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COMMENTARY
Clinical considerations in study designs that use cotinine as a biomarker

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Subjects enrolled in studies are not always screened for routine habits such as smoking. Personal history is not always reliable and therefore an objective biomarker is necessary to screen for smokers. The objectives of this article were to review the metabolism of nicotine and other metabolic considerations associated with smoking; to review some of the routine methods used to assess exposure to nicotine-containing products; to revisit cotinine breakpoints utilized to distinguish smokers from non-smokers during screening for clinical trials; to assess the utility of screening questions regarding smoking practices; and to recommend standards for clinical pharmacology studies. The results indicated that cotinine levels serve as a useful biomarker of tobacco exposure; racial issues may be clinically relevant in determining smoking status; cessation of smoking should occur at least 14 days prior to the start of the study; adverse effects from nicotine withdrawal such as craving, hunger and weight gain may persist for more than 6 months; potential metabolic interactions via cytochrome P2A6 and P1A2 need to be considered when designing a study; and the use of a single calibrator as a breakpoint is acceptable if a categorical outcome such as 'smoker' versus 'non-smoker' is desired. Nicotine from food products is not expected to impact assay sensitivity or to be clinically relevant; a serum cotinine concentration of 10 ng ml^{-1} be employed as a breakpoint for non-smokers versus smokers; other non-invasive alternatives are collection of urine, saliva, or hair (with suggested breakpoints of 200 ng ml^{-1}, 5 ng ml^{-1} and 0.3 ng mg^{-1}, respectively; screening questions be accompanied by testing for cotinine; and the inclusion of smokers in studies should be considered once the impact of smoking on the targeted population is understood.

Keywords: Cotinine, biomarker, assays, screening, questionnaire, clinical study designs.

Introduction

Clinical pharmacology studies typically exclude unsuitable subjects based on medical history, undesirable demographic characteristics, dietary issues, and/or use of drugs, including recreational drugs. However, subjects enrolled in a study are not always screened for routine habits such as smoking. It is well established that tobacco products may alter metabolism as well as response to drugs, as can the adverse events associated with withdrawal from tobacco (Hughes *et al*. 1984, Benowitz 1988, Miller 1989). Adverse effects from nicotine withdrawal such as craving, hunger and weight gain may persist for more than 6 months (Hughes *et al*. 1994).

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As demonstrated by Apseloff *et al*. (1994), self-reported personal history is not always reliable as a means of excluding smokers. Thus an objective marker is needed to screen for smokers.

Cotinine is a major metabolite of nicotine. Many studies have used this substance as a biomarker of tobacco smoking (Ehrlich *et al*. 1992, Apseloff *et al*. 1994, Benowitz 1996, Haufroid and Lison 1998). However, before this biomarker can be successfully employed for assessing exposure to tobacco or other sources of cotinine, it is important to establish a specific range of cotinine levels in the various types of biological samples (e.g. plasma, urine). The objectives of this article are: (i) to review the metabolism of nicotine and other metabolic considerations associated with smoking; (ii) to review some of the routine methods used to assess exposure to nicotine-containing products; (iii) to revisit cotinine breakpoints utilized to distinguish smokers from non-smokers during screening for clinical trials; (iv) to assess the utility of screening questions regarding smoking practices; and (v) to recommend standards for clinical pharmacology studies.

Metabolism of nicotine and cotinine

Nicotine is a major constituent of tobacco and undergoes extensive oxidative metabolism in the human liver. The major pathway of nicotine metabolism is C-oxidation to cotinine. It has been estimated that an average of 70–80% of the nicotine absorbed by a smoker is metabolized to cotinine, and approximately 10–15% of the circulating cotinine is excreted in the urine. The remainder of the cotinine is further converted to other metabolites, including trans-3′-hydroxycotinine, cotinine glucuronide, cotinine-*N*-oxide, norcotinine and trans-3′-hydroxycotinine glucuronide. The other metabolites of nicotine, such as nicotine glucuronide, nicotine-1′-*N*-oxide, and nornicotine, are eliminated in the urine. About 10% of the nicotine is eliminated unchanged in urine (Benowitz 1996). The mean $\pm$SD protein binding values of nicotine and cotinine in the plasma are $4.9 \pm 2.8\%$ and $2.6 \pm 3.5\%$, respectively (Benowitz *et al*. 1982, 1983a). Thus protein binding does not meaningfully alter the pharmacokinetics of nicotine or cotinine. Cotinine is widely distributed throughout the body, with values 25–33% more than the volume of total body water (~ 0.60 l kg^{-1}). The mean plasma half-lives of nicotine, cotinine, and trans-3′-hydroxycotinine in smokers are about 2–3, 17 and 6 h, respectively (Benowitz 1996, Perez-Stable *et al*. 1998).

Other metabolites of nicotine such as trans-3′-hydroxycotinine have been investigated. Although the amount of trans-3′-hydroxycotinine excreted in urine is similar or higher compared with cotinine, the serum concentrations of trans-3′-hydroxycotinine were much lower throughout an observation period of 60 h after infusion of nicotine or cotinine. When trans-3′-hydroxycotinine is infused and the half-life determination post-infusion calculated, the plasma half-life appeared shorter (6 h) than following post-infusion of cotinine (15–17 h) (Scherer *et al*. 1988, Curvall *et al*. 1990, Benowitz and Jacob 2001). There is a need for further research to clarify the relative elimination of trans-3′-hydroxycotinine following cotinine or nicotine administration.

The cotinine level in the blood of smokers is approximately 10 times that of nicotine (Benowitz *et al*. 1983b). Because of the longer half-life and higher plasma concentrations of cotinine, measurement of cotinine is less dependent on the exact timing for blood sampling. Therefore, it is a preferred surrogate measurement to estimate nicotine acquired from tobacco.

In two studies, the clearance and half-life of cotinine have been shown to be ethnically dependent (see Table 1). In the study comparing African Americans to Caucasians, the average clearance and non-renal clearance were about 18% and 23% lower in the African Americans, respectively, (Perez-Stable *et al*. 1998). The half-life appeared to be slightly longer in African Americans, but this difference was not statistically significant. In the study comparing the pharmacokinetics of cotinine in Latinos, Chinese Americans and Caucasians, a statistically significant difference was detected for clearance (total, renal and non-renal) and for half-life (Benowitz *et al*. 2002). Further statistical analysis revealed that both the renal and the non-renal clearance of Chinese Americans was statistically different from that of Caucasians: the average renal clearance was 40% slower and the average non-renal clearance was 30% faster in Chinese Americans. The half-life appears to be race dependent, but not enough data is available to show statistical differences among race comparisons.

There are individual variations in the quantitative relationship between cotinine level and nicotine intake. This variation is thought to be due to individual differences in the percentage of conversion of nicotine to cotinine (ranging from 55–92%) and the clearance rate for cotinine (ranging from 19–75 ml min^{-1}). Several studies have demonstrated the important role of cytochrome P450 2A6 (CYP2A6) in the C-oxidation of nicotine to generate cotinine (Nakajima *et al*. 1996, Murphy *et al*. 1999, Yamazaki *et al*. 1999). The degree of variation in the metabolism of nicotine in humans is possibly due to variable expression of CYP2A6 (Messina *et al*. 1997). Nakajima *et al*. (2000) recently showed that deficient cotinine formation in humans after cigarette smoking is associated with deletion of the CYP2A6 gene.

