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Assessment of nicotine uptake from cigarette smoke: comparison of a colorimetric test strip (NicCheck I™) and gas chromatography/mass selective detector

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Colorimetric test strip assays are a convenient and inexpensive means for the determination of cotinine in human urine because they can be performed in a non-laboratory environment using a trained technician. Four hundred human urine samples were separated into four categories: (1) heavy smokers (>20 cigarettes smoked per day), (2) light smokers (<20 cigarettes smoked per day), (3) non-smokers, and (4) vegetarian non-smokers. Samples were evaluated by a gas chromatography/mass selective detector (GC/MSD) method as a reference and using NicCheck I™ (DynaGen, Inc.). Colour intensity can range from 0 (no colour) to 14 (deep pink). Qualitative values were assigned as negative (0), low (1–6) and high (7–14). Comparison of the test strip and GC/MSD results showed: (1) 43 (10.75%) false negatives using the criterion of a GC/MSD cotinine level above 200 ng ml^{-1} and test strip reading of 0, (2) 31 (7.75%) false positives using the criterion of a GC/MSD cotinine level below 1 ng ml^{-1} and a test strip reading of 1 or greater, and (3) no correlation between the test strip and GC/MSD results ($r = 0.597$, $p < 0.05$). The fact that the colorimetric reaction is sensitive to many nicotine metabolites and/or heterocyclic amine structures whereas the GC/MSD method measures nicotine and cotinine selectively might explain the false positive results. False negative results were likely to be due to a lack of sensitivity of the test strip.

Keywords: GC/MSD

Introduction

There are several approaches to the determination of nicotine and its metabolites in biological fluids. Among these are enzyme immunoassay (Bjercke *et al.* 1986, 1987, Chang *et al.* 1992), radioimmunoassay (Langone *et al.* 1973, Knight *et al.* 1985), gas chromatography (Jacob *et al.* 1981, Vereby *et al.* 1982), high performance liquid chromatography (Hortsman 1985, Lequaug *et al.* 1989) and colorimetry (Peach *et al.* 1985, Barlow *et al.* 1987). Cotinine is a major metabolite of nicotine and has historically been the marker of choice for exposure to tobacco smoke due to its long half-life (Benowitz *et al.* 1983). The matrix generally used to determine cotinine level is urine. Chromatographic assays for cotinine currently require a laboratory setting and highly trained personnel. Immunoassays are more

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convenient but also require a laboratory setting, although less training is needed for this method. Whereas immunoassays are easier to carry out, they have been shown to be non-specific for the determination of cotinine (Schepers and Walk 1988, Anderson and Proctor 1991). They would be expected to yield falsely elevated results due to the presence of other nicotine metabolites, which may demonstrate varying analytical sensitivities. Immunoassay methods may also suffer from falsely elevated results due to structurally related compounds. Chromatographic approaches would therefore be the method of choice and the benchmark.

The colorimetric approach is more convenient than both the chromatographic and immunoassay approaches and has been adapted to a 'test strip' (NicCheck I^{TM}) format in which the colour reaction is read by eye after immersion of the strip into a sample of unmodified urine (Amruta *et al.* 1996). While the test strip assay can be conducted conveniently in a non-laboratory environment, it would be expected to be susceptible to falsely elevated results due to nicotine metabolites. The colour reaction reacts non-specifically with unsubstituted pyridine rings and has been shown to react with aldehydes and ketones (Barlow *et al.* 1987). A number of commonly used drugs and/or their metabolites possess these structures (Moffat *et al.* 1986) and might be expected to interfere at some level with the test strip assay. In addition, dietary sources of heterocyclic amines have been shown to form from cooked beef and might also interfere with the test strip assay at some level (Friesen *et al.* 1996).

