Science of the Total Environment 426 (2012) 132–138

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Chemical fate and genotoxic risk associated with hypochlorite treatment of nicotine

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ARTICLE INFO

Article history:
Received 13 January 2012
Received in revised form 19 March 2012
Accepted 19 March 2012
Available online 19 April 2012

Keywords:
Nicotine
Chlorination
Genotoxicity
Transformation by-products

ABSTRACT

Nicotine, the main alkaloid of tobacco, is a non- prescription drug to which all members of a tobacco-smoking society are exposed either through direct smoke inhalation or through second-hand passive 'smoking'. Nicotine is also commercially available in some pharmaceutical products and is used worldwide as a botanical insecticide in agriculture. Nicotine dynamics in indoor and outdoor environments as well as the human excretions and the manufacturing process are responsible for its entry in the environment through municipal and industrial wastewater discharges. The presence of nicotine in surface and ground waters points out that it survives a conventional treatment process and persists in potable-water supplies. Complete removal of nicotine is instead reported when additional chlorination steps are used.

In this paper a simulation of STP chlorination of nicotine and a genotoxic evaluation of its main degradation products are reported. Under laboratory conditions removal of nicotine seems not to be due to mineralization but to transformation in oxidized and chlorinated products. The by-products have been isolated after fractionation by diverse chromatographic procedures and their structures determined using mass spectrometry and ^{1}H and ^{13}C NMR spectroscopy. Preliminary genotoxic SOS Chromotests with *Escherichia coli* PQ37 evidence no toxicity of the products.

1. Introduction

Nicotine is a non-prescription drug to which all members of a tobacco-smoking society are exposed either through direct smoke inhalation or through second-hand passive 'smoking'. Nicotine is also commercially available in some pharmaceutical products (Buckingham et al., 2009; Cheng et al., 2002) and is used worldwide as a botanical insecticide in agriculture (Casanova et al., 2002).

FAO Projections (2003) foresaw the 7.1 million ton production of leaf tobacco, 1.3 billion smokers and a world production of 5.6 trillion cigarettes in 2010. Nicotine normally makes up about 5% of tobacco plants, by weight (Hossain and Salehuddin, in press; Vlase et al., 2005).

During the manufacturing process of tobacco part of nicotine is released into the atmosphere, part into wastewater which is sent to water treatment facilities, and part is found in the tobacco dust transported to landfills, added to compost or used as a filler in fertilizers. 93% of nicotine released is found in water, 4% in soil, and 3% in the air (Seckar et al., 2008).

As far as the manufacturing of tobacco is concerned, depending on the brand, a cigarette contains 8 to 20 mg of nicotine. Only part of nicotine found in a cigarette is likely to be present in the smoke 'stream' and the smokers may or may not absorb it, while the remaining nicotine is lost in the environment or undergoes thermal degradation (Schmeltz et al., 1979). Approximately 5% of nicotine absorbed is excreted as an unchanged drug into the urine together with its major urinary metabolite cotinine (10%) and its 3′-hydroxy derivative (35%) (Heinrich et al., 2005).

Since 1995 nicotine is in the list of chemicals included in the Toxic Release Inventory Program of the Environmental Protection Agency (2003) and, on the basis of the above data, the year's environmental world load of nicotine is many thousands of tons.

The United States National Library of Medicine (www.toxnet.nlm.nih.gov) and a model of the Canadian Environmental Modeling Center (Seckar et al., 2008) estimate a nicotine half-life of 4.3 h in the atmosphere, 38 days in water, 75 days in soil and 340 days in sediment.

Nicotine dynamics in indoor (Petrick et al., 2010) and outdoor environments as well as human excretion and the manufacturing process are responsible for its entry into surface water as well as into ground water. Gomez et al. (2010) have reported the presence of nicotine in four rivers located in the centre of Spain and, in the same country, Huerta-Fontela et al. (2008) have found nicotine in a further four rivers near Barcelona. The presence of the alkaloid has also been reported in surface water in Canada (Metcalfe et al., 2003), Switzerland (Buerge et al., 2008), Croatia (Grung et al., 2007) and in ground waters of Spain near Barcelona (Albaiges et al., 1986) and in Montana (Godfrey et al., 2007).

According to the presence of nicotine in surface water, analyses on the effluent of many sewage treatment plants (STPs) show that nicotine

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doi:10.1016/j.scitotenv.2012.03.047

survives conventional treatment processes. Amounts in the μg/L order have been found in the effluent from the Missoula plant (Montana), (Godfrey et al., 2007), from the Depurbaix plant (Barcelona, Spain; Teijon et al., 2010), and from 16 plants of northeast Spain (Huerta-Fontela et al., 2007).

Teijon et al. (2010) report however that in the Depurbaix sewage treatment plant the nicotine removal percentage is about 79%, after conventional treatment processes based on flocculation–coagulation, lamellar clarification, filtration, and disinfection, while after an additional treatment of chlorination the removal was about 97%. Huerta-Fontela et al. (2008) report a complete removal of nicotine in a drinking water treatment plant based also on two chlorination steps.

