DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention

MEMORANDUM

Date: September 18, 2006

From: Director, DLS, (F-20)

Subject: Mainstream Smoke Laboratory Results on New Zealand Roll-Your-Own Cigarettes (DLS Study Number #2005-0022)

To: Jefferson Fowles

Please find attached laboratory results on the mainstream smoke deliveries (filtered and unfiltered) from the supplied 5 brands of roll-your-own (RYO) cigarette. Quantitative results on the mainstream levels of tar, nicotine, and CO (TNCO), tobacco specific nitrosamines (TSNAs) and volatile organic compounds (VOCs) were obtained. In addition to this copy of the results, you will also receive the results in an electronic version.

These laboratory results have been reviewed and approved by a quality assurance officer to assure that they conform to acceptable quality standards. If you have any questions concerning these results, please contact Clifford Watson, Ph.D. at 770-488-7638.

Eric J. Sampson

Eric J. Sampson, Ph.D.

1 Attachment:
Summary Report

New Zealand roll-your-own (RYO) cigarette mainstream smoke analyses

Prepared for the New Zealand Ministry of Health

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Background

Most cigarettes, both filtered and unfiltered, consumed worldwide are machine rolled cigarettes produced in bulk by commercial tobacco companies. Governments worldwide tax cigarette sales to provide revenue and this tax can be a significant percentage of the pack's or carton's price (1) sometimes approaching 80% of the sales price. Cigarette taxation also serves as an economic deterrent to smoking, especially among youth (2). However, as taxation increases some smokers may choose alternate tobacco products that are taxed at a lower rate. Many of these tobacco products have different government regulations or are unregulated. Alternative tobacco products include a wide range of products including cigars, pipes, roll-your-own (RYO) cigarettes, and various smokeless tobacco products (chew or snuff).

RYO cigarettes, as their name implies, are hand-rolled by the individual smoker. Depending on the hand-rolling procedure used and smoker's preference these products can either be filtered or unfiltered and contain variable amounts of loose tobacco filler. The recent International Tobacco Control (ITC) Four Country Survey placed RYO usage to be as low as 6.7%, in the United States, to a high of 28.4% in the United Kingdom (3). The popularity of RYO cigarettes is often associated with lower social classes (4) and many users of RYOs see this as a way to save money. The cost savings of RYO can be significant. Each RYO cigarette could cost 75% less than a factory made cigarette depending on differences in taxation rates between loose tobaccos and commercially rolled cigarettes. This discount of RYO cigarettes compared to commercial brands has

been attributed to sharp consumption increases in the UK (5), Australia (6), and the Netherlands (7), especially as taxation increases.

Previous researchers have studied RYO cigarettes worldwide attempting to understand the role RYO cigarettes plays in select countries. For example, data have been generated on RYOs in the UK (4), Europe (8-10), Canada (11), Thailand (12), and the United States (13). While these previous works yield insight on the chemical and physical nature of RYO cigarettes, these studies were performed prior to the implementation of a standardized method. In 2002, the International Standards Organization (ISO) adopted standardized methods for the analysis of products made from fine-cut-tobaccos, which includes RYO cigarettes. Therefore, more work using a standardized approach to analyze the health implications of RYO cigarettes is needed to provide consistent and comparable data sets.

We report a recent study of RYO cigarettes, with and without filters, rolled using 5 tobacco varieties from New Zealand. Mainstream smoke constituents, including nicotine, 4 tobacco specific nitrosamines (TSNAs), and 22 volatile organic compounds (VOCs) were analyzed from 10 permutations of RYO cigarettes (5 tobacco samples, with and without filters) and a typical NZ commercially available filtered cigarette. All RYO cigarettes were hand-rolled with the aid of a personal "rolling machine" and smoked using a standardized smoking machine protocol.