This study evaluated the degree to which selected drugs, drug metabolites and a non-vegetarian diet may interfere with the test strip assay. The purpose of this study was not to investigate the validity of the manufacturer's procedures, but to evaluate the test strip under normal conditions of use. The potentially interfering compounds were selected for their structural potential for interference, their expected concentrations in urine and their availability. These compounds were added to blank urine, *in vitro*, at concentrations representing high and low levels as predicted from pharmacopoeial information (Thomas *et al.* 1990, Sakamoto and Nakamura, 1994, United States Pharmacupia Convention 1997) and tested using the test strips. It is noteworthy that in preliminary literature searches, it was found that several commonly used antihistamines and vitamin supplements have the structural elements necessary for interference. The effect of a non-vegetarian diet was assessed by the comparison of urine samples from non-vegetarian/non-smoking subjects with those from a vegetarian/non-smoking population. Although the heterocyclic amine concentrations in urine have been found to be quite low in those with a non-vegetarian diet (Friesen *et al.* 1996), these levels were studied to ensure that these amines did not possess high sensitivity to the test strip reaction and thus cause interference.

The primary purpose of this study was the analysis of human urine samples to determine the presence of nicotine and cotinine and to evaluate the reliability of the colorimetric test strip in determining nicotine consumption from cigarette smoke. Subjects were classified as heavy smokers (20 or more cigarettes smoked per day), light smokers (less than 20 cigarettes smoked per day), non-smokers, or vegetarian non-smokers. The clinical portion of the study was conducted in The Center for Drug Studies, Virginia Commonwealth University in Richmond, Virginia, USA. Nicotine and nicotine metabolites were determined using a colorimetric strip test (NicCheck I^{TM}) manufactured by DynaGen Inc., Cambridge, Massachusetts, USA. Urine samples were also evaluated using a validated

gas chromatography/mass selective detector (GC/MSD) method to determine nicotine and cotinine levels.

Methods

Subject recruitment and categorization

Four hundred human subjects were recruited by The Center for Drug Studies, Virginia Commonwealth University in Richmond, Virginia, USA, in accordance with the policies and with the approval of the Institutional Committee for the Conduct of Human Research. Subjects were classified as heavy smokers (20 or more cigarettes smoked per day), light smokers (less than 20 cigarettes smoked per day), non-smokers or vegetarian non-smokers. Demographic data collected on the subjects included gender, age, height, weight, and exposure to passive smoke. All data collected was self-reported. There were no exclusion criteria for subjects, but they were required to answer the following questions: (1) Do you use smokeless tobacco products? (2) Are you exposed to environmental tobacco smoke? (3) Are you currently taking any medication? List medication (4) Are you currently taking vitamin supplements? List supplements (5) Are you a vegetarian? (6) How many cigarettes or other tobacco products do you consume per day? Vegetarians were asked if they ate dairy products, eggs or seafood. The exposure to passive smoke category was broken down into exposed to passive smoke including household, exposed to passive smoke with no household exposure, and not exposed to passive smoke.

Sample collection and storage

A urine sample of at least 80 ml was collected from each subject. Sample collections were conducted randomly throughout the day, as would be the case during normal use of the test strips. Subjects were not instructed to alter their normal routine before their scheduled appointment for urine collection. Samples were immediately frozen at approximately $-18^{\circ}C$. Samples were brought in coolers to the analytical laboratory after being frozen, and were stored at $-20 \pm 10^{\circ}C$ until the day they were assayed.

NicCheck I™ testing

Urine samples were thawed and brought to room temperature. NicCheck I™ strips were removed from refrigeration ($3 \pm 3^{\circ}C$) and brought to room temperature. All samples were analysed within 14 day. An aliquot of 2.0 ml of human urine from each sample was centrifuged for approximately 5 min at 2000 r.p.m. An aliquot of 1.0 ml of supernate from the centrifuged aliquot was transferred to a 10×75 mm test tube. A NicCheck I™ test strip at room temperature was placed in each test tube with the arrow pointing downward. Results were observed after 15 min using the colour chart provided with the NicCheck I™ test strips. The results were reported on a scale of 0 (negative result) to 14 (high nicotine consumption).

Quality control. Quality control samples for the test strip assay were prepared with blank human urine, verified using the GC/MSD procedure, and were stored under similar conditions to the study samples. The spiked concentrations of cotinine in the quality control samples were 0.0, 3.0, 10.0 and 40.0 $\mu g\,ml^{-1}$. One quality control sample of each concentration was analysed with each sample set. The order of the quality control samples was from low to high concentration of cotinine. Inter-rater reliability was evaluated by having a second blinded analyst randomly read approximately 10% of the urine samples.

