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Arsenic Concentration in Tobacco Leaves: A Study on Three Commercially Important Tobacco (*Nicotiana tabacum* L.) Types

Nicolas Lugon-Moulin · Florian Martin · Marc R. Krauss · Patrice B. Ramey · Luca Rossi

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Abstract In recent years, arsenic (As) has received increased attention as humans may be exposed to it through occupational and environmental exposure. Tobacco (*Nicotiana tabacum* L.) like other crops can uptake this element from the soil, which may lead to human exposure. Here, we report on a survey on arsenic in cured or processed tobacco leaves obtained from Africa, Asia, Europe, South and North America. A total of 1,431 leaf samples of flue-cured, burley, and Oriental tobaccos were obtained from various sampling locations during 2002 to 2004. Arsenic concentration in the samples averaged 0.4±0.6 μg g^{-1} as determined by inductively coupled plasma-mass spectrometry. Recorded values from most samples showed that concentrations of arsenic were usually found at the lower end of the distribution. Significant differences were found among tobacco types, sampling locations, and crop years. Arsenic concentrations were rather low in the majority of regions investigated, which is compatible with data from the literature. However, sample size was small and sampling geographically restricted. Our results would need to be validated with a larger dataset.

Keywords Arsenic · Metalloids · *Nicotiana tabacum* L. · Tobacco

1 Introduction

In the last decades, arsenic (As) received much attention as humans may be exposed to this toxic element through occupational and environmental exposure (Smith et al. 1998; WHO 2001). The International Agency for Research on Cancer has classified it as carcinogenic to humans (class 1; IARC 1987), and the US Environmental Protection Agency has classified inorganic arsenic as a human carcinogen (class A; EPA 1998). Food (drinking water in some areas) represents the major source of exposure to arsenic for non-occupationally exposed people (WHO 2001; IARC 2004). Agricultural soils and products may become contaminated by arsenic because of natural processes and human activities. Arsenical pesticides and herbicides have been major sources of arsenic in agriculture, but other agronomic practices and pollution arising from nearby industries represent other potential sources of this metalloid in agricultural soils (Smith et al. 1998; WHO 2001; Liao et al. 2005).

N. Lugon-Moulin (✉) · F. Martin · L. Rossi
Philip Morris International R&D,
c/o Philip Morris Products SA,
2000 Neuchâtel, Switzerland
e-mail: nicolas.lugon-moulin@pmintl.com

M. R. Krauss · P. B. Ramey
Philip Morris USA Research Center,
4201 Commerce Road,
Richmond, VA 23234, USA

Tobacco (*Nicotiana tabacum*), like other crops, can absorb arsenic from soil. Numerous reports exist on arsenic levels in cigarette tobacco or smoke (Tso 1990; Smith et al. 1997). About 7–18% of arsenic is volatilized when tobacco is smoked (Chiba and Masironi 1992). To the best of our knowledge, arsenic levels in tobacco leaves from the field (versus tobacco isolated from cigarettes which may be a blend of different tobacco types and origins) have not been recently surveyed on a world-wide basis. Therefore, the goal of the present study is to better characterize arsenic concentrations in conventional cured (and some processed) tobacco leaves from recent crops sampled in various tobacco-producing regions throughout the world. We emphasize that it was not aimed to understand the causes of variation of arsenic concentrations.

2 Materials and Methods

A total of 1,431 cured leaf samples from crop years 2003 and 2004 were obtained from sampling regions located in 20 countries (Table 1; samples obtained from the USA locations were processed tobacco leaves from crop year 2002). Overall, sample sizes were small. Sampling was restricted to some specific tobacco-producing regions and was geographically limited. The samples represented several cultivars regrouped in three major tobacco types: flue-cured (also referred to as Virginia or bright tobacco; n=740), burley (n=452), and Oriental (n=239) tobaccos. Sampling was performed as in Lugon-Moulin et al. (2006). Briefly, 10 (20 for Oriental tobacco) leaves from one farmer's field(s) were randomly sampled either in the curing or storage barn/warehouse (for cured tobacco) or in the processing plant (for processed tobacco), and were pooled to make one leaf sample. Therefore, one sample should consist of leaves from different stalk positions and plants. As such, it should be representative of the tobacco from the field(s) of one farmer. In a few cases, different samples were obtained from the same farmer to give insights in the variability of arsenic concentrations in tobacco from the same farmer (Table 1).

