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## Monitoring of nicotine use in sport

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### Abstract

Nicotine is a psychoactive drug found in the tobacco products which produces physical and mood-altering effects on the brain. Use of nicotine in sports presents a potential health threat for the athlete and may also alter the spirit of sport. The World Anti-Doping Agency (WADA) has included nicotine in the monitoring program from January 2012. The aim of the present work was to reanalyze the data of in-competition samples received during 2011 to study the prevalence of nicotine use or abuse in sports. The data of 2770 routine doping control urine samples received during 2011 for the in-competition testing was reanalyzed for nicotine and its metabolites (cotinine and hydroxy cotinine). The excretion profile of nicotine and its metabolites was also estimated in two regular smoker volunteers. The analysis of routine urine samples ( $n = 2770$ ) showed presence of nicotine & cotinine in 247 (8.9%), out of which, 6 samples showed presence of only cotinine. Out of 247 samples, 215 (87%) showed nicotine concentrations greater than 50 ng/mL; a value assigned by the WADA for monitoring from the year 2012. In 23 (9.3%) samples nicotine concentration was found to be greater than 2000 ng/mL which may be attributed to intentional nicotine abuse amongst sportspersons. Nicotine concentrations ranged from 0.16 - 2.26  $\mu\text{g/mL}$  in urine samples of the regular smokers. The presence of nicotine and its metabolites did not show any variation between the national and international samples. The use (abuse) of nicotine seems to be more popular amongst kabbadi (international) and weightlifting (national) players.

### Introduction

Tobacco consumption is a global issue. Nicotine is a psychoactive drug found in the tobacco products which produces physical and mood-altering effects on the brain and also responsible for addiction issues. Use of smokeless tobacco products like powder, patch etc. have increased in sports due to absence of adverse effects on the respiratory system [1]. However, prevalence of nicotine consumption in sport still suffers from a lack of large-scale comprehensive survey [2]. The World Anti-Doping Agency (WADA) has included nicotine in the monitoring program from January 2012 [3]. The aim of the present work was to monitor the use of nicotine consumption in sports by testing of nicotine and its metabolites in the competition samples received during 2011.

### Experimental

Reagents and reference materials:

Nicotine, cotinine, N-10-methylphenothiazine (NMPZ) and diphenylamine (DPA) were purchased from Sigma-Aldrich (India). All other reagents and chemicals were of analytical or ACS grade.

Urine samples:

The data of 2770 routine doping control urine samples received during 2011 for the in-competition testing were reanalyzed for nicotine and its metabolites (cotinine and hydroxy cotinine). The urine samples were also collected for 48 hrs from two male regular smoker healthy volunteers (aged 40-45 years) who smoked 15-20 cigarettes per day and one male tobacco chewer (aged 27 years) to study the excretion profile of nicotine and its metabolites.

Sample treatment and instrumental analysis:

The urine samples were prepared by a liquid-liquid extraction method. The internal standards (NMPZ and DPA, each 2 µg/mL) were added to each 5.0 mL sample aliquot alkalized with 500 µL of 5N potassium hydroxide. The extraction was performed with 2 mL of tert. butyl methyl ether for 20 minutes on the rotary shaker. The organic layer was separated and injected onto the instrument.

The gas chromatograph equipped with nitrogen-phosphorus and mass selective detector (GC-NPD-MSD) was used for analysis of samples under the following conditions:

Agilent 7890 A GC coupled to 5975C MSD and Front NPD: Column HP Ultra-2; fused silica 0.22 mm x 12.5 m length, column film thickness 0.33 µm; Oven initial temperature 100°C, hold 0 min; rate 20°C/min; final 300°C, hold 4.5 min; Injector temperature 280°C; detector temperature 300°C; split ratio 5:1; Carrier flow 150 kpa He at 100°C; MS mode scan; Injection volume 4 µL. The NPD detector was used for quantification.

Four point calibration curve (range 50-2000 ng/mL) was used to quantify nicotine and cotinine in samples of the volunteers. For routine doping control samples, a single point calibration curve (50 ng/mL) was used for quantification. The method was validated as per WADA ISL (version 7.0) guidelines [4]. However, hydroxy cotinine was identified qualitatively based on library match (NIST 08.L) due to non-availability of the reference standard. Only those peaks which showed library match (main +EI mass spectra) quality of > 95% were considered.

Results and Discussion

Method validation:

The calibration curve for nicotine and cotinine was linear (25 to 5000 ng/mL) with limit of quantification (LOQ) of 25 ng/mL. The method showed very good precision and accuracy (acceptance criteria ± 15 %).

Urine samples of volunteers:

Nicotine and its main metabolite cotinine were detected and quantitated in all samples of the cigarette smokers and the tobacco chewer, whereas hydroxy-cotinine was detected only in two samples (15 hrs and 20 hrs) of one cigarette smoker. Nicotine and cotinine concentrations ranged from 0.16 - 2.26 µg/mL and 71-156 ng/mL in the urine samples of regular smokers, respectively (Figure 1). The maximum concentration of 2.26 µg/mL of nicotine was observed in the regular smoker.

