Pascal Kintz,[1] *Ph.D.; Bertrand Ludes,*[1] *Ph.D.; and Patrice Mangin,*[1] *Ph.D.*

Evaluation of Nicotine and Cotinine in Human Hair

REFERENCE: Kintz, P., Ludes, B., and Mangin, P., **"Evaluation of Nicotine and Cotinine in Human Hair,"** *Journal of Forensic Sciences,* JFSCA, Vol. 37, No. 1, Jan. 1992, pp. 72–76.

ABSTRACT: To validate data on tobacco use, the authors investigated the use of hair samples for quantifying nicotine and cotinine by gas chromatography/mass spectrometry. Hair was taken from 22 nonsmokers and 42 smokers, cut close to the scalp at the back of the head.

The hair (about 100 mg from each subject) was incubated in 3 mL of 1*N* NaOH at 100°C for 1 h. After this, the samples were extracted by diethyl ether. The drugs were separated on a 12-m BP-5 capillary column and detected using selected ion monitoring (nicotine, *m/z* 84; cotinine, *m/z* 98).

Hair from nonsmokers and smokers contains nicotine and cotinine. Although it is difficult to determine an absolute cutoff level, an amount greater than 2 ng of nicotine per milligram of hair can be used to differentiate smokers from nonsmokers. In the population of nonsmokers, the influence of environmental smoke exposure was noted.

KEYWORDS: toxicology, tobacco, nicotine, cotinine, hair

Noninvasive validation of cigarette or cigar smoking behavior data is necessary for large population health studies. It is not always possible or desirable to sample blood from participants in such studies. Urine or saliva samples can be used for confirmation of recent nicotine intake by analysis of cotinine, the major metabolite of nicotine [*1,2*].

However, this test is not suitable for validation of survey data, since the quantification of cotinine in saliva only reflects nicotine exposure during the preceding week [*3*] and does not necessarily indicate the frequency of smoking in subjects who might deliberately abstain for several days before annual biomedical screenings.

Therefore, it is important to have a method for evaluating longer term use of tobacco products. In this report, we present the usefulness of hair samples for quantifying nicotine and cotinine. The presence of these drugs and their amounts should validate information on tobacco use.

Materials and Methods

Hair was collected from smokers and nonsmokers among laboratory personnel. To qualify as a nonsmoker, the individual had to have refrained from cigarette smoking for one year. The studied population included the following:

(a) nonsmokers: 8 men and 14 women, aged from 7 to 59 years, and
(b) smokers: 26 men and 16 women, aged from 22 to 66 years.

Received for publication 12 March 1991; revised manuscript received 27 April 1991; accepted for publication 11 June 1991.

[1]Forensic toxicologist, forensic biologist, and forensic toxicologist, respectively, Institut de Medecine Legale, Strasbourg, France.

All the subjects were Caucasian with naturally colored hair. For each subject, about 100 mg of hair was cut close to the scalp at the back of the head.

Analytical Methods

Since the influence of environmental smoke exposure was being investigated, the hair samples were not washed. Each sample was homogenizated in 3 mL of 1*N* sodium hydroxide (NaOH) for 1 h at 100°C.

The drugs were extracted using 5 mL of diethyl ether in the presence of 20 μL of ketamine (1 mg/L) as an internal standard. After agitation and centrifugation, the organic phase was removed and evaporated to dryness. The residue was dissolved in 20 μL of methanol and 1 μL of the solution was injected into a 12-m by 0.22-mm inside-diameter BP-5 capillary column. The flow rate of the carrier gas (helium N55) through the column was 3.2 mL/min. The column oven temperature was programed from an initial temperature of 60°C (held for 0.9 min) to 280°C at 30°C/min and held at 280°C for the final 5 min. Splitless injection with a split valve-off time of 0.9 min was employed.

The gas chromatograph (GC) system consisted of a Perkin-Elmer (Model 8500) chromatograph with an ion trap detector (ITD). The ITD was operated in the electron impact mode at 70 eV with an ion source temperature of 210 to 220°C. The electron multiplier voltage of the detector was set at 1550 V. For detection and quantification, selected ion monitoring was used as follows: nicotine, *m/z* 84; cotinine, *m/z* 98; and ketamine *m/z* 180.

With this technique, nicotine and cotinine can be quantified in concentrations for approximately 0.01 ng/mg of hair.

Results and Discussion

In the hair of nonsmokers, the nicotine content ranged from 0.06 to 1.82 ng/mg and the cotinine from 0.01 to 0.13 ng/mg. There was significantly more nicotine in the hair of smokers, with a nicotine concentration range from 0.91 to 33.89 ng/mg and a cotinine range from 0.09 to 4.99 ng/mg (Table 1). The distributions are shown by histograms in Figs. 1 and 2.