Chromatographic assay

GC/MSD. The analytical method for the determination of nicotine and cotinine was used as the benchmark for nicotine consumption and consisted of a three-step liquid-liquid extraction and GC/MSD. The method was similar to the method developed by Jacob *et al.* (1991), with minor adjustments. Internal standards were added to buffered urine and the mixture was extracted with toluene:1-butanol. The analytes were back-extracted into dilute sulphuric acid (0.5 m). The organic layer was discarded, and the acid layer was made basic and extracted with toluene:1-butanol (7:3 v/v). Then 2 µl of the final organic layer was injected into the gas chromatograph. The analytes were detected using electron impact selected ion monitoring. The ions monitored were nicotine (84), nicotine-$^{13}C_3$ (87), cotinine (98) and cotinine-d_3 (101). The calibration range for nicotine and cotinine was 1.0 $ng\,ml^{-1}$ to 1000.0 $ng\,ml^{-1}$. The calibration range was similar to the calibration range used by Jacob *et al.* 1991.

Quality control. Quality control samples for the gas chromatography assay were prepared with blank human urine. The concentrations for both nicotine and cotinine in the quality control samples were 2.0, 50.0 and 800.0 $ng\,ml^{-1}$. A 2000.0 $ng\,ml^{-1}$ quality control sample was analysed to determine dilution integrity.

In vitro *interference study*

Fifteen compounds with heterocyclic amine structures (nitrendipine, zimeldine, pyridoxine, pinacidil, iproniazid, broxaldone, cinchophen, clioquinol, bisacodyl, tacrine, ethionamide, torsemide, phenazopyridine, carbinoxamine and brompheniramine) were selected for potential cross-reactivity with the test strip assay. Compounds were prepared in blank human urine; the concentrations tested were 5.0, 10.0, 500.0 and 1000.0 $\mu g\,ml^{-1}$. Ten compounds (bisacodyl, broxaldone, cinchophen, clioquinol, iproniazid, nitrendipine, pinacidil, pyridoxine, tacrine and zimeldine) were not completely soluble in urine and were tested using MeOH (1000.0 $\mu g\,ml^{-1}$) as the solvent.

Results

Test strip analysis of subject's urine

In the heavy smoker category, 23 of the 76 subjects (30%) were not detected as smokers using the test strip (0 result on NicCheck I^{TM}; self-report heavy smoker). Among these 23 subjects, 15 reported taking no drugs except vitamins. The six subjects taking prescribed drugs were all taking different drugs. Two of these subjects reported taking heterocyclic amines that could theoretically interfere with NicCheck I^{TM} (omeprazole and didanosine).

In the light smoker category, 34 of the 95 subjects (36%) were not identified as smokers (0 result on NicCheck I^{TM}). Among these 34 subjects, there was no correlation between any particular drug class for the subjects taking drugs. One of the 34 subjects reported taking a heterocyclic amine (triamterene).

In the non-smoker category, 58 of the 181 subjects (32%) tested positive using the test strip (1–8 result on NicCheck I^{TM}). Among these 58 subjects, there was no correlation with any particular drug class for the subjects taking drugs. In the positive group, 38 of the 58 subjects were exposed to either household or other environmental cigarette smoke. In the non-smoker category that tested negative with the test strip (0 result on NicCheck I^{TM}), 61 of the 123 subjects were exposed to either household or other environmental cigarette smoke. Four of the 58 subjects who tested positive reported taking one or more heterocyclic amine(s) (triamterene, doxazosin and prazosin).

In the vegetarian non-smoker category, 16 of the 48 subjects (33%) were positive (1–8 result on NicCheck I^{TM}). Among the positives results, eight of the 16 were exposed to either household or other environmental cigarette smoke. Both St John's Wort and *Echinacea* were taken by two of the subjects who tested positive; one subject took St John's Wort and one took *Echinacea*. It should be noted that some commercially available preparations of St John's Wort contain *Lobelia*, and lobeline, a nicotine-like compound, is produced from *Lobelia* (Damaj *et al*. 1997).