Table 1. Reported pharmacokinetics of cotinine.

Race	Clearance (ml min^{-1} kg^{-1})	Renal clearance (ml min^{-1} kg^{-1})	Non-renal clearance (ml min^{-1} kg^{-1})	Vss (l kg^{-1})	Half-life (h)
Caucasian[a]	0.68 ± 0.24	0.08 ± 0.04	0.61 ± 0.25	0.80 ± 0.19	15.8 ± 4.9
African American[a]	0.56 ± 0.17	0.10 ± 0.04	0.47 ± 0.18	0.75 ± 0.11	17.7 ± 4.3
p value[c]	0.009	0.10	0.009	0.16	0.07
Latino[b]	0.76 ± 0.32	0.10 ± 0.04	0.66 ± 0.33	0.79 ± 0.21	14.6 ± 5.2
Chinese American[b]	0.60 ± 0.23	0.14 ± 0.06	0.46 ± 0.22	0.83 ± 0.19	18.3 ± 5.4
Caucasian[b]	0.76 ± 0.35	0.09 ± 0.05	0.67 ± 0.34	0.93 ± 0.24	16.1 ± 5.2
p value[c]	0.001	0.006★	< 0.001★	0.12	< 0.001

Values are mean ± SD.
[a]Perez-Stable *et al*. 1998.
[b]Benowitz *et al*. 2002.
[c]$p < 0.05$ indicates statistical significance. ★The value for Chinese Americans is statistically different from that for Caucasians.

Despite interindividual and racial variation, it has been demonstrated that the apparent shape of the disposition profile of nicotine and cotinine are similar in smokers and non-smokers. The half-life and volume of distribution in smokers and non-smokers was not significantly different. However, it is unresolved as to whether the clearance of nicotine in non-smokers is faster or slower compared with smokers (Benowitz and Jacob 1993). After cessation of smoking, blood and urine cotinine concentrations declined to non-smoking levels within 3–4 days (Jarvis *et al*. 1988, Benowitz and Jacob 1993). Therefore, given the population or interindividual variation, a normal range for cotinine should be established to reflect the level of nicotine exposure for individuals before they are enrolled into clinical trials.

Nicotine, smoking and drug interactions

Table 2 provides examples of drugs that are substrates, inhibitors and inducers of CYP2A6 (Pritchard and Wolf 2000). Drugs that are competitive substrates or potent inhibitors of CYP2A6 could result in elevated nicotine concentrations and diminished cotinine concentrations in subjects using tobacco products. Drugs that induce CYP2A6 could result in lower nicotine concentrations and may increase or decrease cotinine levels, since cotinine is both generated by and metabolized by CYP2A6. While nicotine itself is metabolized by CYP2A6, the inhalation of smoke induces cytochrome P450 1A2 (CYP1A2) (Schein 1995, Pelkonen *et al*. 1998, Zevin and Benowitz 1999). CYP1A2 is responsible for the metabolism of numerous drugs (see Table 3) and the activation of some procarcinogens (Ahn *et al*. 2000, Lode 2001, Physicians' Desk Reference 2001, Yew 2002). It has been shown that cigarette smoking can affect the pharmacokinetic and pharmacodynamic properties of many drugs (Miller 1989, 1990, Zevin and Benowitz 1999). The pharmacokinetic interactions between cigarette smoking and certain concomitant medications or xenobiotic exposure may be mediated by the induction of CYP1A2. The polycyclic aromatic hydrocarbons (PAHs) rather than the nicotine in the tobacco smoke are believed to be responsible for this induction. In humans, CYP1A2 is detected only in the liver, where it is regulated by at least two mechanisms: one controlling the constitutive expression levels and the other regulating its inducibility (Butler *et al*. 1992, Landi *et al*. 1999). It has been shown

Table 2. Examples of drugs that are substrates, inducers and/or inhibitors of CYP2A6.

Substrates	Inducers	Inhibitors
Coumarin	Phenobarbitone	Coumarin
Dicoumarol	Dexamethasone	Nicotine
Warfarin	Rifampicin	Methoxsalen
Nicotine		Tranylcypromine
Cotinine		Pilocarpine
Halothane		Menadione
Losigamone		Miconazole
Valproic acid		Clotrimazole
Methoxyflurane		Ketoconazole
Sevoflurane		Psoralen
Methyl *tert*-butyl ether		Metyrapone

Table 3. Examples of drugs that are substrates, inducers and/or inhibitors of CYP1A2.

Substrates	Inducers	Inhibitors
Amitriptyline	Omeprazole	Amiodarone
Carvedilol	Rifampin	Cimetidine
Cilostazol	Ritonavir	Fluorquinolones
Cinnarizine		Ciprofloxacin
Clomipramine		Enoxacin
Clozapine		Gatifloxacin
Cyclobenzaprine		Levofloxacin
Flunarizine		Lomefloxacin
Fluvoxamine		Moxifloxacin
Haloperidol		Norfloxacin
Imipramine		Ofloxacin
Lignocaine (injected)		Sparfloxacin
Mexiletine		Fluvoxamine
Mianserin		Interferon
Mirtazapine		Methoxsalen
Naproxen		Methimazole (thiamazole)
Oestradiol, oestrone		Moclobemide
Olanzapine		Propofol
Propafenone		Ticlopidine
Propranolol		
Riluzole		
Ropinirole		
Ropivacaine		
Tacrine		
Theophylline		
Thiabendazole		
Verapamil		
Zileuton		
Zolmitriptan		

that cigarette smoking upregulates CYP1A2 expression at the transcriptional level and is probably tissue-specific (Quattrochi *et al*. 1994). It may take several days to recover from CYP1A2 induction at the mRNA level, but it may take a much longer time at the protein level. In a study on hepatic cytochrome P450-dependent drug metabolism in rabbits, it was shown that the drug-metabolizing capacity and total P450 enzyme content returned to normal 7 days after the first injection of a stimulating agent (Saitoh *et al*. 1999). In a study assessing the metabolism of theophylline after cessation of smoking for 16 days, the results suggested that clearance of theophylline returned to the level of non-smokers as early as after 7 days (Benowitz *et al*. 1989). In studying the interaction between oltipraz and CYP1A2 in humans, using caffeine as an *in vivo* probe, Sofowora *et al*. (2001) recently reported that the reduction in CYP1A2 activity was reversible: after discontinuing oltipraz, the activity of CYP1A2 returned to 57% and 100% of base activity after 2 and 14 days, respectively. These factors should be taken into consideration when designing studies and interpreting safety, pharmacodynamic and pharmacokinetic data.

Cotinine as a biomarker for tobacco smoking

Chemicals in tobacco, such as carbon monoxide, thiocyanate, cyanide, anabasine and anatabine, can be detected and measured in the blood (SNRT

Subcommittee on Biochemical Verification 2002). Testing for these components of tobacco can require specialized equipment and may be time consuming, and it is expensive to obtain rapid results. In addition, the levels of these chemicals are either non-specific or insensitive (Benowitz 1996, SNRT Subcommittee on Biochemical Verification 2002). Plasma nicotine levels correlate well with smoking exposure, but blood samples must be collected during or immediately after exposure because of the short half-life; 30 min after 6.5 h of smoking, the plasma nicotine concentrations were 0.012–0.044 mg l^{-1} in smokers compared with 0–0.006 mg l^{-1} in non-smokers (Baselt 1995).