It was possible to determine a nicotine cutoff value of 2 ng/mg of hair for distinguishing smokers from nonsmokers when using the unwashed hair samples. In a previous study [*4*], the authors had determined, using 10 nonsmokers and 10 smokers, that the nicotine concentrations for nonsmokers ranged from nondetectable to 11.3 ng/mg and those for smokers from 3.0 to 38.7 ng/mg, which indicated that no clear cutoff value could be established in that study. In another study [*5*], hair from both smokers and nonsmokers contained nicotine in concentrations from 18 to 177.2 ng/mg.

In the nonsmoker population, it was possible to distinguish passive smokers from other nonsmokers (Table 2). The nicotine content was over 0.5 ng/mg in the group of passive smokers and lower in nonexposed nonsmokers. The presence of varying amounts of nicotine in passive smokers' hair can be explained by atmospheric deposition of smoke, which is rich in nicotine; the presence of cotinine is less easily explained, as cotinine is

TABLE 1—*Nicotine and cotinine concentrations, in nanograms per milligram.*

	Nicotine	Cotinine
Nonsmokers	0.06 to 1.82	0.01 to 0.13
Smokers	0.91 to 33.89	0.09 to 4.99

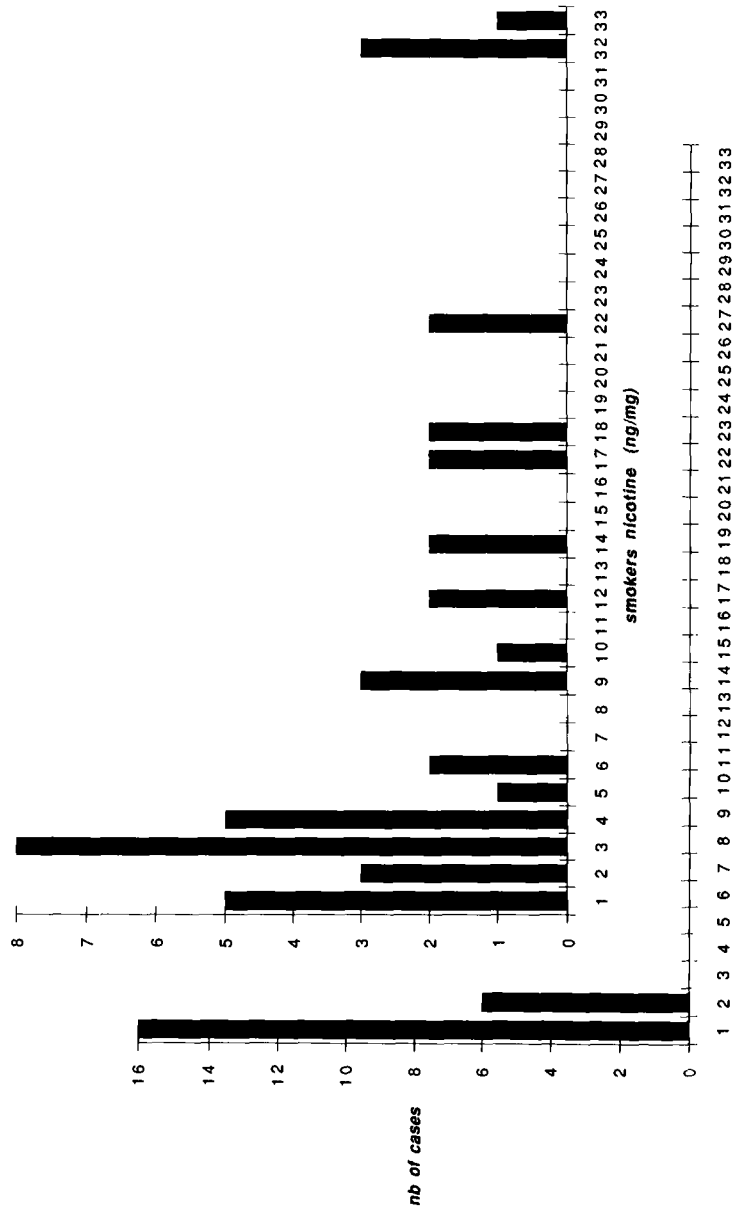

FIG. 1—*Histogram of the nicotine distribution in smokers and nonsmokers.*

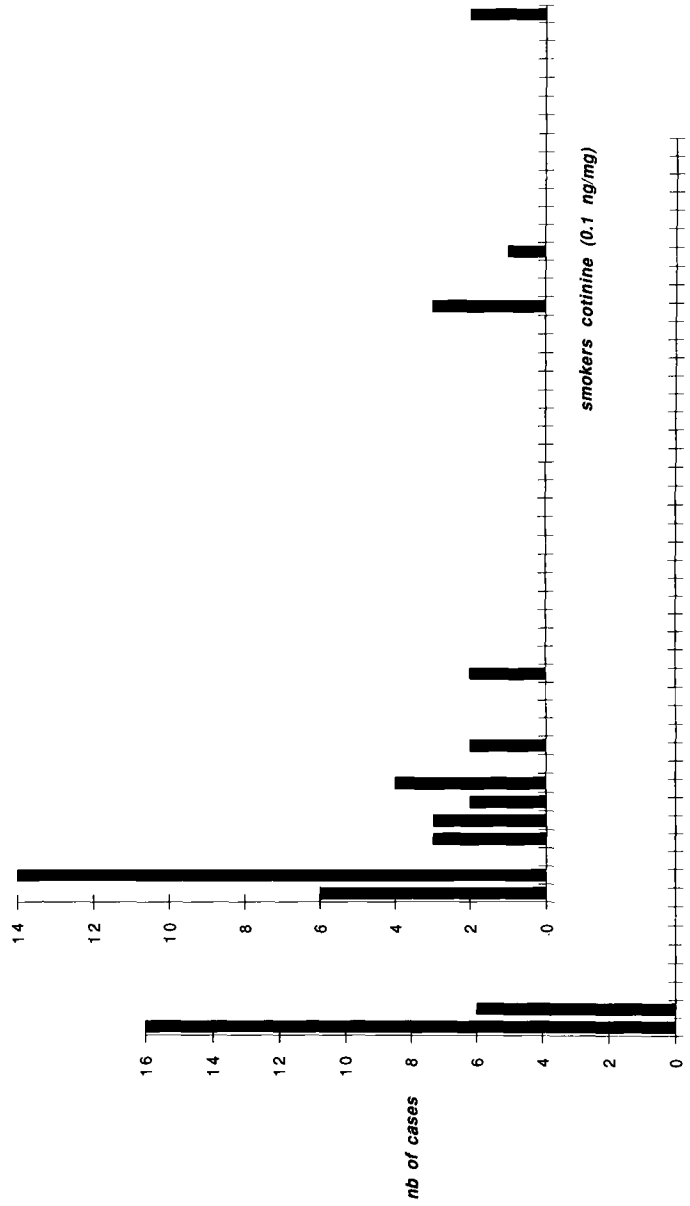

FIG. 2—*Histogram of the cotinine distribution in smokers and nonsmokers.*

TABLE 2—*Nicotine and cotinine concentrations in nonsmokers' hair, in nanogram per milligram.*[a]

	Nicotine	Cotinine
Nonenvironmental exposure	0.06 to 0.33	0.01 to 0.13
Environmental exposure	0.54 to 1.82	0.01 to 0.13

[a]The environmental and nonenvironmental exposures were evaluated by a questionnaire indicating the habits of the nonsmokers (see text).

only produced in vivo [6]. This is why no difference in cotinine concentrations could be noted for passive smokers. Therefore, only the nicotine concentration is the basis for differentiating smokers from nonsmokers and, in the latter population, passive smokers from other nonsmokers.