Quality control

Quality control samples containing cotinine at 0.0, 3.0, 10.0 and 40.0 $\mu g\,ml^{-1}$ were analysed with the study samples. Assay values obtained for the quality control samples are presented in table 1. The relative standard deviations of 37.5, 25.6 and 13.5 for samples containing 3.0, 10.0 and 40.0 $\mu g\,ml^{-1}$ of cotinine indicate that the test strip results are not precise for semi-quantitative assessment. Qualitative assessment was excellent in the quality control samples, since all the negative samples were determined to be negative and all the positive samples were positive.

Table 1. Test strip quality control results for cotinine-spiked blank urine.

Date	Blank	3 μg/ml	10 μg/ml	40 μg/ml
5 November 1997	0	1	3	8
6 November 1997	0	1	4	6
7 November 1997	0	2	5	9
12 November 1997	0	2	4	8
13 November 1997	0	1	3	7
18 November 1997	0	2	4	7
24 November 1997	0	1	3	7
1 December 1997	0	1	2	6
5 December 1997	0	1	3	7
Mean	0.00	1.33	3.44	7.22
SD	0	0.500	0.882	0.972
SEM	0	0.17	0.29	0.32

Inter-rater reliability was used to evaluate experimenter bias; this is the correlation between two independent raters. Computation of the Pearson product moment produced the values $r = 0.990$ and $r^2 = 0.980$ ($p < 0.05$). Of the test strip results that were tested for inter-rater reliability, 88% demonstrated identical results and the remaining 12% were within 1 unit. No consistent directional error appeared to occur between analysts.

The study samples (NicCheck I^{TM}, first assay) were analysed within a 2 week interval after they arrived in the laboratory. The values obtained for the quality control samples, during the course of the sample analyses (over 5 weeks), revealed no trend showing declining or increasing cotinine levels.

Chromatographic analysis and comparison to the test strip results

Thirty-one (7.75%) of the NicCheck I^{TM} results demonstrated false positive results relative to the reference method (a cotinine level below the limit of quantification [BLQ] by GC/MSD and test strip readings of 1 or greater). Forty-three (10.75%) demonstrated false negative results (a cotinine level above 200.0 ng ml^{-1} and a test strip reading of 0). The 200.0 ng ml^{-1} cotinine level is the criterion used by NicCheck I^{TM} to differentiate between smokers and non-smokers (Eswara *et al.* 1996). Using a more conservative 500.0 ng ml^{-1} cotinine level, which falls in the range reported for cigarette smokers by Jacob *et al.* (1999), 33 (8.25%) demonstrated false negative results (test strip reading of 0).

One hundred and four subjects were BLQ by GC/MSD for cotinine, and 31 of these had test strip readings of 1 or greater (29.81%). One hundred and fifty-five subjects had cotinine levels above 200.0 ng ml^{-1}, and 43 of these had test strip readings of 0 (27.74%). These results are summarized in table 2.

A second NicCheck I^{TM} assay of 35 study samples was performed to verify the initial NicCheck I^{TM} results. Only two study samples were greater than ±2 scale points of the original NicCheck I^{TM} results. Degradation of the study samples is not a likely explanation for any observed disagreement since no degradation of the quality control samples was observed over the assay period.

Analysis of the combined data from the GC/MSD and the test strip methods demonstrates a lack of agreement for quantitative (GC/MSD) and semi-quantitative (NicCheck I^{TM}) data. Looking at cotinine levels above 500.0 ng ml^{-1} in

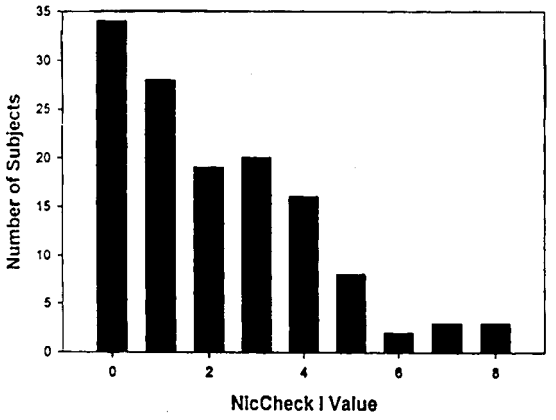

Figure 1. Subjects with GC/MSD levels of cotinine above 500.0 ng ml^{-1}.