Sample preparation used was as described in Lugon-Moulin et al. (2006). In short, all tobacco samples were dried, digested in a microwave accelerated reaction system (MARS 5; CEM Corp., Matthews, NC) and arsenic concentration assessed by inductively coupled plasma-mass spectrometry (ICP-MS; Agilent 7500A; Agilent Technologies, Palo Alto, CA). Blanks and Certified Virginia Tobacco Leaves (CTA-VTL-2; Dybczynski et al. 1997) were analyzed for reference. Data were analyzed using conventional statistics (analysis of variance, ANOVA; Kolmogorov–Smirnov test).

3 Results and Discussion

3.1 Distribution of Arsenic Concentrations

The CTA-VTL-2 reference tobacco was tested twice at several months interval. The arsenic concentration was 0.89±0.02 μg g^{-1} (n=6) and 1.07 μg g^{-1} (n=1), which corresponded to its certified value (0.97± 0.07 μg g^{-1}). To be meaningful, results are presented rounded off to the first digit after the decimal point.

The arsenic concentrations ranged from 0 to 8.5 μg g^{-1} (n=1,431; 71 negative measurements were reported as zero, i.e. none detected). For each tobacco type, concentrations of arsenic in most samples were at the lower limit of the distribution (deviation from a normal distribution, Kolmogorov–Smirnov test, P<0.01 for each tobacco type). Most (91%) samples had concentrations <1 μg g^{-1}, and 98% had less than 2 μg g^{-1}. Overall, average arsenic concentrations were relatively low.

3.2 Arsenic Concentrations in Tobaccos According to Type and Origins

Arsenic concentrations differed between origins and between types (both ANOVA, P<0.0001). Concentrations were higher in Oriental tobacco, but did not differ between burley and flue-cured (Table 1). Relatively higher concentrations were reported for a few leaf samples from some locations in Turkey and China. In Turkey, concentrations differed between the three sampled regions (ANOVA, P=0.03), but this difference was no longer significant when removing the two most extreme samples of the entire dataset, both obtained from the same area in the region of Bergama (8.5 and 8.2 μg g^{-1}). The origin of arsenic in some Turkish samples included in this study is not clear. Relatively low levels of arsenic have been reported (0.1–0.7 μg g^{-1}) in samples of Turkish tobacco from 1949–1950 (Tso 1990).

Table 1 Sample sizes and arsenic concentrations (average±standard deviations, SD; SD not shown if zero) in tobacco leaves according to countries and types

Sampling regions		Tobacco type (*n*)			Tobacco type (As concentrations μg g^{-1})			Grand total
Country	Region[a]	Burley	Flue-cured	Oriental	Burley	Flue-cured	Oriental	
Albania	Korce and Elbasan			40[c]			0.8±1.3	0.8±1.3
Argentina	Provinces of Jujuy and Salta		184			0.4±0.3		0.49±0.42
Argentina	Provinces of Tucuman and Misiones	171			0.5±0.5			
Brazil	States of Rio Grande do Sul and Santa Catarina	54			0.2±0.1			0.2±0.1
Brazil	States of Rio Grande do Sul, Santa Catarina and Parana		146			0.2±0.1		
Bulgaria	Provinces of Blagoevragd, Haskovo, Kardzhali and Smolyan			60			0.3±0.3	0.3±0.3
China	Province of Yunnan[b]		40			1.0±0.7		1.0±0.7
Ecuador	Province of Guayas	8			0.2±0.1			0.4±0.2
Ecuador	Province of Guayas		38			0.4±0.2		
France	Alsace, Midi-Pyrénées, Rhône-Alpes and Pays de la Loire		20			0.1±0.1		0.1±0.1
Greece	Province of Elassona			20[d]			0.2±0.1	0.2±0.1
India	District of Mysore, Karnataka state		77			0.2±0.1		0.2±0.1
Indonesia	Lumajang area, East Java	20			0.1±0.0			0.1±0.1
Indonesia	Province of West Nusa Tenggara, island of Lombok		17					
Italy	Region of Campania	20			0.4±0.1			0.4±0.2
Italy	Regions of Veneto and Umbria		20[d]			0.4±0.2		
Malawi	Southern and Northern regions	40[e]			0		0	0
Malawi	Northern region			20	0		0	
Mozambique	District of Angonia, Tete province	20			0.1			0.1
Philippines	Regions 1 (Ilocos) and 2 (Cagayan valley)	40			0			0
Philippines	Regions 1 (Ilocos)		20			0.1		
Thailand	Province of Sukhothai	20[d]			0.9±0.3			0.7±0.5
Thailand	Province of Lampang		20			0.2±0.1		
Thailand	Province of Roi Et (and Sakhon Nakhon, *n*=1)			39			0.9±0.5	
Turkey	Areas of Bergama, Kale and Karacasu			60			1.3±1.5	1.35±1.46
Uganda	District of Hoima (Western region)	20[f]			0.1			0.1±0.1
Uganda	West Nile Central region		20[f]			0		
USA	Indiana, Kentucky, North Carolina, Tennessee, and Virginia	20			0.5±0.1			0.8±0.4
USA	East & South Carolina, Georgia		58			0.9±0.4		
Zambia	Eastern province	19[g]			0.1			0.05±0.06
Zambia	Southern and Northern provinces		40[h]			0±0.1		
Zimbabwe	Sampling locations could not be obtained		40			0.3±0.3		0.3±0.3
Grand total		452	740	239	0.3±0.4	0.3±0.4	0.7±1.0	0.4±0.6