Results and Discussion

Mainstream smoke nicotine deliveries

Mainstream nicotine smoke deliveries from the 10 RYO varieties ranged from 0.8 mg nicotine/cigarette to 1.6 mg nicotine/cigarette (Tables 1-5). The filtered and unfiltered RYO cigarettes of the same tobacco type yielded nearly equal nicotine deliveries. The similar nicotine levels obtained between the pairs of filtered and unfiltered RYO cigarettes (each being 72 mm in length) largely originates from the constant 0.4 g tobacco used in each. Using 0.4 g of tobacco in both a filtered and unfiltered RYO each having a 72 mm rod length results in the unfiltered RYO cigarette containing 5.55 mg tobacco/mm (400 mg tobacco/ 72 mm rod) and the filtered RYO cigarette having 7.02 mg tobacco/mm (400mg tobacco/ [72 mm rod – 15 mm filter]). The 27 mm burn termination point for all RYO cigarettes results in 45 mm (72 mm rod length – 27 mm butt length) of each rod being consumed during smoking. Therefore, an average of 250 mg of tobacco was consumed for the unfiltered RYO while 316 mg of tobacco was consumed for the filtered RYO. A cigarette filter can reduce nicotine delivery by as much at 48% (4). Thus, the amount of mainstream smoke removed by inclusion of a filter is compensated by the greater mass of tobacco consumed. While the similarities in deliveries obtained here are inconsistent with previously published data (4), ISO 15592-3:2003 protocol states that the delivery reductions due to filtration can be "small compared with the overall variability of the experimental method of measurement." Additionally, we tested both a popular commercially manufactured filtered New Zealand cigarette brand and a 2R4F research cigarette (University of Kentucky, Lexington, KY) for comparison (Table 6). The commercially available NZ

cigarette, Holiday, had a 1.1 mg delivery of nicotine (Table 6) compared to the 1.2 mg average nicotine delivery from the 10 RYO cigarette types.

Mainstream smoke TSNA deliveries

The tobacco-specific nitrosamines (TSNAs), are among the most abundant carcinogens identified in cigarette smoke. We report the levels of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosonornicotine (NNN), N'-nitrosoanabasine (NAB), N'-nitrosoanatabine (NAT) in the 5 RYO cigarette tobaccos (both filtered and unfiltered), the manufactured Holiday cigarette brand, and the 2R4F Kentucky research cigarette (Tables 1-6). Of these TSNAs, NNN and NNK are believed the most carcinogenic (14) and NNAL, the primary metabolite of NNK contributes to the induction of adenocarcinoma, the leading lung cancer in the United States (15). The TSNA deliveries from the RYO cigarettes, both for the filtered and unfiltered pairs, showed brand specific differences (Table 1-6). Higher TSNA levels were measured in the mainstream smoke from the Drum brands (Regular and Mild) compared to the other RYO products or the Holiday brand. Upon visual inspection of the 6 individual tobacco varieties, we attribute the higher TSNA levels in the Drum tobaccos as originating from their higher burley tobacco content compared to the others. Higher TSNAs are typically found in burley tobacco compared to bright or other tobaccos (16,17). The use of high nitrogen content fertilizer and different curing procedures can also influence tobacco's TSNA content (17-19).

Mainstream smoke VOC deliveries

The averaged total VOC deliveries of the 10 RYO cigarettes (2405 ug/cig) were lower than the commercially produced Holiday cigarette brand (2942 ug/cig). This difference is partially attributable to the higher amount of tobacco contained in the commercial brand (0.65 g/cig versus 0.4 g/cig). Analysis of both total and individual VOC levels indicated that the delivery was generally consistent among the 5 RYO brand pairs with the filtered varieties having higher average total deliveries (2571 mg/cig versus 2240 mg/cig). Although the average values are higher for the filtered RYOs, most the variability in this method yielded relatively large standard deviations with overlapping confidence intervals ($p > 0.05$). The averaged levels of individual analytes ranged from low to sub-microgram levels for 3-ethyltoluene to near milligram levels of acetaldehyde and isoprene (Tables 1-5). Relative to the mainstream nicotine deliveries the RYO cigarettes with 0.4 g tobacco deliver less VOCs per mg nicotine than conventional machine made cigarettes when smoked under ISO conditions.

Conclusions

We found that the RYO cigarettes deliver significant amount of nicotine, TSNAs, and VOCs for the amount of tobacco present. Obviously, these RYO mainstream smoke constituent deliveries depend on the tobacco filler amount, inclusion of a filter, and overall construction quality. Unlike conventional manufactured cigarettes where the construction parameters are extremely uniform, an individual's mainstream smoke exposure from a RYO cigarette could vary significantly from cigarette to cigarette and from person-to-person. A switch away from manufactured cigarettes to RYO as a result of economic pressure or regulations aimed at reducing exposure rendering the

commercially rolled tobacco products less palatable in attempts to discourage use could have serious public health consequences. We know that the FTC or ISO machine smoked methods are poor predictors to human smoking exposures (20). This is almost certainly the case for RYO cigarettes. Therefore, the values we found are likely to be lower, rather than upper, exposure limits. The man-made nature of the RYO products makes it difficult to accurately access an individual's exposure to the toxic and carcinogenic chemicals present in tobacco smoke.