Table 2. Comparison of GC/MSD versus NicCheck I^{TM} results.

Cotinine concentration on GC (ng/ml)	No. of subjects	NicCheck I^{TM} value = 0		NicCheck I^{TM} value ≥ 1	
		Number	% of total subjects	Number	% of total subjects
BLQ	104	73	18.25	31	7.75[a]
< 100	235	166	41.50	69	17.25
< 200	245	172	43.00	73	18.25
< 500	267	181	45.25	86	21.50
> 200	155	43	10.75[b]	112	28.00

[a] False positives.
[b] False negatives.

relation to all the observed test strip readings suggests that the distribution of test strip results is skewed to lower semi-quantitative readings (figure 1). This indicates a negative bias for the test strip results compared with the GC/MSD results for cotinine. It would not be expected that the highest frequency among this group of subjects would be a test strip reading of 0. Analysis of test strip results of 1 and 2 produced GC/MSD results with 66 subjects below 100.0 ng ml^{-1}, 13 subjects in the range from 100.0 to 500.0 ng ml^{-1}, 21 subjects in the range from 501.0 to 1000.0 ng ml^{-1} and 26 subjects over 1000.0 ng ml^{-1}. Analysis of test strip results of 3–5 produced GC/MSD results with five subjects below 500.0 ng ml^{-1}, 14 subjects in the range from 500.0 to 1000.0 ng ml^{-1} and 30 subjects over 1000.0 ng ml^{-1}. Analysis of test strip results of 6–8 produced GC/MSD results with one subject below 1000.0 ng ml^{-1} and seven subjects above 1000.0 ng ml^{-1} (table 3). This data indicates that there is little positive bias of the NicCheck I^{TM} results versus the GC/MSD results. A comparison of the GC/MSD results of vegetarian and non-vegetarian non-smokers produced no significant difference between the two groups. Vegetarians had a mean cotinine level of 8.9 ng ml^{-1} and non-vegetarians had a mean cotinine level of 26.7 ng ml^{-1} (including one subject with a level of 2777.6 ng ml^{-1}).

The correlation between NicCheck I^{TM} and cotinine levels (GC-MSD) was determined using the Pearson product moment. The correlation coefficient was

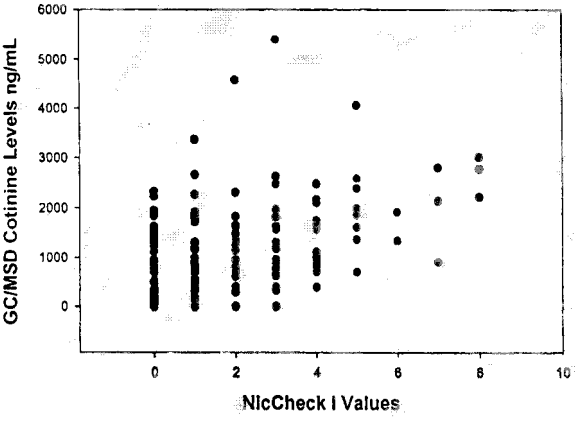

Figure 2. Scatterplot of all subjects: Pearson product moment $r = 0.597$ ($p < 0.05$).

Table 3. Number of subjects with positive NicCheck I™ results according to GC/MSD results.

	Test strip results		
Cotinine concentration on GC (ng/ml)	1–2	3–5	6–8
<100	66 (52.4%)	2 (4.1%)	—
100–500	13 (10.3%)	3 (6.1%)	—
501–1000	21 (16.7%)	14 (28.6%)	1 (12.5%)
>1000	26 (20.6%)	30 (61.2%)	7 (87.5%)
Total	126	49	8

0.597 and is presented graphically as a scatterplot in figure 2; there was no significant correlation between the NicCheck I™ results and the cotinine results measured by GC/MSD ($p < 0.05$).

