	1.5	**16**	1.6	1.4	−9
Carbon monoxide	mg/cig	25	23.8	18.2	−24	18.1	−24	24.6	18.5	−25

The mean of five replicate measurements for each cigarette type are shown. Yield values are given to 1 decimal place and % changes rounded to the nearest whole number. Changes shown in bold type were statistically significant ($P < 0.05$), and were greater than the calculated analytical uncertainty where available.

[a] For values with a "<" symbol, the limit of quantification for the assay is shown.

[b] Benzo(a)pyrene was measured with two analytical approaches: a stand alone and as part of a suite of PAHs.

of formaldehyde and 1,3-butadiene yields for BT1. To understand better the contribution of the blend and the selective filters used in the ECs to the overall reductions in these smoke constituents, direct comparisons were made between the ECs and control cigarettes (SC-BT1, SC-TSS1 and SC-TSS6), which were identical in all aspects to the appropriate EC, except for the use of a mono-stage CA filter without adsorbents which was manufactured with triacetin. The comparisons of the yields from EC and control cigarettes for the carbonyls and other volatile smoke constituents are shown in Table 8.

From these data it is clear that the yields of the carbonyls and the other volatile smoke constituents were reduced by the presence of the triple stage filter containing CR20L and polymer-derived high activity carbon used in ECs BT1 and TSS1 (Table 8). The mean of the percentage change in MS yield across all volatile constituents measured from BT1 was a reduction of 50% compared to the control cigarette SC-BT1, with a range of 23% reduction for acetaldehyde to 79% reduction for crotonaldehyde. Very similar reductions were obtained with TSS1, which also gave a mean percentage reduction of 50%, with a similar range from 20% reduction in acetaldehyde yield to 79% reduction for crotonaldehyde yield in comparison to SC-TSS1.

From Table 8 it is also apparent that the dual filter containing additional polymer derived carbon but without the CR20L resin (as used in TSS6), also reduced the mean percentage yields of the volatile smoke constituents by a mean of 48%, with a range from 35% reduction in ammonia yield to 79% reduction in crotonaldehyde yield.

Together, these data confirm that the selective filters used in the ECs removed substantial quantities of volatile smoke constituents from cigarette MS, confirming previous studies with the filter adsorbents (Branton et al., 2011a,b). For all of the ECs, the MS

yields of both formaldehyde and 1,3-butadiene were lower than measured with the scientific control cigarettes. Thus, it is clear that the greater formaldehyde yield seen when comparing BT1 with the commercial cigarette CC1 (previously shown in Table 7) must be due to differences in blend between these cigarettes. A similar comparison also confirms that the higher 1,3-butadiene yield from BT1 compared to CC1 is not due to the novel filter technologies used in the manufacture of the EC.

3.3. Comparison of EC toxicant yields with those from published cigarette brand data

This paper has focused on a direct measurement comparison of EC toxicant yields with the yields from two commercial comparator cigarettes. However, to fully establish whether the ECs offer reduced machine yields in comparison to conventional commercial cigarettes, it is necessary to compare their yields with those from a wider range of cigarettes. The absolute yield values of the ECs described here can be compared with other published data obtained under HCI smoking conditions (Health Canada, 2004; Counts et al., 2005; Australian Government, Department of Health and Ageing, 2002); although such comparisons must be treated with caution due to the known difficulties based on limited standardisation between laboratories for the analysis of smoke constituents other than NFDPM, nicotine and CO (Gregg et al., 2004; Counts et al., 2005; Intorp et al., 2009).

The three data sources above were compiled into one dataset to provide a reference set of global cigarette yield data with which to

Table 8
Comparison of mainstream smoke yields of carbonyl and miscellaneous volatile constituents across filter types measured under Health Canada Intense smoking machine conditions.