Table 1. Select mainstream deliveries of unfiltered and filtered Drum RYO cigarettes. Values are reported on a per cigarette basis (standard deviation). Number of replicates = 3.

Analyte	Units	Unfiltered	Filtered
Nicotine	mg	1.11 (0.22)	1.04 (0.03)
2,3-Butanedione	µg	81.06 (19.06)	88.62 (19.58)
2,5-Dimethylfuran	µg	41.38 (17.91)	45.22 (4.85)
2-Butanone	µg	72.11 (10.31)	67.80 (4.98)
2-Methylfuran	µg	60.08 (17.98)	72.56 (10.75)
2-Pentanone	µg	7.63 (1.11)	9.71 (2.69)
3-Buten-2-one	µg	33.54 (5.50)	36.91 (8.94)
3-Ethyltoluene	µg	0.53 (0.05)	0.85 (0.63)
3-Petanone	µg	1.83 (0.57)	1.55 (0.21)
Acetaldehyde	µg	833.66 (63.47)	930.79 (101.51)
Acetone	µg	252.95 (50.56)	277.77 (62.63)
Acetonitrile	µg	298.70 (73.96)	242.80 (39.11)
Acrolein	µg	119.38 (9.12)	122.77 (22.59)
Acrylonitrile	µg	14.87 (3.11)	14.19 (1.88)
Benzene	µg	40.61 (8.01)	44.82 (8.26)
Ethylbenzene	µg	12.97 (2.81)	11.82 (1.63)
Isobutyrolnitrile	µg	4.69 (0.91)	5.72 (1.48)
Isoprene	µg	760.35 (195.22)	653.02 (66.26)
Propionaldehyde	µg	60.75 (10.74)	79.49 (40.02)
Propionitrile	µg	41.03 (9.37)	40.51 (19.56)
p-Xylene	µg	7.79 (1.31)	7.31 (0.75)
Styrene	µg	5.73 (0.31)	6.71 (1.59)
Toluene	µg	76.17 (19.06)	67.29 (12.42)
NNN	ng	102.86 (51.49)	72.25 (21.18)
NNK	ng	98.59 (29.47)	91.04 (39.09)
NAT	ng	67.07 (9.15)	54.30 (12.79)
NAB	ng	5.74 (0.76)	4.15 (0.99)

Table 2. Select mainstream deliveries of unfiltered and filtered Drum Mild RYO cigarettes. Values are reported on a per cigarette basis (standard deviation). Number of replicates = 3.

Analyte	Units	Unfiltered	Filtered
Nicotine	mg	1.02 (0.16)	0.83 (0.21)
2,3-Butanedione	µg	72.67 (8.58)	75.97 (21.03)
2,5-Dimethylfuran	µg	35.93 (12.50)	44.05 (16.74)
2-Butanone	µg	56.54 (16.03)	52.10 (2.92)
2-Methylfuran	µg	58.38 (17.39)	67.26 (14.25)
2-Pentanone	µg	6.91 (2.69)	5.65 (0.09)
3-Buten-2-one	µg	30.17 (7.16)	34.21 (9.29)
3-Ethyltoluene	µg	0.46 (0.20)	0.38 (0.08)
3-Petanone	µg	1.74 (0.53)	1.24 (0.09)
Acetaldehyde	µg	835.93 (266.78)	717.66 (110.79)
Acetone	µg	242.57 (104.21)	181.07 (5.90)
Acetonitrile	µg	200.97 (28.62)	190.45 (8.80)
Acrolein	µg	102.94 (36.56)	88.95 (16.24)
Acrylonitrile	µg	11.22 (1.60)	11.65 (2.51)
Benzene	µg	38.19 (15.39)	43.12 (3.36)
Ethylbenzene	µg	11.58 (1.67)	11.67 (1.20)
Isobutyrolnitrile	µg	4.74 (1.50)	3.97 (0.43)
Isoprene	µg	552.53 (96.40)	492.22 (29.04)
Propionaldehyde	µg	46.67 (16.81)	51.62 (3.82)
Propionitrile	µg	26.75 (7.58)	25.64 (5.37)
p-Xylene	µg	6.22 (2.29)	6.95 (1.99)
Styrene	µg	4.34 (0.61)	4.30 (1.01)
Toluene	µg	61.86 (19.98)	66.04 (15.58)
NNN	ng	53.65 (16.37)	52.23 (10.61)
NNK	ng	27.97 (9.95)	26.57 (6.20)
NAT	ng	33.51 (4.74)	27.92 (6.09)
NAB	ng	3.91 (0.76)	3.36 (0.72)