In vitro *interference study*

Twelve of the compounds tested produced NicCheck I™ results of 0, and two compounds produced NicCheck I™ results of 1. No further testing of the two compounds in methanol (pinacidil and zimeldine) was carried out since the concentration of 1000.0 μg ml^{-1} is above the concentrations expected in human urine.

Validation/quality control of the GC/MSD method

The gas chromatography validation results are presented from inter-run samples in table 4. Results for three freeze and thaw cycles demonstrated no significant change in the nicotine or cotinine concentrations.

No interferences for nicotine or cotinine were detected in the blank urine samples analysed by GC/MSD with each analytical run.

The study samples were analysed by GC/MSD within 8 months after the shipments arrived in the laboratory.

Table 4. Inter-run quality control.

	Nicotine concentration (ng/ml)				Cotinine concentration (ng/ml)			
Run	2	50	800	2000	2	50	800	2000
Urinico8	2.01	52.90	785.91	1924.31	1.98	50.67	788.19	1974.90
	2.25	52.30	776.17	1934.47	2.19	49.57	798.13	1946.73
Urinico9	2.19	49.11	683.56	1921.71	2.05	50.28	818.13	1949.53
	2.01	46.94	760.78	1898.35	1.89	50.98	810.17	1941.32
Urinico10	1.90	47.77	791.88	1908.89	1.90	47.91	801.94	1935.24
	1.86	46.90	782.43	1902.89	1.93	48.58	804.71	1913.18
Urinico11	2.22	49.47	790.47		2.30	46.84	839.36	
	2.01	50.91	805.63		2.12	48.30	871.29	
Mean	2.06	49.54		1915.10	1.99	49.67	803.55	1943.48
SD	0.147	2.325		13.942	0.114	1.215	10.253	20.125
%RSD	7.1	4.7		0.7	5.7	2.4	1.3	1.0
%DFN	3.0	0.9		4.2	0.5	0.7	0.4	2.8

The lowest standard concentration for nicotine and cotinine, and thus the limit of quantification (LOQ), was $1.0\,ng\,ml^{-1}$ for GC/MSD. The highest standard concentration for nicotine and cotinine was $1000.0\,ng\,ml^{-1}$. Experimental calibration data was shown to fit the calibration model through analysis of residuals, in which all the percentage relative concentration residuals were less than 10%.

Discussion

It has been demonstrated that nicotine is present in some foods, especially those from the family Solanaceae (Davis *et al.* 1991), and that there are differences in response to nicotine based on the route of administration (Lunell *et al.* 1996). It has been known since the middle of the 1800s that some regularly consumed plants other than the tobacco plant contain nicotine. The concentrations range from $3.8\,ng\,g^{-1}$ for the cauliflower to $100.0\,ng\,g^{-1}$ for the eggplant or aubergine (Domino *et al.* 1993). Davis *et al.* (1991) reported that instant tea had concentrations of nicotine of up to $285\,ng\,g^{-1}$ wet weight. Another important consideration in the potential nicotine content of consumed foods is the use of nicotine-based insecticides in many countries that are key food producers.

There is evidence that nicotine is metabolized differently in different ethnic populations (Ahijevych and Wewers 1994). There is also evidence of differences in the metabolism of nicotine between the sexes (Hee *et al.* 1995). Kyerematen *et al.* (1990) reported that nicotine and cotinine are metabolized differently by smokers and non-smokers, and that the half-life for both compounds is longer in non-smokers than in smokers. The limiting factor in the metabolism of nicotine is hepatic blood flow, while the limiting factor in cotinine metabolism is enzymatic liver activity. Among smokers there is over a two-fold variation in the conversion of nicotine to cotinine (Benowitz and Jacob 1994). Environmental exposure to lead may possibly alter the metabolism of nicotine and/or cotinine. Cory-Slechta and Pokora (1995) suggested that exposure to lead in the rat causes changes in cholinergic sensitivity. The clearance of nicotine is also affected by over-the-counter drugs and prescribed drugs. One example is the ability of cimetidine to decrease the clearance of nicotine by 30% and of ranitidine to decrease the clearance of nicotine by 10% (Bendayan *et al.* 1990). It is evident that some

significant individual differences exist for the metabolism of nicotine and cotinine. Although there is no direct evidence, it might be expected that age-related differences also exist in the metabolism of nicotine.