	Units	Uncertainty (%)	SC-BT1	BT1		SC-TSS1	TSS1		SC-TSS6	TSS6	
			Yield	Yield	Δ (%)	Yield	Yield	Δ (%)	Yield	Yield	Δ (%)
Carbonyls											
Formaldehyde	µg/cig	29	99.8	46.8	**−53**	19.5	17.6	−10	39.1	31.8	−19
Acetaldehyde	µg/cig	16	1048.9	811.3	**−23**	771.7	617.4	**−20**	847.5	751.4	−11
Acetone	µg/cig	17	562.7	311.9	**−45**	374.6	224.6	**−40**	399.9	213.5	**−47**
Acrolein	µg/cig	24	151.9	75.0	**−51**	101.6	52.5	**−48**	116.3	62.3	**−46**
Propionaldehyde	µg/cig	19	103.0	62.3	**−40**	70.6	43.9	**−38**	74.3	44.9	**−40**
Crotonaldehyde	µg/cig	37	52.5	10.9	**−79**	29.0	6.0	**−79**	33.2	7.0	**−79**
Methyl ethyl ketone	µg/cig	19	150.1	48.8	**−67**	89.8	30.5	**−66**	103.6	33.3	**−68**
Butyraldehyde	µg/cig	23	67.7	24.1	**−64**	51.8	22.4	**−57**	58.1	24.8	**−57**
Miscellaneous volatile constituents											
Hydrogen cyanide	µg/cig	18	96.3	59.2	**−39**	166.5	125.5	**−25**	203.7	179.3	−12
Ammonia	µg/cig	23	7.4	4.1	**−45**	18.3	11.9	**−35**	26.1	16.9	**−35**
1,3-Butadiene	µg/cig	32	76.0	53.4	−30	50.7	27.2	**−46**	57.7	36.8	**−36**
Acrylonitrile	µg/cig	32	13.7	6.6	**−52**	17.1	6.0	**−65**	18.4	7.2	**−61**
Isoprene	µg/cig	31	605.8	331.9	**−45**	410.1	126.1	**−69**	514.8	156.3	**−70**
Benzene	µg/cig	27	71.4	22.8	**−68**	56.5	11.9	**−79**	61.8	13.6	**−78**
Toluene	µg/cig	43	105.2	<46.9	**−55**	106.4	<31.4	**−70**	107.4	<38.1	**−65**
Mean% change					−50			−50			−48
NFDPM	mg/cig	10	17.9	17.8	−1	16.2	17.3	7	19.3	20.7	7

The means of 5 replicate measurements for each cigarette type are shown. Change values shown in bold type were statistically significant ($P < 0.05$) and were greater than the analytical uncertainty.
For values with a " < " symbol, the limit of quantification for the assay is shown.