Processes of chlorination in the treatment of raw water therefore seem to be effective in simultaneously removing both pathogens and nicotine.

Aqueous chlorination has been shown to react with natural organic matter and other organic compounds to form disinfection by-products (DBPs) (Rook, 1974; Christman et al., 1980; Richardson et al., 2007) and several studies have shown that these DBPs are more resistant to degradation and can be more toxic than their parent compounds (Petrovic et al., 2003; Glassmeyer and Shoemaker, 2005; Duirk and Collette, 2006; Buth et al., 2009; DellaGreca et al., 2009; Gibs et al., 2007; Boleda et al., 2011; Duirk et al., 2011).

In this paper a simulation of STP chlorination of (−)-nicotine (**1**) and a genotoxic evaluation of the main products obtained after this disinfection process are reported.

2. Material and methods

2.1. Chemicals

Nicotine (**1**) (99.3%) was purchased from Sigma Aldrich. All the other chemicals and solvents were purchased from Fluka (Saint-Quentin Fallavier, France), with HPLC grade and were used as received.

2.2. Chlorination reaction

2.2.1. Apparatus

HPLC was performed on a Shimadzu LC-10 AD by using UV–Vis detector Shimadzu RID-10A. A semipreparative HPLC was performed using RP18 (LiChrospher 10 μm, 250 × 10 mm i.d., Merck) column with a flow rate of 1.2 mL min^{-1}. Column chromatography (CC) was carried out on Merck Kieselgel 60 (230–400 mesh).

Electronic Impact Mass Spectra (EI-MS) were obtained with a QP-5050A (Shimadzu) EI 70 eV spectrometer.

^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA-500 NMR instrument (^{1}H at 499.6 MHz and ^{13}C at 125.62 MHz), referenced with deuterated solvents ($CDCl_3$ or CD_3OD) at 25 °C. Proton-detected heteronuclear correlations were measured using a gradient heteronuclear single-quantum coherence (HSQC), optimized for $^{1}J_{HC} = 155$ Hz, a gradient heteronuclear multiple bond coherence (HMBC), optimized for $^{n}J_{HC} = 8$ Hz.

2.2.2. Chlorination procedure and product isolation

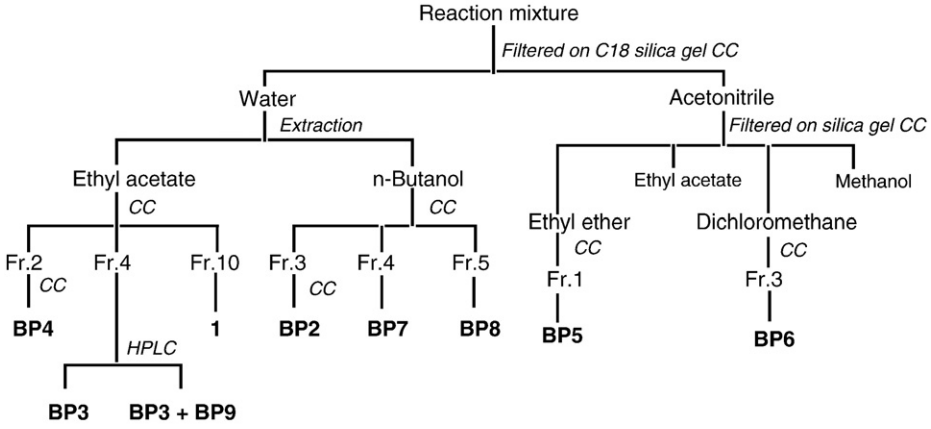
Nicotine (1 g, 6.17 mmol) dissolved in milliQ water (1 L) was treated for 30 min with 10% hypochlorite (molar ratio nicotine: hypochlorite 1:6; concn, spectroscopically determined λ_{max} 292 nm, ε 350 dm^3mol^{-1} cm^{-1}) at room temperature (Bedner and MacCrehan, 2006; DellaGreca et al., 2009). The pH of the solution, measured by a pH-meter at five minute intervals, rose from the initial pH 8.5 to 9.8, after 5 min, and it remained at this value during the reaction time. After 30 min, the solution, quenched by sodium sulfite excess, was filtered on C18 silica gel CC with water and acetonitrile (Scheme 1). The aqueous eluate was concentrated by lyophilization and extracted with ethyl acetate and successively with *n*-butanol.