Table 3. Select mainstream deliveries of unfiltered and filtered Holiday RYO cigarettes. Values are reported on a per cigarette basis (standard deviation). Number of replicates = 3.

Analyte	Units	Unfiltered	Filtered
Nicotine	mg	1.53 (0.27)	1.44 (0.10)
2,3-Butanedione	µg	55.59 (21.51)	77.74 (40.66)
2,5-Dimethylfuran	µg	23.32 (9.64)	30.76 (1.44)
2-Butanone	µg	54.82 (18.61)	81.35 (38.96)
2-Methylfuran	µg	45.71 (5.06)	60.52 (20.59)
2-Pentanone	µg	6.95 (2.85)	7.74 (1.89)
3-Buten-2-one	µg	15.74 (7.37)	26.10 (14.69)
3-Ethyltoluene	µg	1.57 (0.46)	1.24 (0.17)
3-Petanone	µg	1.29 (0.35)	1.64 (0.34)
Acetaldehyde	µg	391.70 (124.67)	768.57 (467.51)
Acetone	µg	220.44 (53.05)	285.92 (101.34)
Acetonitrile	µg	164.61 (36.51)	299.17 (114.53)
Acrolein	µg	62.93 (16.84)	81.76 (12.45)
Acrylonitrile	µg	10.49 (4.06)	10.08 (1.91)
Benzene	µg	30.10 (12.40)	50.19 (18.74)
Ethylbenzene	µg	9.98 (3.24)	12.59 (2.91)
Isobutyrolnitrile	µg	3.75 (2.41)	4.10 (0.30)
Isoprene	µg	403.27 (78.63)	613.40 (121.45)
Propionaldehyde	µg	30.11 (10.37)	43.42 (16.53)
Propionitrile	µg	22.03 (9.32)	30.15 (15.58)
p-Xylene	µg	5.53 (1.35)	6.33 (1.06)
Styrene	µg	4.58 (1.17)	4.78 (1.73)
Toluene	µg	53.30 (20.44)	59.50 (12.78)
NNN	ng	18.13 (5.25)	14.63 (3.50)
NNK	ng	18.69 (4.32)	19.21 (6.10)
NAT	ng	20.59 (6.90)	21.81 (7.17)
NAB	ng	3.08 (1.09)	2.42 (0.98)

Table 4. Select mainstream deliveries of unfiltered and filtered Park and Drive RYO cigarettes. Values are reported on a per cigarette basis (standard deviation). Number of replicates = 3.