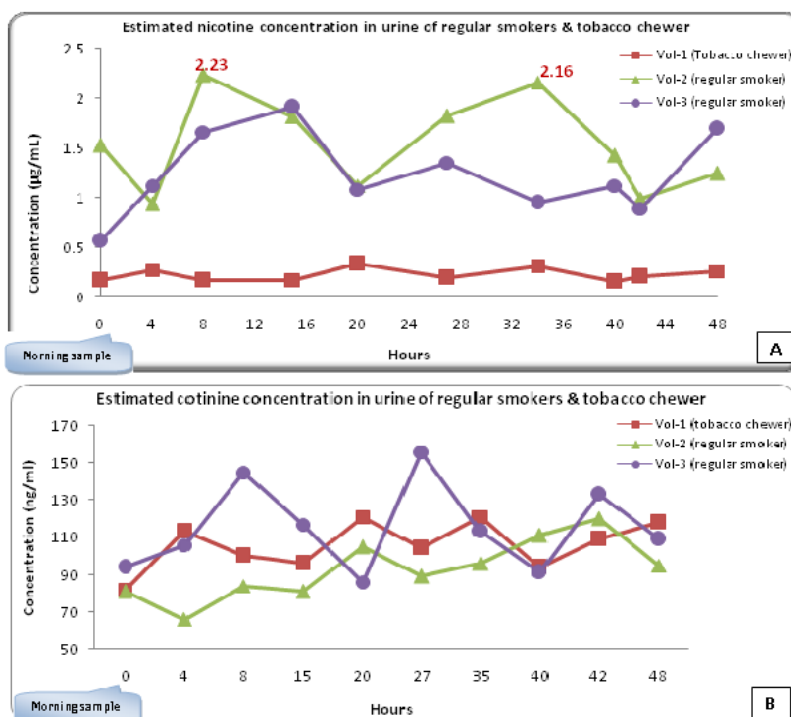


Figure 1: Estimated nicotine (A) and cotinine (B) concentrations in urine samples of volunteers

Routine doping control samples:

The analysis of the data of 2770 urine samples received during 2011 for the in competition testing showed presence of nicotine and cotinine in 247 (8.9%) samples and cotinine was detected only in 6 samples. Hydroxy-cotinine was detected in 47 samples along with nicotine and cotinine. Out of these 47 samples with hydroxy cotinine, 16 samples (6.5%) were from female athletes.

Nicotine : Out of 247 samples, 215 samples (87%) showed values of nicotine greater than 50 ng/mL, a value assigned by the WADA to monitor from the year 2012 [3]. Out of 215, 23 (9.3%) samples showed concentrations greater than 2000 ng/mL and 7 (3%) showed concentrations greater than 4000 ng/mL which may be attributed to the intentional nicotine abuse amongst sportspersons.

Cotinine: Out of 247 samples, 29 samples showed cotinine concentrations in the range of 50-2000 ng/mL and 7 samples showed cotinine concentrations above 2000 ng/mL.

The comparison of national and international samples showed almost an equal distribution of nicotine in the entire concentration range (Figure 2). Focusing the study on the selected sports, maximum nicotine consumption was found in kabaddi and hockey in the international samples and weightlifting and athletics in the national samples (Figure 3 & 4). Further work is in progress to monitor nicotine and its metabolites in routine in-competition samples and regular smokers.

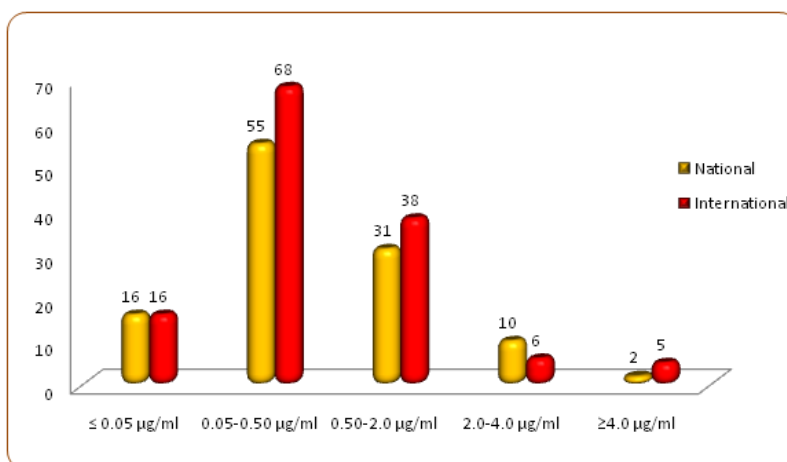


Figure 2: Distribution of nicotine concentrations in national and international samples (in-competition) tested at NDTL (2011)

## Conclusions

Nicotine consumption during competition is an ethical issue apart from being a health hazard. The results of the preliminary study reveal the prevalence of nicotine consumption amongst 9 % of sportspersons. Further work is in progress with higher number of samples to arrive at more decisive findings which may facilitate in considering/establishing a cut off level of nicotine and/or its metabolites in urine so that intentional and unintentional use of nicotine products could be differentiated.