The fraction obtained by extraction with ethyl acetate was chromatographed on silica gel CC using a gradient of dichloromethane: methanol (100:0 to 0:100), to give ten fractions. The 2nd, eluted with 96:4 dichloromethane:methanol, was rechromatographed on silica gel CC eluting with 95:5 ethyl acetate:acetone. The fractions 4–5 contained the by-product (BP) **4** (55 mg). The 4th, eluted with 90:10 dichloromethane:methanol, was analyzed by HPLC using a reversed phase column and eluting with 3:2:5 methanol:acetonitrile:water, to give by-product **3** (40 mg) and a mixture of by-products **3** and **9** (9 mg). The 10th, eluted with 80:20 dichloromethane:methanol contained the unreacted nicotine **1** (387 mg).

The fraction obtained by extraction with *n*-butanol was again fractioned by silica gel CC, using a gradient of ethyl acetate:methanol, to give six fractions. The 3rd, eluted with methanol, was rechromatographed on silica gel CC eluting with a gradient of dichloromethane: methanol. Fraction 5, eluted with 90:10 dichloromethane:methanol, contained the by-product **2** (71 mg). The 4th, eluted with 50:50 dichloromethane:methanol, contained the by-product **7** (89 mg). The 5th, eluted with methanol, contained the by-product **8** (51 mg).

The fraction eluted with acetonitrile was filtered on silica gel CC with ethyl ether, ethyl acetate, dichloromethane, and methanol, to give four fractions. The 1st, eluted with ethyl ether, was rechromatographed on silica gel CC using a gradient of dichloromethane:acetone. Fraction 1, eluted with 95:5 dichloromethane:acetone, was analyzed by HPLC using a reversed phase column and eluting with 4:4:2 methanol:acetonitrile: water, to give pure by-product **5** (7 mg). The 3rd fraction, eluted with dichloromethane, was rechromatographed on silica gel CC using a gradient of dichloromethane:acetone. Fraction 3, eluted with 95:5 dichloromethane:acetone, contained by-product **6** (10 mg).

Structures of all the substances are shown in Fig. 1.



Scheme 1. Isolation of the different identified compounds.

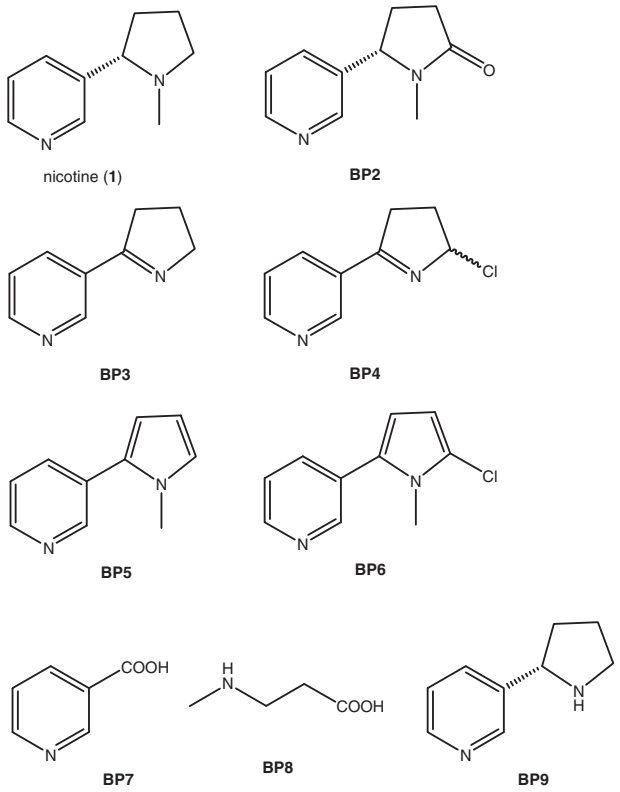


Fig. 1. Nicotine and its transformation products by chlorination.

2.3. Genotoxicity assay

The SOS-Chromotest uses the strain *Escherichia coli* PQ37 that is constitutive for alkaline phosphatase synthesis. This strain exhibits *sfiA::lacZ* fusion and has a deletion of the normal *lac* region, so that β-galactosidase activity is strictly dependent on *sfiA* expression. An uvrA mutation renders the strain deficient in excision repair and accordingly increases the response to certain DNA-damaging agents. An rfa mutation renders the strain lipopolysaccharide deficient and allows better diffusion of certain chemicals into the cell. Two genes play a key role: *lexA* encodes a repressor for all the genes of the system and *recA* encodes a protein able to cleave the *lexA* repressor upon activation by a SOS inducing signal. The assay is quantitative and dose–response curves present a linear region. Nicotine and its products were dissolved in dimethylsulfoxide (DMSO) and further diluted in double-deionized water to make stock solutions. The DMSO concentration in the test solutions, including controls, was 0.01% (v/v) which is a non-effective dose as estimated in preliminary tests. Compound concentrations used were between 1 μg/mL and 10^{-6} μg/mL. All compounds were tested in seven concentrations, three replicates and for each compound three independent assays were performed. Cultures were grown overnight from frozen stocks (−80 °C) in LA-medium and diluted in 5 mL of fresh medium and incubated for 2 h at 37 °C to reach 2×10^8 bacteria/mL. Then, 1 mL of the culture was diluted in 9 mL of fresh L-medium. Culture fractions (600 μL) were distributed between test tubes containing 20 μL of the sample and positive controls (4-nitroquinoline-N-oxide) and negative control (physiological saline). After 2 h of incubation at 37 °C with shaking, two 300 μL aliquots were withdrawn from each tube for β-galactosidase and alkaline phosphatase assays. For the β-galactosidase assay, 2.7 mL of β-galactosidase buffer (pH 7.7) was added to one of the tube series followed by 600 μL of 4-nitrophenyl-β-D-galactopyranoside (ONPG) solution (4 mg/mL). For the alkaline phosphatase assay, 2.7 mL of alkaline phosphatase buffer (pH 8.0) was added to the other tube series followed by 600 μL of 4-nytrophenyl phosphate (PNPP) solution (4 mg/mL). The two tube series were incubated for 10 min at 37 °C and the β-galactosidase activity was measured as the *o*-nitrophenol concentration by photometric measurement at 420 nm.