Analyte	Units	Unfiltered	Filtered
Nicotine	mg	1.40 (0.23)	1.55 (0.02)
2,3-Butanedione	µg	61.30 (28.39)	74.61 (10.24)
2,5-Dimethylfuran	µg	28.64 (2.00)	37.24 (1.79)
2-Butanone	µg	60.85 (24.09)	68.37 (1.47)
2-Methylfuran	µg	48.70 (13.21)	56.06 (13.54)
2-Pentanone	µg	7.26 (2.43)	7.46 (0.82)
3-Buten-2-one	µg	24.31 (7.01)	25.25 (7.12)
3-Ethyltoluene	µg	0.56 (0.32)	1.17 (0.35)
3-Petanone	µg	1.47 (0.19)	1.88 (0.31)
Acetaldehyde	µg	603.65 (161.23)	726.01 (156.70)
Acetone	µg	245.66 (112.58)	282.92 (50.68)
Acetonitrile	µg	165.69 (48.71)	238.67 (37.80)
Acrolein	µg	87.99 (25.03)	101.54 (48.63)
Acrylonitrile	µg	9.27 (1.08)	10.78 (2.03)
Benzene	µg	37.02 (3.70)	39.73 (7.71)
Ethylbenzene	µg	10.42 (1.28)	12.10 (1.62)
Isobutyrolnitrile	µg	3.99 (1.47)	3.85 (0.16)
Isoprene	µg	533.34 (187.51)	657.81 (131.14)
Propionaldehyde	µg	41.73 (11.45)	55.69 (17.52)
Propionitrile	µg	26.30 (10.81)	32.81 (8.71)
p-Xylene	µg	5.24 (1.39)	7.72 (2.65)
Styrene	µg	4.40 (0.87)	4.45 (1.40)
Toluene	µg	53.67 (16.69)	63.18 (4.84)
NNN	ng	14.87 (3.44)	16.27 (7.36)
NNK	ng	19.14 (5.43)	17.82 (5.30)
Nicotine	mg	1.40 (0.23)	1.55 (0.02)
NAT	ng	13.84 (3.33)	13.97 (2.27)
NAB	ng	1.55 (0.37)	1.15 (0.36)

Table 5. Select mainstream deliveries of unfiltered and filtered Park and Drive Mild RYO cigarettes. Values are reported on a per cigarette basis (standard deviation). Number of replicates = 3.

Analyte	Units	Unfiltered	Filtered
Nicotine	mg	1.09 (0.14)	1.21 (0.07)
2,3-Butanedione	µg	77.65 (30.37)	82.34 (44.60)
2,5-Dimethylfuran	µg	22.50 (9.78)	27.92 (9.06)
2-Butanone	µg	66.21 (18.20)	94.33 (72.03)
2-Methylfuran	µg	43.82 (8.08)	56.09 (34.49)
2-Pentanone	µg	6.56 (1.73)	7.27 (0.35)
3-Buten-2-one	µg	27.19 (10.84)	31.80 (9.86)
3-Ethyltoluene	µg	0.81 (0.56)	0.77 (0.27)
3-Petanone	µg	1.70 (0.65)	1.66 (0.32)
Acetaldehyde	µg	735.20 (229.70)	909.88 (280.78)
Acetone	µg	285.71 (155.96)	293.88 (47.65)
Acetonitrile	µg	162.40 (53.24)	229.77 (125.25)
Acrolein	µg	88.16 (42.38)	87.68 (6.88)
Acrylonitrile	µg	11.17 (2.90)	11.99 (3.38)
Benzene	µg	38.81 (11.55)	39.06 (22.02)
Ethylbenzene	µg	7.66 (1.89)	10.51 (5.38)
Isobutyrolnitrile	µg	4.34 (1.52)	4.79 (2.09)
Isoprene	µg	571.83 (154.36)	735.34 (399.31)
Propionaldehyde	µg	46.35 (14.50)	51.48 (8.83)
Propionitrile	µg	27.25 (7.61)	36.09 (9.60)
p-Xylene	µg	5.41 (1.69)	6.26 (3.03)
Styrene	µg	4.50 (2.19)	5.46 (1.82)
Toluene	µg	46.41 (5.98)	61.11 (18.03)
NNN	ng	13.69 (2.65)	12.82 (3.83)
NNK	ng	18.61 (6.10)	16.31 (2.22)
NAT	ng	11.42 (2.78)	10.79 (3.22)
NAB	ng	1.44 (0.56)	1.24 (0.30)

Table 6. Select mainstream deliveries of the factory produced 2R4F and Holiday cigarettes. Values are reported on a per cigarette basis (standard deviation). Number of replicates = 3.