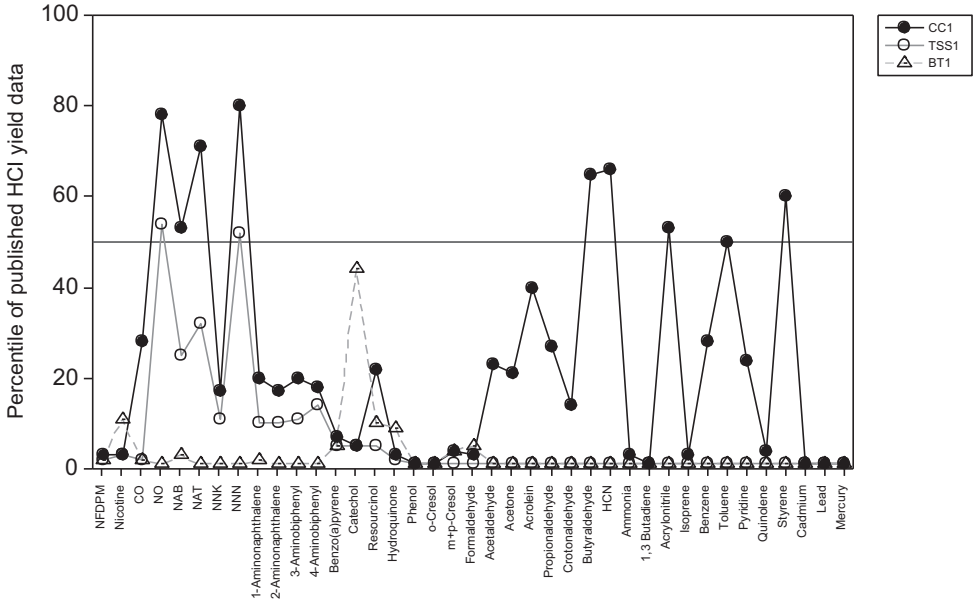

Fig. 1. Comparison of HCI machine toxicant yields from ECs (1 mg ISO) with those from published data sources. (Lines are used to connect data for a single product and do not show trends or relationships.)

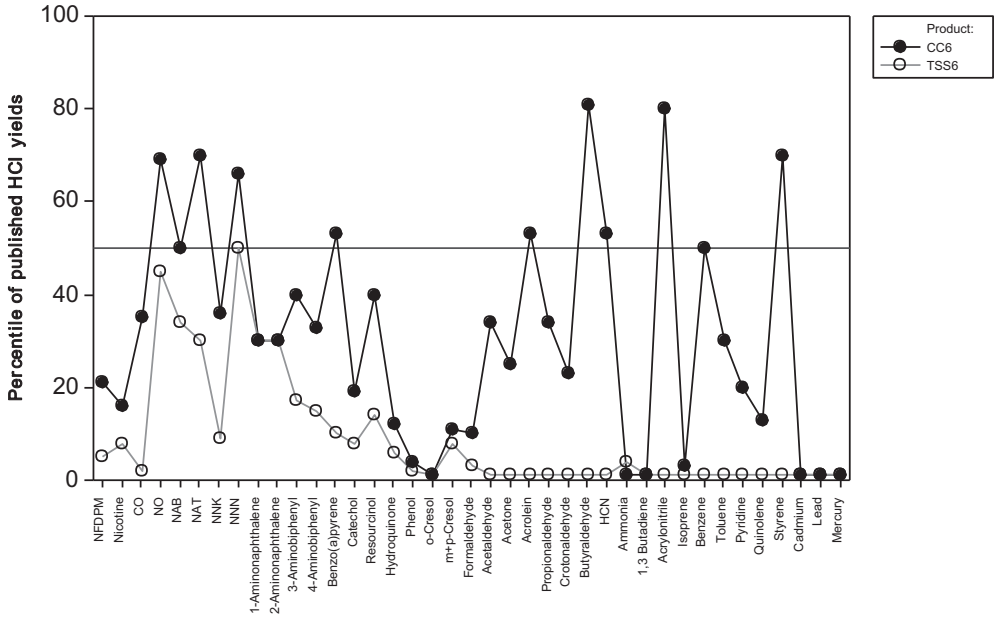

Fig. 2. Comparison of HCI machine toxicant yields from ECs (6 mg ISO) with those from published data sources. (Lines are used to connect data for a single product and do not show trends or relationships.)

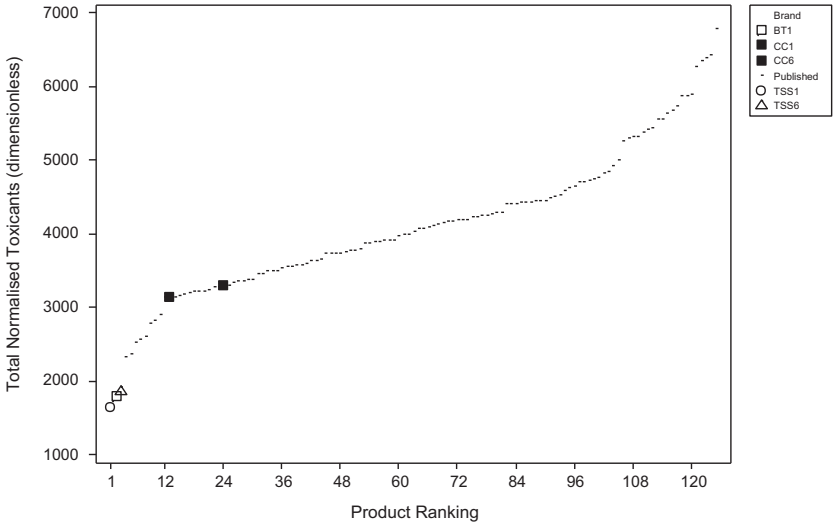

Fig. 3. Comparison of total normalised toxicant yields between ECs and published HCI yield data.