3. Results and discussion

3.1. Chlorination data

Chlorination of nicotine produced by-products BP2–BP9 which were isolated by chromatographic processes and identified on the basis of their physical features.

BP2 (cotinine) and BP8 (*N*-methyl-β-alanine) were identified by the comparison of their spectroscopic data (EI mass spectrum, ^{1}H and ^{13}C NMR spectra) with those of authentic standard.

BP3 had an apparent molecular ion at *m/z* 146 in the EI mass spectrum and nine carbon signals in the ^{13}C NMR spectrum, which could correspond to the molecular formula $C_9H_{10}N_2$.

In the ^{1}H NMR the four protons of the pyridine ring were present besides three methylene signals at δ 4.08, 2.96 and 2.07 correlated in the HSQC spectrum to the carbons at δ 61.5, 22.4 and 34.6, respectively. The ^{13}C NMR spectrum, besides the signals of the protonated carbons, showed two quaternary carbons signals at δ 170.9 and 130.5. HMBC experiments allowed the assignment of the first signal to C-2 and the latter to C-2′. All the data were consistent with the myosmine structure. Myosmine is already known to be present in cigarette smoke (Schmeltz et al., 1979) and to be formed by ozone action on nicotine (Destaillats et al., 2006).

BP5 had an apparent molecular ion at *m/z* 158, which could correspond to the molecular formula $C_{10}H_{10}N_2$, in the EI mass spectrum. ^{1}H NMR spectrum showed a methyl signal at δ 3.67 and seven protons in the aromatic region. On the basis of HMQC and HMBC experiments the signals at δ 8.69, 8.53, 7.72 and 7.34 have been assigned to the H-2, H-6, H-4 and H-5 protons of the pyridine ring, while the signal at δ 6.77, 6.30 and 6.22 have been assigned to the H-5′, H-3′, and H-4′ of a pyrrole ring. All the data are consistent with the nicotyrine structure, a minor alkaloid present in the tobacco leaf (Graca et al., 2000).

The structure of 5-chloro-myosmine was attributed to the first chlorinated by-product BP4. It had an apparent molecular ion at *m/z* 180 with its isotopic peak at *m/z* 182 (ratio 3:1, Fig. 2) in the EI mass spectrum and nine carbon signals in the ^{13}C NMR spectrum, which could correspond to the molecular formula $C_9H_9ClN_2$. The DEPT (Distorsionless Enhancement by Polarization Transfer) experiment defined the carbons as five methines, two methylenes and two quaternary carbons. ^{1}H NMR spectrum showed the four pyridine proton signals at δ 9.11, 8.66, 7.36, and 8.24. These signals, correlated to the carbons at δ 152.1, 150.0, 124.0 and 135.9, respectively, were attributed to the H-2, H-6, H-4, and H-5 protons. ^{1}H NMR also showed four signals at δ 5.20, 4.20, 2.50 and 2.40; the first two were correlated in the HSQC spectrum to the carbons at δ 59.0 and 60.0 and the last to the same carbon at δ 35.6. H–H COSY (COrrelated SpectroscopY) evidenced that both the signals at δ 5.20 and 4.20 were correlated to the methylene protons at δ 2.50 and 2.40. The cross peak between the pyridine carbon at δ 128.0 and the proton at δ 4.20 allowed the assignment of this signal at H-3′ and that of the proton at δ 5.20, geminal with the chlorine, to H-5′.