Analyte	Units	Holiday	2R4F
Nicotine	mg	1.10 (0.16)	0.79 (0.04)
2,3-Butanedione	µg	114.42 (16.52)	72.57 (9.15)
2,5-Dimethylfuran	µg	38.54 (2.46)	21.22 (2.50)
2-Butanone	µg	104.74 (16.92)	72.14 (9.75)
2-Methylfuran	µg	67.09 (4.64)	46.90 (8.38)
2-Pentanone	µg	11.64 (1.36)	7.37 (1.20)
3-Buten-2-one	µg	33.24 (3.12)	27.13 (5.97)
3-Ethyltoluene	µg	0.94 (0.18)	0.47 (0.41)
3-Petanone	µg	2.77 (0.30)	2.06 (0.43)
Acetaldehyde	µg	827.65 (71.03)	697.20 (37.36)
Acetone	µg	408.38 (34.79)	315.06 (17.51)
Acetonitrile	µg	190.41 (19.41)	178.02 (51.02)
Acrolein	µg	86.56 (7.15)	72.51 (22.13)
Acrylonitrile	µg	15.69 (2.62)	12.72 (1.46)
Benzene	µg	49.70 (2.38)	42.20 (3.94)
Ethylbenzene	µg	12.68 (0.77)	8.44 (1.44)
Isobutyrolnitrile	µg	5.13 (0.12)	4.42 (0.36)
Isoprene	µg	799.27 (79.01)	483.96 (30.74)
Propionaldehyde	µg	62.32 (9.21)	48.33 (5.73)
Propionitrile	µg	36.49 (5.20)	29.32 (5.29)
p-Xylene	µg	7.74 (1.11)	5.11 (0.88)
Styrene	µg	5.06 (0.75)	3.96 (1.44)
Toluene	µg	62.01 (6.80)	55.79 (4.34)
NNN	ng	25.10 (0.26)	131.59 (14.67)
NNK	ng	21.07 (0.40)	112.37 (24.78)
NAT	ng	18.43 (0.21)	118.78 (11.40)
NAB	ng	2.07 (0.12)	16.28 (2.10)

Methods

Materials.

The isotopically labeled tobacco-specific nitrosamine (TSNA) internal standards (ISs) (14) were purchased from Cambridge Isotopes (Cambridge, MA). Hexane, 2-propanol (low water), methanol, and ethyl acetate were obtained from Burdick and Jackson (Muskegon, MI). Cambridge filter pads (CFP), 44 mm, were purchased from Whatman (Maidstone, England). The ultra-high purity gas helium was obtained from AirGas, Inc. (Atlanta, GA). Tedlar® brand polyvinylfluoride (PVF) bags, carboxen-polydimethylsiloxane (Carboxen-PDMS) SPME fibers, and Mininert® septum caps were purchased from Supelco (Bellefonte, PA). All other chemicals were obtained through Sigma-Aldrich (St. Louis, MO) in the highest purity commercially available and used without further purification.

RYO preparation and smoking.

All RYO cigarettes were prepared as described in ISO 15592-3:2003. The brands of tobacco used were obtained in New Zealand and consisted of Drum, Drum Mild, Holiday, Park and Drive, and Park and Drive Mild. All RYO cigarettes were made by rolling 0.4 g tobacco in Beehive® (Swedish Match, NZ) rolling paper using a Rizla+® automatic rolling box. Filtered RYO cigarettes, provided by the New Zealand Ministry of Health, contained a 15mm cellulose acetate filter inserted into one end. Tobacco paper glue was activated with the application of distilled water. Preparation of RYO cigarettes for machine smoking was fairly straightforward; following the ISO 15592-3:2003 protocol, 0.4g of tobacco was used in the construction of each RYO cigarette. This

tobacco amount is comparable, though slightly lower than, the 0.49 g to 0.5 g typically used by RYO smokers (4). The hand-made preparation of RYO cigarettes makes achieving consistent tobacco density throughout the cigarette rod more difficult. Subjective measures (visual uniformity and firmness) were used to select appropriate representative cigarettes deemed acceptable for analysis.

Prior to smoking all RYO cigarettes were conditioned as specified in ISO 15592-2:2003 in a controlled humidity and temperature enclosure (Labline, Boston, MA). The smoking machines were located inside a room-sized environmental chamber (Parameter Generation Control, Inc, Black Mountain, NC) that was maintained at 22°C and 60% relative humidity. Unfiltered RYO cigarettes were inserted 13mm into holders having a 7.2 mm opening and meeting the specifications of ISO 15592-3:2003. Filtered RYO cigarettes were inserted into holders used for the smoking of filtered cigarettes (ISO 3308:2000). Cigarettes were smoked with a 35 mL puff of 2 second duration every 60 seconds (ISO 15592-3:2003) on an automated linear 16-port ASM 500 smoking machine (Cerulean, Milton Keynes, UK) to a butt length of 27mm as specified in ISO 15592-3:2003.