Cotinine is highly specific and sensitive for tobacco use in the absence of nicotine replacement therapy (SNRT Subcommittee on Biochemical Verification 2002). Cotinine concentrations have been shown to correlate well with nicotine exposure, and plasma cotinine levels of 2–10 ng ml^{-1} was considered to indicate environmental tobacco smoke exposure (Eskenazi *et al*. 1995). Another study determined that steady-state cotinine levels were linearly and directly related to the daily available nicotine ($r = 0.919$), with an increase in correlation coefficient directly related to the increase in tar and nicotine yield (Rosa *et al*. 1992). However, other research has indicated that nicotine intake per cigarette smoked, as estimated from salivary cotinine levels, does not correspond with expected machine-smoked yields of nicotine. Machine-smoked nicotine yields are poor predictors of nicotine intake in smokers. Cotinine levels in blood, saliva, semen and urine were correlated quantitatively during passive and active smoking (Curvall *et al*. 1990, Vine *et al*. 1993). Cotinine levels in semen and blood were similar in magnitude, but levels were an order of magnitude higher in urine. For non-smokers, smokers of 1–19 cigarettes and smokers of >20 cigarettes, the median cotinine concentrations were 0.8, 220 and 602 ng ml^{-1}, respectively, in semen, 0.8, 137 and 467 ng ml^{-1}, respectively, in blood, and 17, 3516 and 7179 ng ml^{-1}, respectively, in urine. Cotinine levels increased significantly with the increase in the number of cigarettes smoked per day (Vine *et al*. 1993). Since cotinine concentrations are substantial in these three body fluids, any of them can be used to assess smoking status. The advantage of urine collection is that it is non-invasive.

Besides tobacco exposure, some foods, including aubergine, potato, tomato, cauliflower, bell pepper and black tea, contain small amounts of nicotine (Castro and Monji 1986, Davis *et al*. 1991). For the population of countries for which consumption data were available, the mean daily dietary nicotine intake was approximately 1.4 μg per day (2.25 μg per day at the 95th percentile) (Siegmund *et al*. 1999). Davis *et al*. (1991) estimated the average daily intake of nicotine from food sources is about 9 μg, and that the maximum daily consumption of nicotine from these foods would be about 100 μg. They also reported average and maximal daily urinary cotinine levels from food consumption of 0.6 and 6.2 ng ml^{-1}, respectively. Most of these calculations are based on the ingestion of black tea; however, the necessary volume to achieve these amounts would be about 4 l per day. This would result in diuresis, dilute urine and concentrations below 6.2 ng ml^{-1} (Benowitz 1996). Therefore, for most people, nicotine intake from food sources is insignificant compared with cigarette smoking, and is not expected to impact assay sensitivity or be clinically relevant. Dietary restrictions of food sources

of nicotine are not considered necessary in designing a study. In summary, cotinine is a specific and sensitive biomarker for cigarette smoking.

Determination of cotinine levels

Plasma/serum

A typical plasma cotinine level for a smoker is 300 ng ml^{-1}, which corresponds to a daily intake of 24 mg of nicotine. On average, a cigarette smoker absorbs about 1 mg of nicotine per cigarette smoked (Benowitz 1996). Multiple methodologies (enzyme immunoassay, high performance liquid chromatography, radioimmunoassay, Immulite, gas chromatography, liquid chromatography-mass spectrometry) have been employed in the determination of plasma and serum cotinine levels. Examples of some of these methodologies used for screening the smoking status of subjects are discussed briefly below; detailed consideration of very sensitive and specific research tools such as gas chromatography and liquid chromatography-mass spectrometry are beyond the scope of this review.

A competitive-inhibition enzyme immunoassay (EIA) technique was used to analyse serum samples from 30 current smokers, 34 former smokers and 15 non-smokers collected over a 1 year period (Gonzalez *et al*. 1996). Since the mean cotinine level did not change in an individual over time, it was concluded that it could be used reliably to assess the level of smoking. In non-smokers the maximum serum cotinine level was 19.7 ng ml^{-1}, with a mean of 11.2 ng ml^{-1}, and in current smokers the minimum serum cotinine level was 47.8 ng ml^{-1}, with a mean of 564.9 ng ml^{-1}. These results were consistent with radioimmunoassay results (Vine *et al*. 1993).

Lawson *et al*. (1998) determined plasma cotinine concentrations using high performance liquid chromatography (HPLC) and found cotinine levels of 179, 266 and 304 ng ml^{-1}, respectively, in light (10–15 cigarettes per day), moderate (16–30 cigarettes per day) and heavy (>30 cigarettes per day) smokers. Pirkle *et al*. (1996) summarized the distribution of serum cotinine levels based on a survey in the US population aged 4 years and older. The highest cotinine levels were 0.1, 0.8 and 600 ng ml^{-1} in those without reported environmental tobacco smoking exposure, those with reported environmental exposure, and tobacco smokers, respectively.

In our own research, Immulite assays from Diagnostic Products Corporation (Los Angeles, California, USA) were used for the measurement of cotinine levels in serum. Immulite assays are chemiluminescent immunoassays performed on an automated analyser that measure cotinine quantitatively. The detection limit for the method is 2 ng ml^{-1} with 20 μl of serum. The lower and upper limits of quantification are 10 and 500 ng ml^{-1}, respectively. Samples were assayed in duplicate during the validation period of 20 days, two runs per day, for a total of 40 runs and 80 replicates. The precision was 6.3% within run and 16% overall. The antibody used was highly specific to cotinine, with non-detectable cross-reactivity to other naturally occurring compounds that might be present in patient samples. Cross-reactivity to 3-hydroxycotinine was 28%. Values of ≤ 20 ng ml^{-1} were used to distinguish non-smokers from smokers. This assay was used to screen 30 healthy

Table 4. Screening questions for tobacco use.

- Are you exposed to tobacco products by handling them (e.g. farming tobacco, manufacturing cigars, etc.)?
- Do you use tobacco products of any form (e.g. cigarettes, pipes, cigars, chewing tobacco, etc.)?
- Do you smoke?
  If so, in what form: □ cigarettes □ pipe □ cigars
- Approximately how may cigarettes, cigars, or pipes do you smoke per day?
- Are you an ex-smoker? If so, about when [month/year] did you quit? Have you used tobacco products within the last 6 months?
- Do you use any products to help quit or prevent smoking (e.g. gum, patch, etc.)?
- Do you live with a smoker?
- Do you frequent establishments where smoking is allowed?
- Are there any circumstances where you are routinely (at least once a day) exposed to 'second hand' smoke?

subjects who claimed not to smoke. These subjects were asked several screening questions (Table 4) prior to serum cotinine testing. All the enrolled subjects had serum cotinine levels of < 10 ng ml^{-1} (the lower limit of quantification). Thus, appropriate screening questions are effective, and the serum cotinine concentrations in non-smokers should be < 10 ng ml^{-1}.