Analogously, BP6 (5-chloro-nicotyrine) was characterized by the presence of a chlorine atom. It had an apparent molecular ion at *m/z* 192 with its isotopic peak at *m/z* 194 (ratio 3:1, Fig. 3), which could correspond to the molecular formula $C_{10}H_9ClN_2$, in the EI mass spectrum. ^{13}C NMR spectrum showed ten carbon signals identified by a DEPT experiment as one methyl, seven methines and two quaternary carbons. The ^{1}H NMR spectrum, besides the signals at δ 8.66, 8.60, 7.76 and 7.38 identified as the H-2, H-6, H-4 and H-5 protons of the pyridine ring, showed a methyl at δ 3.67 and two coupled protons at δ 6.67 and 6.22. The long range correlations in the HMBC spectrum of the proton at δ 6.67 with the C-3 carbon at δ 133.0 and the C-2′ carbon at δ 142.0 fixed the chlorine at the C-5′ position.

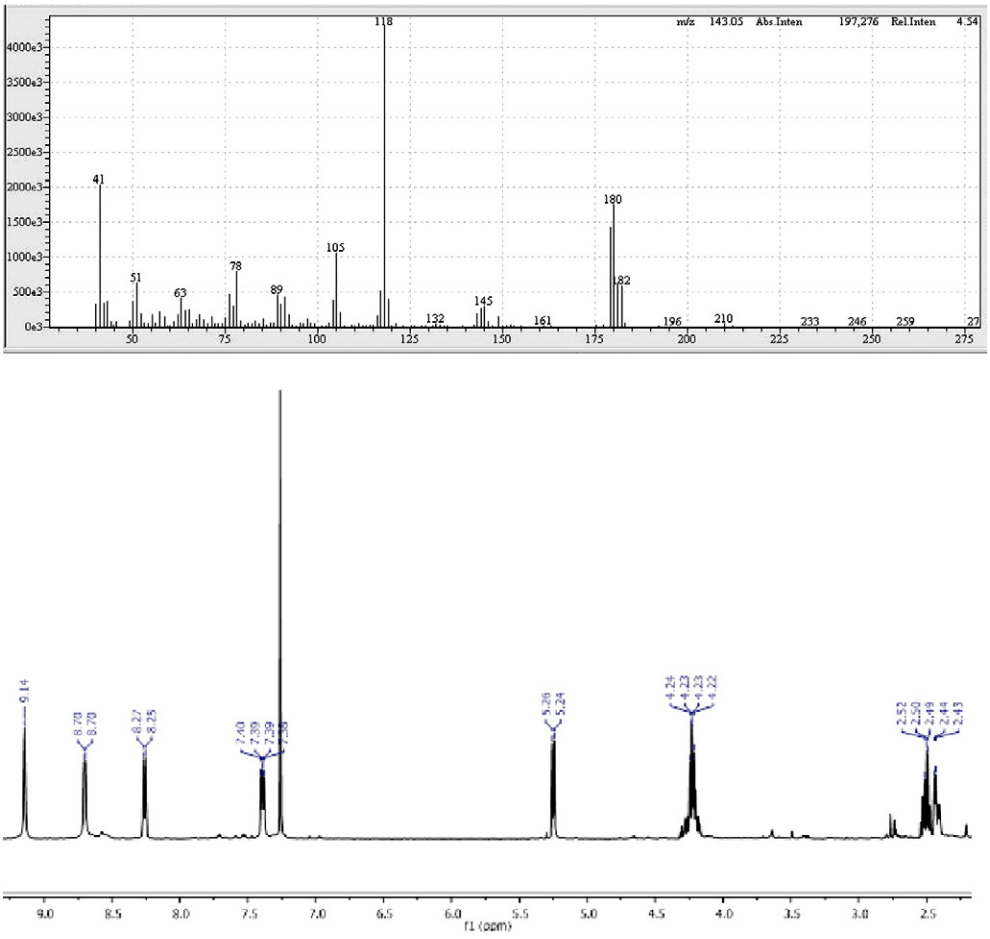


Fig. 2. MS spectrum and ^{1}H NMR of BP4.

BP7 was identified as nicotinic acid by comparison of its physical features (^{1}H and ^{13}C NMR spectra) with those reported (Koczon et al., 2003).

Finally the nornicotine structure was attributed to BP9. This one was isolated in mixture with BP3 and all efforts to separate them failed. From the best of our conditions a mixture 9:1 (based on ^{1}H-NMR) of BP9 ((*S*)-3-(pyrrolidin-2-yl)pyridine) and BP3 was obtained. It had an apparent molecular ion at *m/z* 148, which could correspond to the molecular formula $C_9H_{12}N_2$, in the EI mass spectrum.

In the ^{1}H NMR spectrum, besides the signals of the aromatic protons at δ 8.60, 8.45, 7.74 and 7.25, there was also present a double doublet at δ 4.14 attributable at H-2′, two protons as a multiplet at δ 3.11 attributable to H-5′ and the remaining four protons H-3′–H-4′ in the ppm range of 1.9–2.2, partly overlapped to the H-4′ of myosmine. ^{13}C NMR, besides the pyridine carbons at δ 148.3, 148.0, 140.1, 134.2, and 123.2, evidenced four carbon signals at δ 59.7, 46.7, 34.0, and 25.2. All the chemical shifts were in agreement with those reported for nornicotine (Ayers et al., 2005). The negative specific rotation of the mixture, $[\alpha]_D^{25} = -28.2°$, agreed with the proposed mechanism of formation (see below) that excludes loss of chirality at C-2′.