## References

1. Marclay F, Saugy, M. (2010) Determination of nicotine metabolites in urine by LC-MS/MS: trends of smoke and smokeless products exposure in ice hockey. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) Recent Advances in Doping Analysis (18), Köln, pp 147-151.

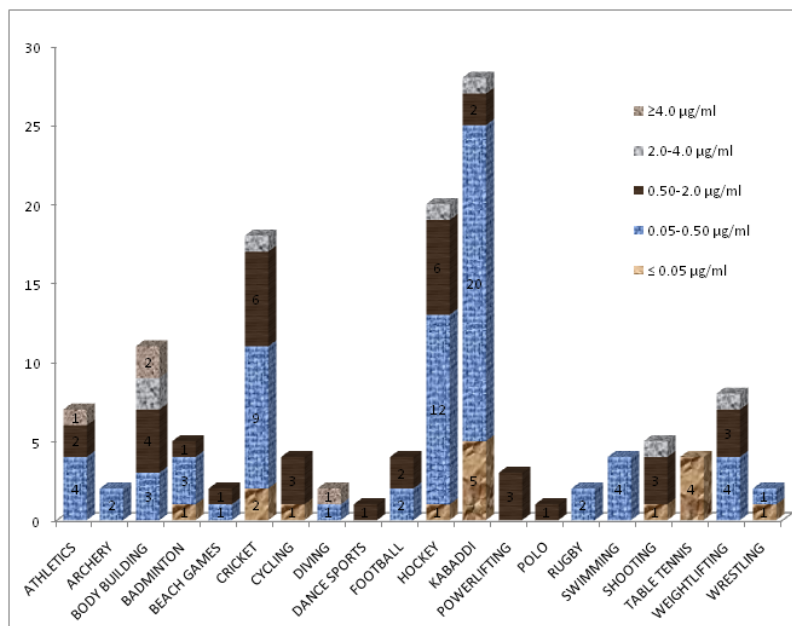


Figure 3: Distribution of nicotine concentrations amongst various sports disciplines (international samples)

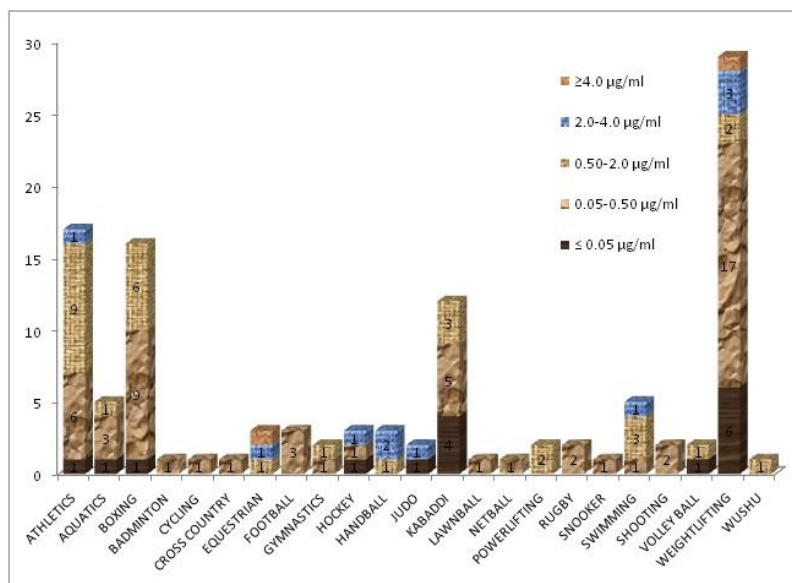


Figure 4: Distribution of nicotine concentrations amongst various sports disciplines (national samples)

2. Schweizer C, Cardis C, Saugy M, Rivier L. (1998) Nicotine in Sport: Use or Abuse. In: In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) Recent Advances in Doping Analysis (5), Köln, pp 269-277.
3. The World Anti-Doping Agency. The 2012 prohibited List. International Standard, Montreal (2012) [http://www.wada-ama.org/rtecontent/document/list\\_2012.pdf](http://www.wada-ama.org/rtecontent/document/list_2012.pdf) (access date 06.01.2012).
4. The World Anti-Doping Code. The WADA International Standard for Laboratories Version 7.0. Available online at [http://www.wada-ama.org/rtecontent/document/International\\_Standard\\_for\\_Laboratories\\_v7\\_0\\_January\\_2012.pdf](http://www.wada-ama.org/rtecontent/document/International_Standard_for_Laboratories_v7_0_January_2012.pdf)