Urine

The correlation between urinary cotinine and daily tobacco smoke is approximately 75% or greater (Haufroid and Lison 1998). Urinary cotinine reaches peak levels at 6 h after stopping environmental tobacco smoke exposure, remains at an apparent plateau for about half a day, and then decreases with a mean half-life of 19 h (Willers *et al.* 1995). For urinary cotinine analysis, urine samples are stable for up to 30 h at room temperature or for up to 8 days at -25°C (Lequang *et al*. 1994). Another study showed that cotinine measurements made based on the urine samples stored at -20°C for 10 years still allowed a clear separation of smokers and non-smokers, with 92% sensitivity and 100% specificity (Riboli *et al*. 1995).

Urine cotinine levels can be used to follow occupational exposure in tobacco pickers. The urinary cotinine level averaged 35 ng g^{-1} creatinine in unexposed persons and 890 ng g^{-1} creatinine in 16 persons who were heavily exposed at work (Gehlbach *et al*. 1975). In another study, researchers used a urinary cotinine/creatinine ratio (CCR) ≥ 30 ng mg^{-1} to identify children exposed to passive smoking at home (Ehrlich *et al*. 1992).

Table 5 summarizes the results of three different assays routinely used to screen subjects for urine cotinine. The first method is a double antibody radioimmunoassay (RIA) kit from DPC (Diagnostic Products Corporation). In a liquid-phase RIA, ^{125}I-labelled cotinine competes for a fixed time with cotinine (and other nicotine metabolites) in the urine sample for antibody sites. After incubation, separation of bound sites from free is achieved using the phosphoethylene glycol (PEG)-accelerated double antibody method. The antibody-bound fraction is then precipitated and counted. The samples were analysed in duplicate and the concentrations were read from a calibration curve. The detection limit of the method was 5 ng ml^{-1} using 50 μl of urine. The lower and upper limits of quantification were 100 and 15 000 mg ml^{-1}, respectively. Correlation coefficients

Table 5. Screen failures attributed to smoking identified by post-screening questions in various studies.

				Screen failures identified by screening questions		Subjects passing screening questions but positive for cotinine		Cotinine in non-smokers (ng ml^{-1})	
Study	Assay type	Breakpoint used (ng ml^{-1})	Total no. screened	No.	%	No.	%	Mean ± SD	Range
European study 1	RIA	≥ 300	40	8	20	4	10	89 ± 51	16–201
European study 2	RIA	≥ 300	14	0	0	0	0	< 100[b]	
European study 3	RIA	≥ 300	31	0	0	0	0	115 ± 46	99–297
European study 4	RIA	≥ 300	37	3	8.1	0	0	104 ± 17	99–173
European study 5	RIA	≥ 300	51	0	0	0	0	99 ± 1	99–106
European study 6	Immulite	≥ 300	57	2	3.5	2	3.5	101 ± 10	100–163
European study 7	Immulite	< 20[a]	57	2	3.5	0	0	< 10[b]	
US study 1	EIA	≥ 300	12	0	0	0	0	ND	
US study 2	RIA	≥ 300	850	58	6.8	–	–	ND	

RIA, radioimmunoassay; EIA, enzyme immunoassay; ND, not determinable.
[a]Cut-off point in serum.
[b]All values less than the value indicated.

of calibration curves recorded during the validation period were above 0.9930. According to the manufacturer's information, the anti-serum is highly specific for nicotine metabolites, with no cross-reactivity detected with other drugs.

The second method is a chemiluminescent immunoassay called Immulite (Diagnostic Products Corporation) that can also be used for the measurement of cotinine level in serum as described above.

The third method is a cotinine EIA developed by Diagnostic Reagents, Inc. This cotinine assay is a liquid, ready-to-use homogeneous EIA based on the competition of a cotinine-labelled enzyme glucose-6-phosphate dehydrogenase (G6PDH) and the free cotinine in the sample for a fixed amount of cotinine-specific antibody binding sites. In the absence of cotinine, the cotinine-labelled G6PDH is bound by the cotinine-specific antibody and the enzyme activity is inhibited. The enzyme G6PDH activity is determined spectrophotometrically at 340 nm by measuring its ability to convert nicotinamide adenine dinucleotide (NAD) to reduced NAD (NADH). Acceptable control ranges are established with the selected controls supplied. The negative, 100 ng ml^{-1}, 250 ng ml^{-1}, 500 ng ml^{-1}, 1000 ng ml^{-1} and 2000 ng ml^{-1} cotinine calibrators should be used for calibration of the assay. Controls should be used at least once a day to validate the assay performance. The cross-reactivity in the assay was 50% for 3′-hydroxycotinine and 0.2% for theophylline, but no cross-reactivity was reported for other drugs.

Proper advertising and pre-screening by phone can reduce the potential for screen failures when recruiting non-smokers. European studies 1–6 were performed sequentially. As can be seen in Table 5, the greatest number of screen failures, as well as subjects passing screening questions but determined to be positive for cotinine, occurred in European study 1. After retraining staff on the importance of smoking habits to our studies, the percentage of failures during screening and subsequent testing of cotinine decreased considerably. In a large US trial investigating a drug metabolized by CYP1A2 (US study 2), proper screening of smokers was important. Even then, as shown in Table 5, there was screen failure of about 7% due to no phone screening prior to subjects arriving at the clinic. This reinforces the necessity for proper phone screening and confirmation of smoking status by testing for cotinine. This table re-emphasizes the usefulness of proper screening questions.

The advantage of using calibrators to define a standard curve is that actual concentrations of cotinine can be obtained. Using a single calibrator for a breakpoint will only provide 'yes' or 'no' answers with regard to cotinine; if the clinical study already has a predefined breakpoint, 'yes' and 'no' answers are quicker to obtain.

The cotinine EIA kit insert published in March 1997 (Diagnostic Reagents, Inc.) states, 'The urine cotinine concentration for non-smokers is normally below 500 ng ml^{-1}, as reported by Jarvis' (Jarvis *et al.* 1987). Urine cotinine levels in non-smokers have been reported to be < 100 ng ml^{-1}, with the majority of subjects having levels less than 80 ng ml^{-1} in urine (Biber *et al*. 1987, Kolonen and Puhakainen 1991, Eremin *et al*. 1992). Riboli *et al*. (1995) suggested 150 ng mg^{-1} creatinine as a cut-off point for tobacco exposure, with 1.5% of alleged non-

smokers being light smokers in their study involved 1369 women in 10 countries. To be sure there is no enrolment of smokers in phase I studies, Apseloff *et al*. (1994) suggested a threshold value of 50 ng ml^{-1}. However, this suggestion may eliminate a substantial number of subjects who have no smoking history and who are not exposed to environmental tobacco smoke, as shown by our own research in Figures 1 and 2. Fifteen out of 19 subjects who were not screen failures had values >50 ng ml^{-1}, and four subjects were screen failures in terms of having urinary cotinine levels of >300 ng ml^{-1} but were allowed to continue in the study. Upon revisiting the screening questions, it was determined that these screen failure subjects either lived with a smoker or travelled on trains where smoking was allowed. In three of the four screen failures the cotinine levels plateaued off to levels <299 ng ml^{-1} within 8 days of confinement. The other subject left the facility and returned again after exposure to environmental tobacco smoke. Only two subjects consistently had cotinine concentrations of <50 ng ml^{-1} throughout the confinement. Excluding the screen failures, only three subjects had cotinine concentrations on day 1 that were >50 ng ml^{-1}; these fell to <50 ng ml^{-1} and stabilized below this breakpoint. Table 5 shows the mean $\pm$ SD and range of urine cotinine concentrations in non-smokers from five of the studies. Of the 216 subjects from these five studies, only two (0.93%) had cotinine concentrations >200 ng ml^{-1}. Based on these results, a breakpoint separating smokers from non-