All the products are derived from nicotine through transformations of the pyrrolidine moiety by addition, substitution and oxidation reactions (Boyce and Horning, 2000). According to the results of Abia et al. (1998) on the oxidation of aliphatic amines by hypochlorite, it can be assumed that in the key step a positive charge is developed on the nitrogen atom of the pyrrolidine ring by an attack of the lone pair of nitrogen on the HOCl molecule. Subsequent dehydrohalogenation (Guillemin et al., 1988) of the intermediate (I) cation 10 (Fig. 4) gives the iminium cations I11–I13.

BP2 and BP5 have already been proposed as oxidative derivatives of imine salt I11 by Hubert-Brierre et al. (1975). Electrophilic substitution on BP5 should lead to 5′-chloronicotyrine BP6. Iminium cation I12 could justify the formation of BP9, BP3 and BP4. In particular, BP9 is likely formed by hydrolysis of I12 and undergo *N*-chlorination (Abia et al., 1998) leading to compound I15. The latter by dehydrohalogenation at C-2′ carbon could give BP3 while by dehydrohalogenation at C-5′ carbon it leads to BP4 via imine I16.

Finally, hydrolysis of I13 could produce the not isolated 4-(methylamino)-1-(3-pyridinyl)-1-butanone I14 that by oxidation forms BP7 and BP8.

Iminium cations I11–I13 have already been postulated by Joice and Leete (1989) as intermediates in the formation of BP2, BP3, BP5, BP9 and I14 when nicotine 1′-oxide was treated with ferric nitrate. In the work the authors proposed the initial formation of a radical cation on the pyrrolidine nitrogen followed by the loss of a hydrogen atom. This oxidative step could be an alternate route in the formation of I11–I13.

3.2. Genotoxicity data

Although there is a large amount of data and literature on the risks of direct and passive smoking, only a little data is available on the environmental toxicity of nicotine and its derivatives.

In connection with its use as an insecticide, the effects of nicotine on zooplankton (Savino and Tanabe, 1989) and on crustaceans and amphibians (Lone and Javaid, 1976) have been studied: the results showed that there is no acute toxicity unless at very high concentrations incompatible with the environment. In 2009 Sanderson and Thomsen