compare the toxicant yields from the ECs described in this study. The full dataset was truncated as follows: first, arsenic, methyl ethyl ketone, nickel and selenium yields were removed from the dataset because yields were not provided by all three sources–leaving 39 toxicants for the comparison, second, a number of brands were removed from the dataset due to incomplete, duplicated or erroneous data (two brands in the HC dataset appear to have erroneously exchanged toluene and styrene yields; tar, nicotine and CO yields were not provided in the HC dataset for one brand and multiple instances of the same yield data were observed in the HC dataset). Finally, reference products were removed from the dataset to ensure that only commercial brands were included. This resulted in a dataset of 120 cigarette brands covering 16 countries or regions. While extensive, it is unlikely that this dataset is fully representative of the range of cigarette products on-sale globally, either with respect to the range of design features, or as a representative sample of global brands. However, while it is limited in these respects, it does constitute a valid comparator set for the toxicant yields from these ECs.

The data was examined to see if it was normally distributed; while a number of toxicants in the dataset were normally distributed the majority (and in particular nitrogenous toxicants such as TSNAs and aromatic amines) were not. Consequently the reference dataset was subject to an empirical cumulative distribution analysis, producing a percentile distribution within the toxicant yields. Yields from the ECs were then compared to the empirical cumulative distribution to identify the position of these yields in comparison to the commercial brands (Figs. 1 and 2). In these comparisons, the yields of the ECs described here fall at the low end of the range for numerous toxicants and often give lower

Table 9
Sidestream smoke yields under ISO smoking machine conditions.

	Uncertainty (%)	Units	CC1 Yield	BT1		TSS1	
				Yield	Δ (%)	Yield	Δ (%)
Ammonia	17	µg/cig	6971	4005	**−43**	5669	−19
1-Aminonaphthalene	33	ng/cig	165	130	−21	144	-13
2-Aminonaphthalene	34	ng/cig	152	119	−22	121	−20
3-Aminobiphenyl	30	ng/cig	39	25.3	**−35**	38.4	−2
4-Aminobiphenyl	29	ng/cig	27.7	16.7	**−40**	27.1	−2
Benzo(a)pyrene	22	ng/cig	144	184	**28**	119	−17
Formaldehyde	16	µg/cig	453	552	**22**	537	**19**
Acetaldehyde	21	µg/cig	1393	1629	17	1401	1
Acetone	8	µg/cig	801	1038	**30**	754	−6
Acrolein	10	µg/cig	325	386	**19**	328	1
Propionaldehyde	9	µg/cig	132	170	**29**	125	−5
Crotonaldehyde	16	µg/cig	59	92.7	**57**	53	−10
Methyl ethyl ketone	14	µg/cig	162	264	**63**	144	−11
Butyraldehyde	19	µg/cig	100	104	4	93	−7
HCN	16	µg/cig	127	67.6	**−47**	91	**−28**
NNN	31	ng/cig	220	40.6	**−82**	106	**−52**
NAT	38	ng/cig	83	19.3	**−77**	43	**−48**
NAB	41	ng/cig	16.5	3.63	**−78**	11.8	−28
NNK	30	ng/cig	204	141	**−31**	186	−9
Hydroquinone	24	µg/cig	96	122	**28**	82	−15
Catechol	22	µg/cig	75	132	**77**	53	**−29**
Phenol	16	µg/cig	209	273	**31**	188	−10
m + *p*-Cresols	17	µg/cig	61.9	85.3	**38**	56.6	−9
o-Cresol	19	µg/cig	24	42.3	**76**	23	−4
Pyridine	15	µg/cig	333	239	**−28**	267	**−20**
Quinoline	19	µg/cig	17.8	14.5	**−19**	14	**−21**
Styrene	37	µg/cig	119	122	3	124	4
1,3-Butadiene	24	µg/cig	446	551	24	391	−12
Isoprene	23	µg/cig	3130	3779	21	3284	5
Acrylonitrile	26	µg/cig	143	100	**−30**	139	−3
Benzene	26	µg/cig	344	413	20	297	−14
Toluene	29	µg/cig	697	712	2	637	−9
Mercury	22	ng/cig	11.2	12.8	14	10.6	−5
Cadmium	13	ng/cig	351	460	**31**	239	**−32**
NO	11	µg/cig	2139	1389	**−35**	2018	−6
NO_x	11	µg/cig	2321	1510	**−35**	2182	−6
NFDPM	12	mg/cig	22.8	27.7	**21**	21.8	−4
Nicotine	10	mg/cig	4.26	5.76	**35**	4.42	4
CO	15	mg/cig	40.4	50.6	**25**	32.9	**−19**
CO_2	27	mg/cig	367	395	8	330	−10