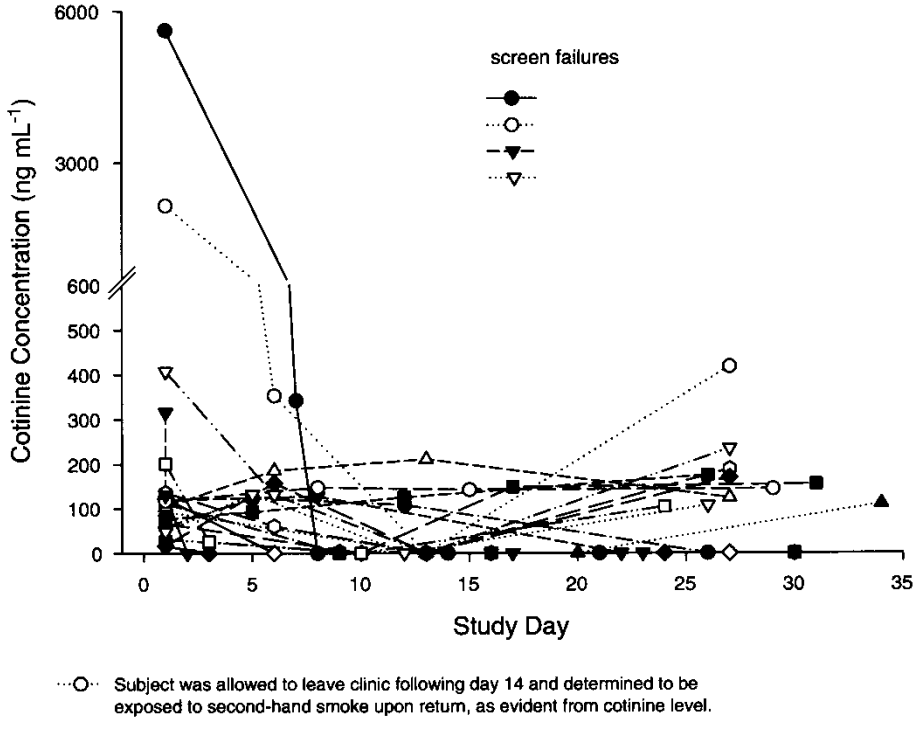

Figure 1. Urine cotinine concentrations in healthy subjects maintained in a smoke-free study unit for up to 35 days in a supervised, contained, controlled environment.

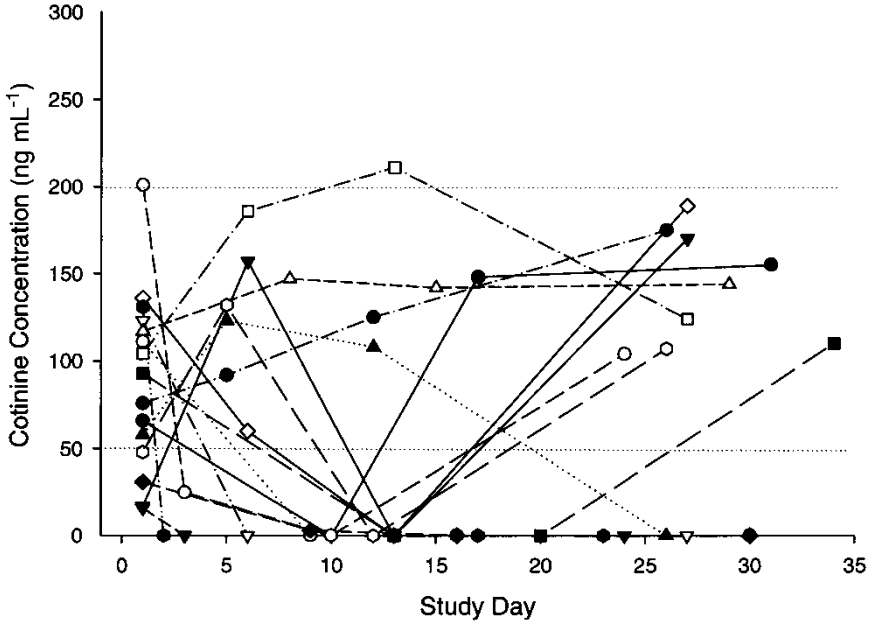

Figure 2. Urine cotinine concentrations in healthy subjects (with a urinary cotinine < 300 ng ml^{-1}) maintained in a smoke-free study unit for up to 35 days in a supervised, contained, controlled environment.

smokers of 200 ng ml^{-1} in urine is recommended when utilizing kits such as the cotinine EIA kit for routine screening of potential study volunteers.

Saliva

Levels of cotinine in saliva have been a somewhat useful measure of recent cigarette consumption. Similar terminal half-lives were seen in saliva and plasma, as the clearance and distribution values in saliva were directly proportional to the corresponding values in plasma. Thus, salivary concentrations would give the same information about cotinine disposition in the body as plasma concentrations (Curvall *et al*. 1990). Saliva cotinine concentrations are about 10–40% higher than in plasma, and can vary depending on stimulation of saliva production (SNRT Subcommittee on Biochemical Verification 2002). Greater accuracy was obtained by correcting for age and a cubic non-linear component (Swan *et al*. 1993). Passive smokers usually have saliva cotinine concentrations < 5 ng ml^{-1}, but heavy passive exposure can result in saliva cotinine levels ≥ 10 ng ml^{-1}. Levels between 10 and 100 ng ml^{-1} may result from infrequent active smoking or regular active smoking with a low nicotine intake. Levels greater than 100 ng ml^{-1} are probably the result of regular active smoking (Etzel 1990). Parazzini *et al*. (1996) suggested a breakpoint of 10 ng ml^{-1} to distinguish non-smokers (≤ 10 ng ml^{-1}) from smokers (> 10 ng ml^{-1}). The breakpoint used will depend on whether infrequent active smoking or regular smoking with a low nicotine intake as well as the use of saliva stimulants would affect the experimental design under investigation.

Hair

While plasma, serum, urine and saliva cotinine levels can be used to detect recent exposure to tobacco smoking, cotinine in hair may reveal information on long-term cigarette smoking. It may indicate tobacco smoking history in subjects who might deliberately abstain for several days before a sample was taken. Hair analysis is a sensitive marker of chemical exposure over a prolonged period of time because the substance is incorporated into the growing hair shaft. Cotinine within the hair shaft could not be removed by hexane washing and thereby must be extracted for analysis by a strong solvent (Haley and Hoffmann 1985).