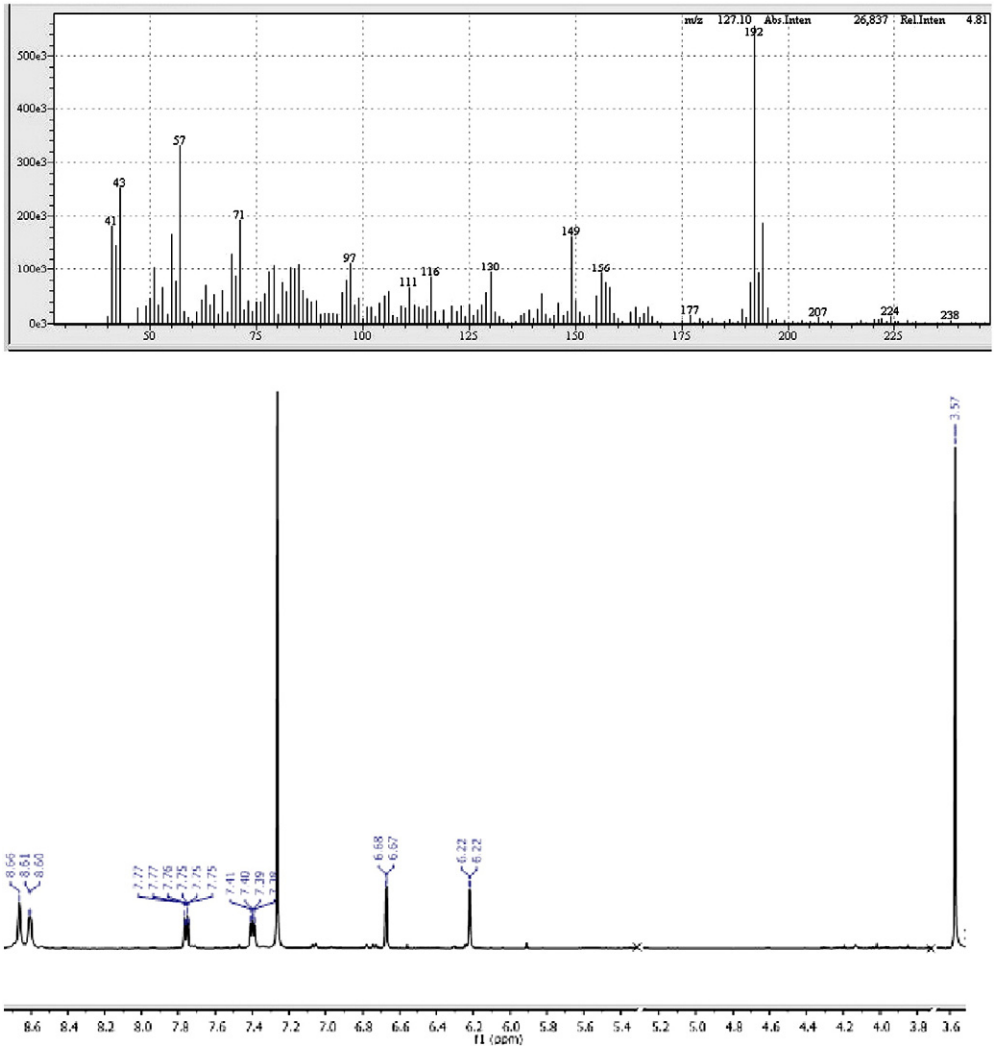


Fig. 3. MS spectrum and ^{1}H NMR of BP6.

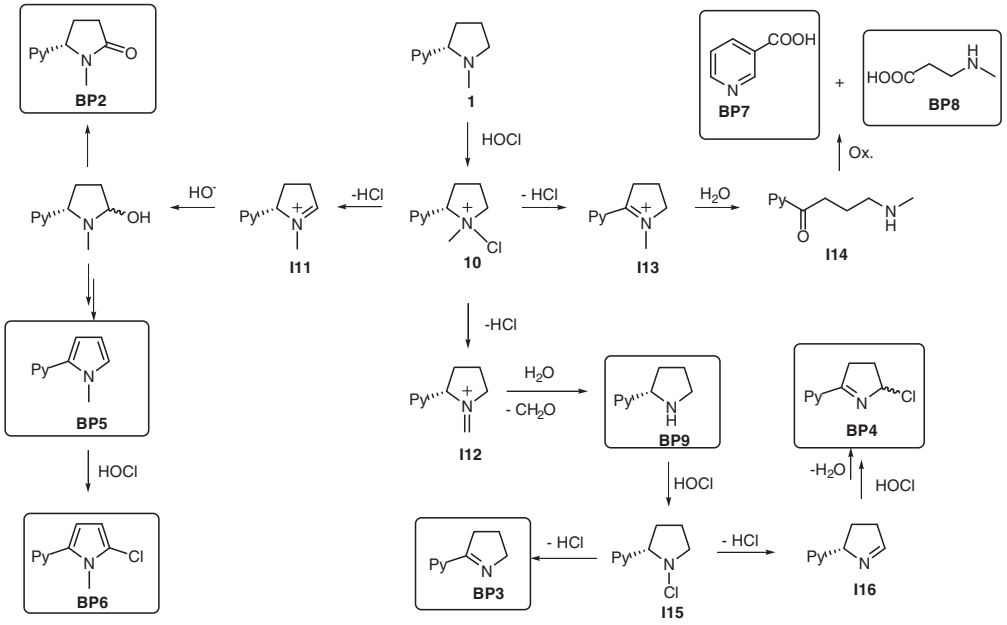


Fig. 4. Suggested pathways for the reaction of nicotine with hypochlorite. Boxed structures represent isolated products.

Table 1
Genotoxicity on *Escherichia coli* PQ37 (±SD) of nicotine and its main transformation products.