Mean of three replicates for each cigarette are shown. The expanded uncertainty was calculated from these data and presented as a % of the overall mean value. All values for resorcinol, arsenic, chromium, lead, nickel and selenium were either below limits of quantification or were not measured and are not shown.
Values shown in bold are statistically significant ($P < 0.05$) and greater than the analytical uncertainty.

values for specific toxicants than any of the products in the commercial brand dataset. Exceptions to this are catechol yields from BT1, NO and TSNA yields from TSS1 and from TSS6, where the yields are approximately equivalent to the median values for the commercial product dataset. In contrast, the yields of the commercial comparator cigarettes CC1 and CC6 are generally distributed over the range of yields observed with the commercial dataset.

A further comparison was conducted, examining the total toxicant levels from the ECs and each of the commercial products in the dataset. This was conducted in three ways. The first method was to sum the yields of the 39 toxicants for each cigarette to give a total toxicant yield for each brand. This approach is of limited utility because the total toxicant yield value for each brand is dominated by NFDPM, CO and nicotine, and many other toxicants do not contribute significantly to the total value. A second approach was to sum the yields of all toxicants (but excluding NFDPM, nicotine and CO yields) for each cigarette to give a total for the toxicant subset of yields (data not shown). A third, normalisation method gave greater insight into the contribution of all toxicants, wherein a median value was calculated for each toxicant in the commercial dataset. The median value was normalised to 100 for each toxicant, and the yields of toxicants scaled against this value of 100. Totalling the scaled values for all toxicants gave a total normalised toxicant value for each brand. The total normalised toxicant values for the ECs are compared to and ranked against the values for all of the brands in the commercial dataset in Fig. 3. The comparison shows that the ECs were at the low end of the ranking order. The 1 mg ECs were found to have the lowest total normalised toxicant yields under each of the three approaches, and the 6 mg EC was also lower than any of the commercial brands for the toxicant subset yields and the total normalised toxicant value.

These analyses show that the ECs offer some of the lowest machine toxicant yields of cigarettes for which published HCI smoke chemistry is available, confirming that the ECs generate reduced machine toxicant yields in comparison to published yields from commercial cigarettes.

3.4. Comparisons of EC yields as a ratio to nicotine yields

The analysis described above is restricted to assessment of machine yields of toxicants. Assessment of smokers' exposure to toxicants from these cigarettes is discussed in another paper (Shepperd et al., submitted for publication). However, it has been proposed that the ratio of smoke toxicants to the MS nicotine yield of cigarettes gives a better predictor of smokers' exposure to the

toxicant than the MS yield value alone (Laugesen and Fowles, 2006). Therefore, the ratio of MS constituents yields measured in this study to the MS nicotine yields, all measured under HCI smoking machine conditions, has been calculated and is given as a Supplemental Table S2 (available online). Under Health Canada Intense machine smoking conditions, the NFDPM yields from BT1, TSS1 and CC1 were comparable, but the nicotine yield from BT1 was slightly higher than from CC1, and the nicotine yields from TSS1 and TSS6 slightly lower than from CC1 and CC6 respectively (Tables 4 and 7). When the yield values for the EC were calculated as a ratio to the nicotine yield, and compared to those from CC1 and CC6, they followed the same trends as found when comparing the yields per cigarette, but with slightly greater reductions from BT1 (compared to CC1), slightly lower reductions from TSS1 (compared to CC1), and also slightly lower reductions from TSS6 (compared to CC6).