The high nicotine concentrations in the hair of the passive smokers were confirmed by a questionnaire indicating their habits (the presence of smokers at home, at the workplace, and during spare-time activities; the duration of the exposure; the frequency of exposure).

Testing human hair for nicotine offers the possibility of revealing an individual's recent history of drug exposure, beginning at the sampling time and dating back over a period from months to years.

References

[1] Wald, N. J., Boreham, J., Bailey, A., Ritchie, C., Haddou, J. E., and Knight, G., "Urinary Cotinine as a Marker of Breathing Other People's Tobacco Smoke," *The Lancet*, 28 Jan. 1984, pp. 230–231.

[2] Jarvis, M. J., Russell, M. A. H., and Feyerabend, C., "Passive Exposure to Tobacco Smoke: Saliva Cotinine Concentrations in a Representative Population Sample of Nonsmoking School Children," *British Medical Journal*, Vol. 291, 1985, pp. 927–929.

[3] Haley, N. J., Axelrad, C. M., and Tilton, K. A., "Validation of Self-Reported Smoking Behavior: Biochemical Analyses of Cotinine and Thiocyanate," *American Journal of Public Health*, Vol. 73, 1983, pp. 1204–1207.

[4] Haley, N. J. and Hoffmann, D., "Analysis of Nicotine and Cotinine in Hair to Determine Cigarette Smoker Status," *Clinical Chemistry*, Vol. 31, No. 10, 1985, pp. 1598–1600.

[5] Ishiyana, I., Nagai, T., and Toshida, S., "Detection of Basic Drugs (Methamphetamine, Antidepressants, and Nicotine) from Human Hair," *Journal of Forensic Sciences*, Vol. 28, No. 2, April 1983, pp. 380–385.

[6] Jaffe, J. H. and Kanzler, M., "Nicotine: Tobacco Use, Abuse, and Dependence," *Substance Abuse: Clinical Problems and Perspectives*, Williams and Wilkins, Baltimore, MD, 1981, p. 269.

Address requests for reprints or additional information to
Pascal Kintz, Ph.D.
Institut de Medecine Legale
11 Rue Humann
67085 Strasbourg, France