[a] Samples from any given region may be geographically restricted to some areas.

[b] Five sampling locations, two in the region of Dali, one of Luliang, and two of Yuxi

[c] From 32 farmers; eight farmers sampled twice (6 farmers in Korce and 2 in Elbasan region)

[d] Four farmers, five samples each in Italy and Thailand; in Greece: 2,3,3,12 samples, respectively

[e] Five samples were obtained from a same farmer; sometimes, exact farm origin could not be verified

[f] From one farmer

[g] Seven farmers; two to four samples per farmer

[h] Northern: one farmer; Southern; two farmers from the same area

Sampled locations in Yunnan province (China) also yielded relatively higher arsenic concentrations, but significant differences were found between the five locations (ANOVA, $P<0.0001$). For example, samples from Pai Yin Binchuan Dali averaged 2.7±0.6 μg arsenic g^{-1} ($n=5$), while in Xia Zhuang Dali, the average was 0.2±0.1 μg arsenic g^{-1} ($n=5$). Sample numbers are too limited to draw definitive conclusions, but it appears that variability in arsenic concentrations may be fairly important. It is known that contamination from As may affect some areas in China, including fields used for crop production (e.g. Liao et al. 2005).

Other sampled countries had average arsenic concentrations below 1 μg g^{-1}, and most had averages below 0.5 μg g^{-1} (Table 1). Tobacco from sampled locations in the USA had relatively low arsenic levels which is in line with literature data. Indeed, a strong reduction in arsenic levels has been reported in tobacco in North America since the arsenic peak years in the early 1950, due to the phasing-out of As-containing pesticides in tobacco agronomy (Labstat Inc. 1995; Rodgman and Green 2002). Our results, though based on a limited sample, give further evidence that arsenic levels in US tobacco from recent crops appear to be relatively low. However, this would need to be verified using a larger sample covering many fields throughout the US tobacco producing regions.

Arsenic concentrations in tobaccos from sampled locations in Mysore (India) were low (average: 0.2 μg g^{-1}; Table 1). This result is in accordance with published results for India, reporting low arsenic levels for tobacco leaves from Andhra Pradesh, a state adjacent to the one sampled here (Purkayastha and Bhattacharyya 1975). Despite low arsenic levels, significant differences were found between crop years (ANOVA, $P=0.0099$; crop 2002: 0.2 μg g^{-1}; crop 2003: 0.1 μg g^{-1}).

Tobacco leaves from sampling sites in the southernmost states of Brazil had a low average arsenic concentration (0.2 μg g^{-1}). Differences were found between burley and flue-cured tobacco (ANOVA, $P<0.0001$), but not for either tobacco type according to state of origin.

In the few locations sampled in Africa, average arsenic concentrations in tobaccos were low (Table 1). Samples from Zimbabwe had a slightly higher mean value than those from sampling locations in the other African countries (Table 1), although no information on sampling location could be obtained from Zimbabwe and that several samples were obtained from the same farmer in African countries (see below).