Cotinine levels in the hair have been shown to reflect systemic exposure to tobacco (Uematsu 1993, Knight *et al*. 1998). Eliopoulos *et al*. (1996) reported a significant correlation between the number of cigarette smoked and measurement of cotinine levels in hair ($r = 0.57$, $p = 0.0008$). Cotinine concentrations in hair were also correlated to those in plasma ($r = 0.42$, $p = 0.02$). The average hair cotinine levels were 1.18 and 1.95 ng mg^{-1} hair in smoking women and men, respectively, while the plasma cotinine concentrations were 297 and 330 ng ml^{-1}, respectively. A RIA method has a lower limit of sensitivity of 0.1 ng mg^{-1} hair for cotinine, based on a 2 mg sample of hair (Klein *et al*. 1994). It has been shown that the average hair cotinine concentrations are 6.3 and 0.3 ng mg^{-1} hair for smoking and non-smoking mothers, respectively (Eliopoulos *et al*. 1994, Klein and Koren 1999). This was very close to the results of Haley and Hoffmann (1985), who reported mean cotinine concentrations of 0.5 and 0.12 ng mg^{-1} hair for smokers and non-smokers, respectively (Haley and Hoffmann 1985). Using a gas chromatographic method, Kintz (1992) reported that cotinine concentrations as low as 0.01 ng mg^{-1} hair could be detected and quantified. In this study the average hair cotinine concentrations were 2.54 and 0.07 ng mg^{-1} hair for smoking and non-smoking mothers, respectively. In summary, measurement of hair cotinine is an economic, non-invasive way to detect tobacco smoking. A cotinine concentration of 0.3 ng mg^{-1} hair may be used as a cut-off to distinguish non-smokers from smokers.

It has been reported that the hair colour may affect nicotine uptake by the hair. White or fair hair has lower nicotine levels than black hair. In order to minimize inter-individual variability, it is advisable to include hair colour as a covariate in the analysis of hair cotinine (Al-Delaimy 2002).

Other considerations in determining nicotine exposure

Geographical location may be important in studies where smoking status is a concern. In the US there is a heightened awareness of exposure to 'second hand' smoke. In fact, many public facilities do not allow smoking or have restricted areas. In Europe, there currently appears to be a higher prevalence of 'second hand' smoke, even though there is a move towards facilities for non-smokers. However, analysing data from studies involving patients enrolled in US and European trials, the prevalence of smoking is about the same (41% in the US versus 42% in Europe). Specific trial information is proprietary, but these transatlantic studies had the same design. The prevalence of smoking may be higher than in the general

population since the disease population was prone to lifestyle compliance issues. The prevalence of tobacco smoking in many countries around the world, such as Canada, Australia, New Zealand, the UK, Africa, Latin America, many parts of Asia, Norway, Chile, Ecuador, Venezuela, Cuba and France, need to be taken into consideration when providing medical treatment.

Conclusions

Cotinine levels may serve as a useful biomarker of tobacco exposure. Cotinine clearance and elimination half-life are race dependent and these factors must be considered in defining breakpoints.

Potential metabolic interactions via CYP2A6 and CYP1A2 need to be considered when designing a study. From a metabolic standpoint, cessation of smoking should occur at least 14 days prior to the start of the study.

There are well-established assays and kits available for detecting cotinine. The choice of the assay depends on the sensitivity and specificity needed for the research being conducted. As a screening tool, the use of calibrators to define a breakpoint is acceptable. Nicotine from food products is not expected to impact assay sensitivity or be clinically relevant. Thus, dietary restrictions related to nicotine are not considered necessary when determining exposure to environmental tobacco smoke or smoking status.

Generally, blood cotinine measurements are chosen only when venipuncture is being performed for another purpose such as part of clinical chemistry testing. Since urine collection is easier and non-invasive, urinary cotinine is often measured. The investigators' comfort level in defining breakpoints to differentiate smokers from non-smokers depends on assay sensitivity and specificity, and the potential clinical impact on the study. The choice of assay must be balanced with costs and the specificity and sensitivity necessary to assess clinical outcomes.

It is our recommendation that a serum cotinine concentration of 10 ng ml^{-1} and a urinary cotinine concentration of 200 ng ml^{-1} be employed as breakpoints to aid in the confirmation of smoking status during study screening periods. Other non-invasive alternatives are salivary cotinine levels (smoker > 10 ng ml^{-1}) and hair cotinine levels (smoker ≥ 0.3 ng mg^{-1} hair). Since concentrations in urine are substantially greater and thus require a less sensitive assay, urine testing is the best alternative. It is highly recommended that screening questions be accompanied by testing for cotinine where smoking or nicotine-containing products may affect the study outcome. Also, geographical location may affect the ability to effectively recruit non-smokers if required for the study.

In the early phases of drug development, a full understanding of metabolism in humans nor the expected adverse event profile of the drug are not known. Hence, it is critical for most early phase clinical trials to exclude the tobacco-smoking population in order to avoid capturing 'noisy data' that will interfere with the interpretation of pharmacokinetic and drug safety results. The inclusion of smokers in studies should be considered once the impact of smoking on the targeted population is understood.

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References

Ahn, H. C., Yang, J. H., Lee, H. B., Rhee, Y. K. and Lee, Y. C. 2000, Effect of combined therapy of oral anti-tubercular agents on theophylline pharmacokinetics. *International Journal of Tuberculosis and Lung Disease*, **4** (8), 784–787.

Al-Delaimy, W. K. 2002, Hair as a biomarker for exposure to tobacco smoke. *Tobacco Control*, **11**, 176–182.

Apseloff, G., Ashton, H., Friedman, H. and Gerber, N. 1994, The importance of measuring cotinine levels to identify smokers in clinical trials. *Clinical Pharmacology and Therapeutics*, **56** (4), 460–462.

Baselt, R. C. 1995, *Disposition of Toxic Drugs and Chemicals in Man*, 4th edition (Chicago: Year Book Medical Publishers), pp. 543–547.

Benowitz, N. L. 1988, Pharmacologic aspects of cigarette smoking and nicotine addiction. *New England Journal of Medicine*, **319** (20), 1318–1330.

Benowitz, N. L. 1996, Cotinine as a biomaker of environmental tobacco smoke exposure. *Epidemiologic Reviews*, **18** (2), 188–204.

Benowitz, N. L. and Jacob, P. 1993, Nicotine and cotinine elimination pharmacokinetics in smokers and nonsmokers. *Clinical Pharmacology and Therapeutics*, **53** (3), 316–323.

Benowitz, N. L. and Jacob, P. 2001, Trans-3′-hydroxycotinine: disposition kinetics, effects and plasma levels during cigarette smoking. *British Journal of Clinical Pharmacology*, **51**, 53–59.

Benowitz, N. L., Jacob, P., Jones, R. T. and Rosenberg, J. 1982, Interindividual variability in the metabolism and cardiovascular effects of nicotine in man. *Journal of Pharmacology and Experimental Therapeutics*, **221** (2), 368–372.

Benowtiz, N. L., Kuyt, F., Jacob, P., Jones, R. T. and Osman, A. 1983, Cotinine disposition and effects. *Clinical Pharmacology and Therapeutics*, **34** (5), 604–611.

Benowitz, N. L., Hall, S. M., Herning, R. I., Jacob, P., Jones, R. T. and Osman, A. 1983, Smokers of low-yield cigarettes do not consume less nicotine. *New England Journal of Medicine*, **309** (3), 139–142.