The results produced in this study demonstrated no correlation between the test strip results and the GC/MSD cotinine results. This could be due to the fact that the colorimetric reaction of the test strip is sensitive to not only the cotinine marker but also to other nicotine metabolites. One would expect this test to obtain a positive bias with the test strip results if the test strip approach had a similar sensitivity to the GC/MSD approach. This was not observed, probably due to a lack of sensitivity of the test strip approach relative to the GC/MSD assay. This lack of sensitivity of the test strip assay combined with the positive interactions from non-cotinine metabolites may have caused the lack of correlation between the methods. The sensitivities of the test strip to various metabolites is not known, but considerable variation in the test strip results would be expected in the event that these sensitivities differ significantly. In addition, any significant variation in metabolic pattern between individuals would be expected to yield different test strip results for similar levels of cotinine from nicotine consumption.

Another significant result of this work is the apparent unreliability of self-reported information on nicotine consumption. There were 31% false negatives and 32% false positives for the test strip relative to self-reported information on smoking practices. These were reduced to 10.75% and 7.75%, respectively, when considered relative to the benchmark GC/MSD results. Possible explanations for self-reported false positives are subjects misrepresenting their smoking habits, positive interference with the test strip, exposure to nicotine from other than cigarette smoke, or metabolites besides cotinine being detected by the test strip. Possible explanations for the false negative results relative to self-reported information would include misrepresentations of smoking habits, lack of sensitivity of the test strip method, or negative interference with the test strip. False negative results for GC/MSD would require nicotine and/or cotinine concentrations below $1.0\,\mathrm{ng\,ml^{-1}}$.

A number of reports in the literature describe very high incidences of detectable cotinine levels due to environmental tobacco smoke (Jarvis *et al.* 1992, Weaver *et al.* 1996). In our study, the 7.75% false positives (GC/MSD) included six subjects exposed to household smoke, eight subjects exposed to non-household smoke, and 17 subjects exposed to no environmental smoke according to self-reported information. The analytical methodology used in many previous assessments were immunoassays, which are more sensitive than the test strip but are still susceptible to potentially variable interference from non-cotinine metabolites and perhaps other structurally related compounds. The apparent high rates of nicotine and nicotine metabolite biomarkers among the non-smoking public creates doubts about the validity of non-selective testing for nicotine consumption from cigarette smoke. Domino (1995) states, 'I have had volunteers insist that they are non-smokers who actively avoid smoking areas, and yet they have detectable cotinine levels in their body fluids. Why? I find it hard to believe that 25% of people who volunteer for clinical trials who claim to be non-smokers of tobacco are really smokers'. Selective analytical alternatives need to be more convenient and available so that these types of studies provide meaningful results for the identification of smokers and non-smokers. In order for this to occur, cases of non-selectivity need to be carefully studied to determine the cause of such

discrepancies. This is important in routine testing for cotinine, since test subjects could potentially be denied health insurance benefits based on such results.

Another concern with cotinine testing is that nicotine and nicotine metabolites may be present in biological samples from sources other than tobacco products. It is possible that the source of nicotine or the route of administration may be distinguished by using selective analytical methodology and by looking at metabolite ratios. The evidence that exists to indicate that there are differences in the way nicotine is metabolized in different ethnic or age groups further indicates the importance of the implementation of analytical approaches that are not susceptible to interference from substances other than the intended metabolite. If the sensitivity of the assay is significantly different for different nicotine metabolites and metabolic differences exist between populations, then testing may be different in one ethnic or age group compared with another.

An unexpected result was the lack of significant demonstrable interference from non-nicotine derived heterocyclic amines. Although pinacidil and zimeldine produced positive test strip readings, they had to be added to urine at relatively high concentrations to achieve these results. It is not known what the effect of the methanol matrix was on the results of the amines that could not be dissolved in water. These insoluble amines are not likely to be excreted unchanged in urine. A complete study of exogenous heterocyclic amine interference would involve *in vitro* studies with metabolites of these amines or an assessment of subjects dosed with these compounds who were determined to be negative for nicotine metabolites prior to dosing.

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