Nicotine Determination.

Mainstream smoke nicotine deliveries were determined from extraction of the CFP in 2-propanol. Nicotine levels in the extracts were analyzed and quantified using gas chromatography with flame ionization detection as previously reported (21).

Tobacco-Specific Nitrosamine (TSNA) Determination

The TSNAs in mainstream smoke particulate matter captured on CFPs were measured using an 1100 high performance liquid chromatographer (Agilent, Palo Alto, CA) and API-4000 tandem mass spectrometer (Sciex , Toronto, ON) as previously described (22). Sample preparation was streamlined with direct extraction of the CFPs in the mobile phase (23), 20 mM ammonium acetate containing 5% methanol. One hour before smoking the CFPs were treated with 2 mL 50 mM ascorbic acid in methanol to reduce TSNA artifact formation (22,24).

VOC Determination.

The vapor-phase portion of mainstream cigarette smoke was collected in individual 1-L PVF bags attached directly to individual ASM 500 puffing engines as previously described (25) and analyzed by gas chromatography / mass spectrometry (GC/MS). To reduce background and sample carryover, the smoking machine was programmed to take 30 blank puffs per port to clear any residual smoke before attaching the PVF bags. Internal standards were added to each 1-L PVF bag prior to smoking. Following smoking the PVF bags were sealed closed and a portion of the smoke sample transferred via cannula to an evacuated 20-mL headspace vial (Microliter Corporation, Atlanta, GA). Vials, containing the analytical samples were loaded on a Combi-Pal auto sampler (LEAP Technologies, Carrboro, NC) equipped with SPME sampling arm for quantitative analysis using a 6890/5973 GC/MS (Agilent, Palo Alto, CA). Vapor phase smoke samples were warmed to 30°C for 2 min then sampled for 30 sec with a 75-μm Carboxen-PDMS SPME fiber. Analytes were desorbed from the fiber at 260°C in the GC's heated inlet and focused on the head of a DB-624 capillary column (30.0 m x 320

μm x 1.80 μm). Helium carrier gas was maintained in constant flow mode at an average linear velocity of 46 cm/sec. For separation and analysis the GC oven was programmed to start at -20°C, hold for 2 min, and ramp to 200°C at 8°C/min, for a total run time of 29.50 min. The GC transfer line was maintained at a temperature of 255°C and the ion source and quadrupole temperatures were 230°C and 150°C, respectively. Data were acquired in full-scan mode over 30 - 200 amu.

Quality Control Materials.

Kentucky 2R4F research cigarettes (University of Kentucky, Lexington, KY) were used as the quality control (QC) material. The QC cigarettes were smoked with each smoking run. All analytes were measured in the 2R4F cigarette smoke and thoroughly characterized to determine the mean and the 95th and 99th confidence intervals for each analyte. Acceptance criteria for QC and blank samples followed the criteria prescribed by Taylor (26).

References

1. Mackay, J; Eriksen, M; Shafey, O, *The Tobacco Atlas*, 2nd ed. Atlanta, GA: American Cancer Society; 2006. pp. 106-113.

2. Cokkinides V, Bandi P, Ward E, Jemal A, Thun M, Progress and opportunities in tobacco control, *CA Cancer J Clin.* (2006);56:135-42.

3. Young D, Borland R, Hammond D, Cummings KM, Devlin E, Yong HH, O'Connnor RJ; ITC Collaboration. Prevalence and attributes of roll-your-own smokers in the International Tobacco Control (ITC) Four Country Survey. *Tob Control* (2006) Suppl 3:iii.pp. 76-82.

4. Darrall KG, Figgins JA, Roll-your-own smoke yields: theoretical and practical aspects. *Tob Control* (1998);7:168-75

5. Lader D, Meltzer H. Smoking related behaviour and attitudes. London: Office for National Statistics, 2001.

6. Scollo M, Younie S, Wakefield M, et al. Impact of tobacco tax reforms on tobacco prices and tobacco use in Australia. Tob Control 2003;12 (suppl II) :ii59–66.