Benowitz, N. L., Lee, B. L. and Jacob, P. 1989, Nicotine gum and theophylline metabolism. *Biomedicine and Pharmacotherapy*, **43**, 1–3.

Benowitz, N. L., Pérez-Stable, E. J., Herrera, B. and Jacob, P. 2002, Slower metabolism and reduced intake of nicotine from cigarette smoking in Chinese-Americans. *Journal of the National Cancer Institute*, **94** (2), 108–115.

Biber, A., Scherer, G., Hoepfner, I., Adlkofer, F., Heller, W., Haddow, J. E. and Knight, G. 1987, Determination of nicotine and cotinine in human serum and urine: an interlaboratory study. *Toxicology Letters*, **35**, 45–52.

Butler, M. A., Lang, N. P., Young, J. F., Caporaso, N. E., Vineis, P., Hayes, R. B., Teitel, C. H., Massengill, J. P., Lawsen, M. F. and Kadlubar, F. F. 1992, Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. *Pharmacogenetics*, **2**, 116–127.

Castro, A. and Monji, N. 1986, Dietary nicotine and its significance in studies on tobacco smoking. *Biochemical Archives*, **2**, 91–97.

Curvall, M., Vala, E. K., Enzell, C. R. and Wahren, J. 1990, Simulation and evaluation of nicotine intake during passive smoking: cotinine measurements in body fluid of nonsmokers given intravenous infusions of nicotine. *Clinical Pharmacology and Therapeutics*, **47**, 42–49.

Davis, R. A., Stiles, M. F., DeBethizy, J. D. and Raynolds, J. H. 1991, Dietary nicotine: a source of urinary cotinine. *Food and Chemical Toxicology*, **29** (12), 821–827.

Ehrlich, R., Kattan, M., Godbold, J., Saltzberg, D. S., Grimm, K. T., Landrigan, P. J. and Lilienfeld, D. E. 1992, Childhood asthma and passive smoking. Urinary cotinine as a biomarker of exposure. *American Review of Respiratory Disease*, **145** (3), 594–599.

Eliopoulos, C., Klein, J., Phan, M. K., Knie, B., Greenwald, M., Chitayat, D. and Koren, G. 1994, Hair concentrations of nicotine and cotinine in women and their newborn infants. *JAMA*, **271** (8), 621–623.

Eliopoulos, C., Klein, J. and Koren, G. 1996, Validation of self-reported smoking by analysis of hair for nicotine and cotinine. *Therapeutic Drug Monitoring*, **18** (5), 532–536.

Eremin, S. A., Coxon, R. E., Colbert, D. L., Landon, J. and Smith, D. S. 1992, Urinary cotinine fluoroimmunoassay for smoking status screening adapted to an automated analyser. *Analyst*, **117**, 697–699.

Eskenazi, B., Prehn, A. W. and Christianson, R. E. 1995, Passive and active maternal smoking as measured by serum cotinine: the effect on birthweight. *American Journal of Public Health*, **85** (3), 395–398.

Etzel, R. A. 1990, A review of the use of saliva cotinine as a marker of tobacco smoke exposure. *Preventive Medicine*, **19**, 190–197.

Gehlbach, S. H., Perry, L. D., Williams, W. A. and Freeman, J. I. 1975, Nicotine absorption by workers harvesting green tobacco. *Lancet*, **i**, 478–480.

Gonzalez, Y. M., Nardin, A. D., Grossi, S. D., Machtei, E. E., Genco, R. J. and Nardin, E. D. 1996, Serum cotinine levels, smoking, and periodontal attachment loss. *Journal of Dental Research*, **75** (2), 796–802.

Haley, N. J. and Hoffmann, D. 1985, Analysis for nicotine and cotinine in hair to determine cigarette smoker status. *Clinical Chemistry*, **31** (10), 1598–1600.

Haufroid, V. and Lison, D. 1998, Urinary cotinine as a tobacco-smoke exposure index: a mini review. *International Archives of Occupational and Environmental Health*, **71**, 162–168.

Hughes, J. R., Hatsukami, D. K., Pickens, R. W., Krahn, D., Malin, S. and Luknic, A. 1984, Effect of nicotine on the tobacco withdrawal syndrome. *Psychopharmacology*, **83**, 82–87.

Hughes, J. R., Higgins, S. T. and Bickel, W. K. 1994, Nicotine withdrawal versus other drug withdrawal syndromes: similarities and dissimilarities. *Addiction*, **89**, 1461–1470.

Jarvis, M. J., Tunstall-Pedoe, H., Feyerabend, C., Vesey, C. and Saloojee, Y 1987, Comparison of tests used to distinguish smokers from non-smokers. *American Journal of Public Health*, 77 (11), 1435–1438.

Jarvis, M. J., Russell, M., Benowitz, N. L. and Feyerabend, C. 1988, Elimination of cotinine from body fluids: implications for noninvasive measurement of tobacco smoke exposure. *American Journal of Public Health*, **78** (6), 696–698.

Kintz, P. 1992, Gas chromotagraphic analysis of nicotine and cotinine in hair. *Journal of Chromatography*, **580**, 347–353.

Klein, J. and Koren, G. 1999, Hair analysis – a biological marker for passive smoking in pregnancy and childhood. *Human and Experimental Toxicology*, **18**, 279–282.

Klein, J., Forman, R., Eliopoulos, C. and Koren, G. 1994, A method for simultaneous measurement of cocaine and nicotine in neonatal hair. *Therapeutic Drug Monitoring*, **16**, 67–70.

Knight, J. M., Eliopoulos, C., Klein, J., Greenwald, M. and Koren, G. 1998, Pharmacokinetic predisposition to nicotine from environmental tobacco smoke: a risk factor for pediatric asthma. *Journal of Asthma*, **35** (1), 113–117.

Kolonen, S. A. and Puhakainen, E. V. J. 1991, Assessment of automated colorimetric and the high-performance liquid chromatographic methods for nicotine intake by urine samples of smokers' smoking low- and medium-yield cigarettes. *Clinica Chemica Acta*, **196**, 159–166.

Landi, M. T., Sinha, R., Lang, N. P. and Kadlubar, F. F. 1999, Human cytochrome P4501A2. In *Metabolic Polymorphisms and Susceptibility to Cancer*, edited by P. Vineis, N. Malats, M. Lang, A. d'Errico, N. Caporaso, J. Cuzick and P. Boffetta, IARC Scientific Publications no. 148 (Lyon: IARC), pp. 173–195.

Lawson, G. M., Hurt, R. D., Lowell, D. C., Offord, K. P., Croghan, I. T., Schroeder, D. R. and Jiang, N. S. 1998, Application of serum nicotine and plasma cotinine concentrations to assessment of nicotine replacement in light, moderate, and heavy smokers undergoing transdermal therapy. *Journal of Clinical Pharmacology*, **38**, 502–509.

Lequang, N. T., Roussel, G., Roche, D., Migueres, M. L., Chretien, J. and Ekindjian, O. G. 1994, Urine collection for nicotine and cotinine measurements in studies on nicotine addicts. *Pathologie-Biologie*, **42** (2), 191–196.

Lode, H. 2001, Evidence of different profiles of side effects and drug–drug interactions among the quinolones – the pharmacokinetic standpoint. *Chemotherapy*, **47** (3), 24–31.