3.5. Sidestream smoke yields

To complete the chemical analysis of smoke emissions from the EC, SS yields for the expanded list of smoke constituents were measured, under ISO smoking parameters for BT1, TSS1 and CC1; TSS6 and CC6 were not measured. The ISO smoking parameters were chosen because they generate higher SS yields than any of the other smoking regimes. In general, under any smoking regime, the quantity of sidestream smoke can be expected to be dependent on the amount of tobacco consumed in the static burn or smoulder phase of cigarette smoking. The SS yield results are presented in Table 9.

Statistically significantly higher yields of sidestream NFDPM, nicotine and CO (21–35%) and several constituents such as benzo(a)pyrene (28%), phenolics (28–77%), most carbonyls (19–63%) and cadmium (31%) constituents were measured from BT1 than from CC1 (all $P < 0.05$). In contrast lower yields of nitrogenous SS smoke constituents such as TSNAs (31–82%), HCN (47%), some aromatic amines (35–40%) nitrogen oxides, pyridine and quinoline (19–35%) were measured from BT1 than from CC1. Most of these changes were described previously (Liu et al., 2011); however, the higher SS phenolic yields and lower than anticipated TSNA yields from BT1 suggest that chemical differences between Virginia and US-blended tobaccos also influence the SS yields of individual constituents. Finally, the 15% higher tobacco weight from BT1 than from CC1 will also contribute across all measured endpoints to the observed increases.

A number of SS smoke constituent yields were lower from the EC cigarette TSS1 than from CC1. The greatest numerical differences in SS yields were observed for NNN and NAT which were around 50% lower from TSS1 than CC1; these observations are consistent with the observed trends in MS yields of these species. Significant reductions were also found in the sidestream yields of CO, cadmium, catechol, HCN, pyridine and quinoline (19–32%). One constituent with a statistically significantly higher sidestream yield from TSS1 than from CC1 was formaldehyde (19% higher, $P < 0.05$). Higher SS formaldehyde yields were also observed with higher levels of TSS inclusion in the blend (McAdam et al., 2011), suggesting that formaldehyde might be a combustion by-product of the organic materials used in TSS manufacture.

4. Conclusions

Three ECs were made using a combination of technological approaches, and chemical testing under four different machine smoking parameters has confirmed overall reductions of MS toxicants yields from the ECs. When compared with published values of MS toxicant yields from conventional cigarettes, despite a small number of elevated yields with BT1, the performance of these ECs appears to be superior, even if they are ranked on a nicotine ratio basis. The data presented in this study support a designation of these ECs as reduced machine-yield prototypes, and previous data with EC made using the TSS approach suggest that lower biomarkers of exposure to MS toxicants should be achieved if these ECs were to be smoked by human volunteers (McAdam et al., 2011).

Despite the low overall machine yields of toxicants obtained from the current ECs and their performance against commercial comparators and other published toxicant yield data, substantial amounts of scientific data would need to be acquired, including biomarkers of exposure and biomarkers of biological effect, to determine whether such products might be associated with lower health risks, and therefore there is no certainty that these ECs will meet the IOM definitions of a PREP.

Nonetheless, we believe that the results from this study are sufficient to encourage further work, including human biomarker studies in volunteers smoking these ECs and further application and refinement of the technologies used in their manufacture.

Conflict of interest statement

The authors are employees of British American Tobacco, except for Dr. Evan Gregg who acts as a consultant to BAT and who was paid for his contribution to this manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.yrtph.2011.11.007.

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