7. Mindell J, Whynes D. Cigarette consumption in the Netherlands 1970–1995: does tax policy encourage the use of hand-rolling tobacco. Eur J Public Health 2000;10:214–

8. de Kok A, Besamusca EW, Vreeker CP, et al. Roll-your-own: a delicate subject. A study of the tar and nicotine content of the 42 roll your own brands sold in the Netherlands. Report AL TAB-1992-1. Alkmaar, Netherlands: Inspectie Gezondheidsbescherming, 1993.

9. Hauknes A. Analysis of hazardous substances in rolling tobaccos. Oslo, Norway: National Council on Tobacco and Health, 1994.

10. Dymond H. Making habits of roll-your-own smokers in the Netherlands and tar and nicotine yields from the resultant products. Tobacco Sci 1996;40:87-91.

11. Rickert WS, Robinson JC, Bray DF, et al. Characterization of tobacco products: a comparative study of the tar, nicotine, and carbon monoxide yields of cigars, manufactured cigarettes, and cigarettes made from fine-cut tobacco. Prev Med 1985;14:226-233.

12. Mitacek EJ, Brunnemann KD, Polednak AP, et al. Composition of popular tobacco products in Thailand, and its relevance to disease prevention. Prev Med 1991;20:764-773.

13. Appel BR, Guirguis G, Kim IS, Garbin O, Fracchia M, Flessel CP, Kizer KW, Book SA, Warriner TE, Benzene, benzo(a)pyrene, and lead in smoke from tobacco products other than cigarettes, *Am J Public Health.* (1990):80(5):560-4.

14. Hecht S. S Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. (1998) *Chemical Research in Toxicology*, 11, 559–603.

15. Thun MJ, Lally CA, Flannery JT, Calle EE, Flanders WD, Heath CW Jr., Cigarette smoking and changes in the histopathology of lung cancer. *J Natl Cancer Inst.* (1997);89(21):1580-6

16. Hoffmann D, Hoffmann I, The changing cigarette, 1950-1995 J Toxicol Env Health, 1997:50(4):307-64.

17. Fisher S, Spiegelhalder B, Preussmann R, Preformed Tobacco-Specific Nitrosamines in Tobacco – Role of Nitrate and Influence of Tobacco Type. *Carcinogenesis* **1999**;10(8): 1511-17.

18. Chamberlain W, Severson R and Stephenson M. G. Levels of N-nitrosonornicotine in tobaccos grown under varying agronomic conditions. (1984) *Tobacco Science*, 28, 156–158.

19. Bush L, Cui M, Shi H, Burton H, Fannin F, Lei L and Dye N. Formation of tobacco-specific nitrosamines in air-cured tobacco. (2001) *Recent Advances in Tobacco Science*, 21, 23–46.

20. Kozlowski, L. T.; Rickert, W. S.; Pope, M. A.; Robinson, J. C.; Frecker, R. C. Estimating the yield to smokers of tar, nicotine, and carbon-monoxide from the lowest yield ventilated filter-cigarettes. *Br. J. Addiction* **1982,** *77*, 159.

21. Calafat AM, Polzin GM, Saylor J, Richter P, Ashley DL, Watson CH, Determination of tar, nicotine, and carbon monoxide yields in the mainstream smoke of selected international cigarettes, *Tobacco Control,* **2004**;13(1): 45-51

22. Wu W, Ashley DL, Watson CH. Simultaneous determination of five tobacco-specific nitrosamines in mainstream cigarette smoke by isotope dilution liquid chromatography/electrospray ionization tandem mass spectrometry, *Anal Chem.* **2003**;75(18):4827-32.

23. Wagner, KA; Finkel, NH; Fossett, JE; Gillman, IG Development of a Quantitative Method for the Analysis of Tobacco-Specific Nitrosamines in Mainstream Cigarette Smoke Using Isotope Dilution Liquid Chromatography/Electrospray Ionization Tandem Mass Spectrometry, *Anal. Chem* **2005**;77(4):1001-6.

24. Caldwell, W. S.; Conner, J. W. Artifact formation during smoke trapping: an improved method for determination of N-nitrosamines in cigarette smoke. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 783-9.

25. Polzin, GM; Kosa-Maines, RE; Ashely, DL; Watson, CH. Analysis of volatile organic compounds in mainstream cigarette smoke. *Env. Sci. Tech.* (2006) in press

26. Taylor, J. K. *Quality Assurance of Chemical Measurements;* CRC Press: Boca Raton, 1987.