Messina, E. S., Tyndale, R. F. and Sellers, E. M. 1997, A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. *Journal of Pharmacology and Experimental Therapeutics*, **282** (3), 1608–1614.

Miller, L. G. 1989, Recent developments in the study of the effects of cigarette smoking on clinical pharmacokinetics and clinical pharmacodynamics. *Clinical Pharmacokinetics*, **17** (2), 90–108.

Miller, L. G. 1990, Cigarettes and drug therapy: pharmacokinetic and pharmacodynamic considerations. *Clinical Pharmacy*, **9**, 125–135.

Murphy, S. E., Johnson, L. M. and Pullo, D. A. 1999, Characterization of multiple products of cytochrome P450 2A6-catalyzed cotinine metabolism. *Chemical Research in Toxicology*, **12** (7), 639–645.

NAKAJIMA, M., YAMAMOTO, T., NUNOYA, K., YOKOI, T., NAGASHIMA, K., INOUE, K., FUNAE, Y., SHIMADA, N., KAMATAKI, T. and KUROIWA, Y. 1996, Role of human cytochrome P450 2A6 in C-oxidation of nicotine. *Drug Metabolism and Disposition*, **24** (11), 1212–1217.

NAKAJIMA, M., YAMAGISHI, S., YAMAMOTO, H., YAMAMOTO, T., KUROIWA, Y. and YOKOI, T. 2000, Deficient cotinine formation from nicotine is attributed to the whole deletion of the CYP2A6 gene in humans. *Clinical Pharmacology and Therapeutics*, **67**, 57–69.

PARAZZINI, F., DAVOLI, E., RABAIOTTI, M., RESTELLI, S., STRAMARE, L., DINDELLI, M., VECCHIA, C. L. and FANELLI, R. 1996, Validity of self-reported smoking habits in pregnancy: a saliva cotinine analysis. *Acta Obstetricia et Gynecologica Scandinavica*, **75**, 352–354.

PELKONEN, O., MAENPAA, J., TAAVITSAINEN, P., RAUTIO, A. and RAUNIO, H. 1998, Inhibition and induction of human cytochrome P450 (CYP) enzymes. *Xenobiotica*, **28** (12), 1203–1253.

PEREZ-STABLE, E. J., HERRERA, B., JACOB, P. and BENOWITZ, N. L. 1998, Nicotine metabolism and intake in black and white smokers. *JAMA*, **280** (2), 152–156.

Physicians' Desk Reference, 2001, 55th edition (Montvale: Medical Economics), p. 1614.

PIRKLE, J. L., FLEGAL, K. M., BERNERT, J. T., BRODY, D. J., ETZEL, R. A. and MAURER, K. R. 1996, Exposure of the US population to environmental tobacco smoke. *JAMA*, **275** (16), 1233–1240.

PRITCHARD, M. P. and WOLF, C. R. 2000, Other CYP: 2A6, 2B6, 4A. In *Metabolic Drug Interactions*, edited by R. H. Levy, K. E. Thummel, W. F. Trager, P. D. Hansten and M. Eichelbaum (Philadelphia: Lippincott Williams & Wilkins), pp. 145–150.

QUATTROCHI, L. C., VU, T. and TUKEY, R. H. 1994, The human CYP1A2 gene and induction by 3-methylcholanthrene: a region of DNA that supports Ah-receptor binding and promoter-specific induction. *Journal of Biological Chemistry*, **269** (9), 6949–6954.

RIBOLI, E., HALEY, N. J., TRÉDANIEL, J., SARACCI, R., PRESTON-MARTIN, S. and TRICHOPOULOS, D. 1995, Misclassification of smoking status among women in relation to exposure to environmental tobacco smoke. *European Respiratory Journal*, **8** (2), 285–290.

ROSA, M., PACIFICI, R., ALTIERI, I., PICHINI, S., OTTAVIANI, G. and ZUCCARO, P. 1992, How the steady-state cotinine concentration in cigarette smokers is directly related to nicotine intake. *Clinical Pharmacology and Therapeutics*, **52** (3), 324–329.

SAITOH, T., KOKUE, E. and SCHIMODA, M. 1999, The suppressive effects of lipopolysaccharide-induced acute phase response on hepatic cytochrome P450-dependent drug metabolism in rabbits. *Journal of Veterinary Pharmacology and Therapeutics*, **22**, 87–95.

SCHEIN, J. R. 1995, Cigarette smoking and clinically significant drug interactions. *Annals of Pharmacotherapy*, **29**, 1139–1148.

SCHERER, G., JARCZYK, L., HELLER, W. D., BIBER, A., NEURATH, G. B. and ADLKOFER, F. 1988, Pharmacokinetics of nicotine, cotinine and 3′-hydroxycotinine in cigarette smokers. *Klinische Wochenschrift*, **66** (11), 5–11.

SIEGMUND, B., LEITNER, E. and PFANNHAUSER, W. 1999, Determination of the nicotine content of various edible nightshades (solanaceae) and their products and estimation of the associated dietary nicotine intake. *Journal of Agricultural and Food Chemistry*, **47** (8), 3113–3120.

SNRT SUBCOMMITTEE ON BIOCHEMICAL VERIFICATION 2002, Biochemical verification of tobacco use and cessation. *Nicotine and Tobacco Research*, **4**, 149–159.

SOFOWORA, G. G., CHOO, E. F., MAYO, G., SHYR, Y. and WILKINSON, G. R. 2001, In vivo inhibition of human CYP1A2 activity by oltipraz. *Cancer Chemotherapy and Pharmacology*, **47**, 505–510.

SWAN, G. E., HABINA, K., MEANS, B., JOBE, J. B. and ESPOSITO, J. L. 1993, Saliva cotinine and recent smoking evidence for a nonlinear relationship. *Public Health Reports*, **108** (6), 779–783.

UEMATSU, T. 1993, Utilization of hair analysis for therapeutic drug monitoring with a special reference to ofloxacin and to nicotine. *Forensic Science International*, **63**, 261–268.

VINE, M. F., HULKA, B. S., MARGOLIN, B. H., TRUONG, Y. K., HU, P., SCHRAMM, M., GRIFFITH, J. D., MCCANN, M. and EVERSON, R. B. 1993, Cotinine concentrations in semen, urine, and blood of smokers and nonsmokers. *American Journal of Public Health*, **83** (9), 1335–1338.

WILLERS, S., SKARPING, G., DALENE, M. and SKERFVING, S. 1995, Urinary cotinine in children and adults during and after semiexperimental exposure to environmental tobacco smoke. *Archives of Environmental Health*, **50** (2), 130–138.

YAMAZAKI, H., INOUE, K., HASHIMOTO, M. and SHIMADA, T. 1999, Roles of CYP2A6 and CYP2B6 in nicotine C-oxidation by human liver microsomes. *Archives of Toxicology*, **73**, 65–70.

YEW, W. W. 2002, Clinically significant interactions with drugs used in the treatment of tuberculosis. *Drug Safety*, **25** (2), 111–133.

ZEVIN, S. and BENOWITZ, N. L. 1999, Drug interactions with tobacco smoking. *Clinical Pharmacokinetics*, **36** (6), 425–438.