3.3 Arsenic Concentrations in Tobaccos from Argentina

Sampling effort was more extensive in Argentina, where burley and flue-cured tobaccos were each sampled in two provinces (Table 1; $n=355$). For the sampled locations, burley in Tucuman yielded significantly higher arsenic concentrations than burley from Misiones (0.8±0.5 μg g^{-1}, $n=111$; and 0.1±0.2 μg g^{-1}, $n=60$, respectively; ANOVA, $P<0.0001$). In Tucuman, significant differences were found between crop years, but the fields sampled were not the same for the two years (ANOVA, $P<0.0001$; crop 2003: 0.6±0.5 μg g^{-1}, $n=64$; crop 2004: 1.0±0.4 μg g^{-1}, $n=47$). In Misiones, samples were all from the 2002 crop. For flue-cured tobacco, differences were also found between the two provinces sampled (ANOVA, $P=0.0005$; Jujuy, 0.5±0.4 μg g^{-1}, $n=91$; Salta, 0.4±0.2 μg g^{-1}, $n=93$). Similarly, in both provinces, significantly higher values were found for crop year 2004 as compared to 2003, although the fields sampled were not the same for the two years (ANOVA, both $P<0.0001$). For instance, in Jujuy, crop 2003 averaged 0.2±0.1 μg g^{-1} and crop 2004, 0.8±0.4 μg g^{-1}.

3.4 Variations in Arsenic Concentration for Samples from the Same Farmer

Variation was found between samples obtained from the same farmer. In Africa, samples were obtained from a same farmer in several instances. Arsenic concentrations were typically quite low and variations among samples from a same farmer (e.g. burley from Uganda) or from different farmers (e.g. burley from Mozambique) were essentially the same (Table 1). Five samples of Italian flue-cured tobaccos were sampled from each of four farmers. There was a two to fivefold difference between the extreme concentrations among samples from the same farmer. The ranges (averages±SD) in arsenic concentrations were: 0.2–1.0 (0.5±0.3), 0.2–0.6 (0.3±0.2), 0.3–0.7 (0.4±0.2) and 0.3–0.6 (0.3±0.1) μg g^{-1}. A similar sampling was done for Thai burley. The ranges in arsenic concentrations were (averages±SD): 0.5–0.9

(0.6±0.2), 0.7–1.4 (1.1±0.3), 0.7–1.1 (0.9±0.1) and 0.8–1.1 (0.9±0.1) μg g^{-1}. In Albania, 8 farmers were sampled twice. One pair of samples showed a twofold difference in concentrations (0.9 and 0.4 μg g^{-1}), while other pairs of values differed by <40%. Taken together, our results suggest that arsenic concentration of different tobacco leaf samples from one farmer may vary, and this variation may be as large as that found by obtaining leaf samples from several farmers.

4 Conclusion

Most tobacco leaf samples contained <1 μg arsenic g^{-1}. Variations in arsenic concentrations were found at all levels considered in this survey (region, farmer, crop year, tobacco type). The causes of this variation are not known. There are several possible sources of arsenic in agricultural soils. Besides, a number of variables like soil pH, presence of Fe oxide/hydroxide, clay content, phosphate availability in the soil or phosphorus demand by the plant, may affect arsenic behaviour in the soil, its uptake from the soil and its translocation to the leaves (Smith et al. 1998; Gulz et al. 2005). The mechanisms governing arsenic uptake by roots and translocation to the leaves in tobacco are not known and their study would have been well beyond the scope of this work. Our sample size, even though consisting of several hundreds samples, remains very small when considering all areas cultivated with tobacco worldwide. For example, one of the largest sample of this survey was obtained from Brazil (n=200). For the 2004/05 season, there were close to 200,000 farmers cultivating tobacco in south Brazil (Corrêa et al. 2005). Hence, in the southernmost states of this country, only about 0.1% of tobacco farmers have been sampled. Therefore, we emphasize that due to limited sample size and geographically restricted sampling, our results cannot be used to draw general conclusions on arsenic concentrations that may be found in tobacco leaves from the countries included in this survey and that our results are only valid for the fields and crop years from which tobacco leaves were sampled.

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