Compound	Concentrations (μg/mL)	I.F.[a]	Compound	Concentrations (μg/mL)	I.F.[a]
p-Nitroquinoline (positive control)	0	1 ± 0.1			
	1	2.23 ± 0.31			
	5	10.82 ± 0.42			
1	1×10^{-6}	1.03 ± 0.31	**2**	1×10^{-6}	1.05 ± 0.11
	1×10^{-5}	1.12 ± 0.27		1×10^{-5}	1.10 ± 0.15
	1×10^{-4}	1.22 ± 0.18		1×10^{-4}	1.11 ± 0.14
	1×10^{-3}	1.26 ± 0.14		1×10^{-3}	1.18 ± 0.12
	1×10^{-2}	1.36 ± 0.28		1×10^{-2}	1.20 ± 0.23
	1×10^{-1}	1.45 ± 0.24		1×10^{-1}	1.23 ± 0.18
	1	1.47 ± 0.19		1	1.31 ± 0.17
3	1×10^{-6}	1.12 ± 0.18	**4**	1×10^{-6}	1.10 ± 0.15
	1×10^{-5}	1.17 ± 0.13		1×10^{-5}	1.15 ± 0.19
	1×10^{-4}	1.17 ± 0.15		1×10^{-4}	1.17 ± 0.17
	1×10^{-3}	1.18 ± 0.12		1×10^{-3}	1.19 ± 0.21
	1×10^{-2}	1.22 ± 0.19		1×10^{-2}	1.20 ± 0.23
	1×10^{-1}	1.28 ± 0.26		1×10^{-1}	1.37 ± 0.25
	1	1.56 ± 0.24		1	1.44 ± 0.26
5	1×10^{-6}	1.06 ± 0.09			
	1×10^{-5}	1.07 ± 0.07	**6**	1×10^{-6}	0.89 ± 0.08
	1×10^{-4}	1.12 ± 0.12		1×10^{-5}	0.98 ± 0.07
	1×10^{-3}	1.27 ± 0.15		1×10^{-4}	1.12 ± 0.11
	1×10^{-2}	1.35 ± 0.18		1×10^{-3}	1.20 ± 0.15
	1×10^{-1}	1.37 ± 0.21		1×10^{-2}	1.25 ± 0.18
	1	1.85 ± 0.22		1×10^{-1}	1.28 ± 0.21
				1	1.32 ± 0.19
7	1×10^{-6}	1.02 ± 0.21	**8**	1×10^{-6}	1.09 ± 0.11
	1×10^{-5}	1.05 ± 0.18		1×10^{-5}	1.12 ± 0.23
	1×10^{-4}	1.09 ± 0.17		1×10^{-4}	1.20 ± 0.24
	1×10^{-3}	1.15 ± 0.23		1×10^{-3}	1.37 ± 0.19
	1×10^{-2}	1.34 ± 0.22		1×10^{-2}	1.45 ± 0.18
	1×10^{-1}	1.38 ± 0.19		1×10^{-1}	1.51 ± 0.17
	1	1.52 ± 0.21		1	1.60 ± 0.21

[a] IF (induction factor) = R/R_0.

(2009) in a QSAR study on acute aquatic toxicity from pharmaceuticals reported for nicotine a LC_{50} of 3 mg/L on daphnids, 4 mg/L on fish and no algal toxicity. The authors also reported no toxicity on algae and daphnids from nicotinic acid and a LC_{50} greater than 10 mg/L on fish. In the same time Seckar et al. (2008) reported that nicotine exhibited a low toxicity toward algae and inhibited seed germination and root elongation of *Lactuca sativa* at concentrations of several mg/L. Finally Waiser et al. (2011) expressed an EC_{50} greater than 1 mg/L in inhibiting growth of the *Lemna minor* by cotinine.

In this framework, the present study aims to investigate the effects of nicotine and its products on the environment by evaluating their genotoxic properties.

The genotoxic potential was assessed using the SOS Chromotest with *E. coli* PQ37, a colorimetric assay based on the detection of damage to DNA measured through the induction of the SOS DNA repair system (Quillardet and Hofnung, 1993). The test was performed on nicotine and BP2–BP8, after purification by HPLC, at concentrations between 10^{-6} and 1 μg/mL. The concentration range was chosen considering the environmental relevance of the compounds investigated. The results of three independent tests are reported in Table 1 and they indicate that all compounds tested were not able to activate the SOS system. In fact, a compound is considered as a SOS inducer if the induction factor, defined as IF = R/Ro in which Ro is the spontaneous ratio measured in the negative control and R = β/p (β = galactosidase activity and p = phosphatase alkaline activity) in the sample concentrations, is higher than 2, the β-galactosidase activity is significantly increased compared to the negative control and the induction factor versus concentration shows a clear dose–effect relationship. None of the tested compounds exceeded the IF value of 2, demonstrating no genotoxic potential of nicotine and its products in this experimental system based on repair mechanisms. Our findings on nicotine agree with those obtained by Yim and Hee (1995) who tested this compound using the Mutatox system as well as with those by Doolittle et al. (1995) who evaluated the mutagenicity of nicotine and its metabolites in the Ames Test on *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 and in sister-chromatid exchange assay (SCE) in a Chinese Hamster Ovary at concentrations of up to 1000 μg/mL. On the other hand, opposite results were found utilizing other genotoxicity testing such as Comet assay and chromosome aberrations in Chinese Hamster Ovarian cells based on single-strand breaks, alkali labile and excision repair sites (Ginzkey et al., 2009).

4. Conclusions

Nicotine in contact with sodium hypochlorite excess for 30 min, undergoes more than a 60% degradation. This result could account for the reported removal of nicotine by chlorination in a sewage treatment plant (Teijon et al., 2010) and a drinking treatment plant (Huerta-Fontela et al., 2008). However, here it has been showed that the treatment does not give rise to mineralization of nicotine but induces its transformation into several products, two of them chlorinated. These compounds show no genotoxic activity in the SOS Chromotest with *E. coli* PQ37.

Despite our result, concerning environmental risk, a battery screening approach will be necessary in the future if the large but often conflicting toxicity data reported on nicotine and its metabolites are also be taken into consideration.

Acknowledgments

NMR Spectra were performed on a 500 Varian Inova apparatus of Consorzio INCA.

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