

# Chemical, Pharmacological, and Toxicological Assessment of 6-Methylnicotine

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## Abstract

There is interest in nicotine-related alkaloids for both recreational use and pharmaceutical applications such as smoking cessation and central nervous system disorders conditions such as Parkinson's, Tourette's, ADHD. Nicotine is one of many alkaloids produced by the tobacco plant (*Nicotiana tabacum* species) and more recently synthesized for commercial use. The compound 6-methylnicotine (CAS# 101540-79-8) has been identified as a nicotine analog of interest based on its chemical structure, sensorial properties, and commercial availability. Chemical, pharmacological, and toxicological assessments were conducted on 6-methylnicotine and compared to pharmaceutical grade (S)-nicotine. Samples of 6-methylnicotine analyzed included both freebase and salt forms, as well as in e-liquid formulations containing propylene glycol (PG) and vegetable glycerin (VG) for use in an electronic nicotine delivery system (ENDS). Chemical analysis confirmed the sample was 6-methylnicotine, racemic, and ~98% pure utilizing <sup>1</sup>H NMR, chiral UPLC-UV, and GC-MS. The aerosol transfer efficiency of 6-methylnicotine was similar to that of nicotine (82.5 ± 0.6 % vs. 85.6 ± 2.9 % for freebase forms). Archival pharmacological data indicates that 6-methylnicotine is similar in potency and binding affinity to that of (S)-nicotine in *in vivo* and *ex vivo* models. Regulatory *in vitro* toxicology testing (Neutral Red, Ames, and Micronucleus) demonstrated 6-methylnicotine salt e-liquid formulations have similar cellular cytotoxicity and mutagenicity/genotoxicity responses to the analogous (S)-nicotine salt e-liquid formulation. The totality of available evidence indicates that 6-methylnicotine has comparable chemical, pharmacological, and toxicological properties to the more widely used nicotine.

## Introduction

(S)-nicotine is the primary active ingredient in a range of tobacco and nicotine consumer products and in smoking cessation drug therapies such as Nicorette<sup>™</sup> gum, lozenge, and mini-lozenges. Since April 2022, when the "synthetic nicotine loophole" was closed by US Congress, all nicotine-containing products are now required to submit a PMTA to FDA to receive marketing approval, regardless of the nicotine source (tobacco-derived or synthetic). This process is a costly, time-consuming, unpredictable, and uncertain. Consequently, there is interest in identifying alternative agents that act in a manner similar to nicotine. One such molecule, 6-methylnicotine, was identified during tobacco industry research conducted between 1977 and 1982 as having similar pharmacological effects in animal models, though it was not incorporated into any marketable products. Recent publications<sup>1-3</sup> seem to indicate that interest in alternate nicotine analogs is rising again. Herein, we present the results of chemical, pharmacological, and toxicological assessments of 6-methylnicotine conducted to fill the existing knowledge gap.

## Materials & Methods

### Materials

All nicotine and 6-methylnicotine-containing materials were donated by SS Vape Brands, with the exception of freebase nicotine which was sourced from MilliporeSigma (St. Louis, MO). ENDS devices used for aerosol studies were also provided by SS Vape Brands.

### Methods

- GC-MS EI: Agilent HP-5ms, 15 m × 250 μm × 0.25 μm; 70 to 300 °C, 20 °C/min.
- Chiral UPLC-UV: AM-271, AZYP NicoShell SPP, 100 mm × 4.6 mm, 2.7 μm; 0.2 % NH<sub>4</sub>HCO<sub>2</sub> in methanol.
- <sup>1</sup>H NMR: Bruker NanoBay AVANCE III 400 MHz NMR spectrometer, conducted at the Virginia Commonwealth University (VCU) NMR Center.
- Aerosol Transfer Efficiency: *Devices* – Vapresso<sup>®</sup> Tarot Nano (tank-based) for freebase formulations; Vapresso<sup>®</sup> Zero (pod-based) for benzoate salt formulations; *Collection* – ISO 20768 conditions, pad collection and extraction into isopropanol; *Analysis* – AM-224, GC-FID, Restek Stabilwax-DA, 30 m × 320 μm × 1 μm, 80 to 240 °C, 20 °C/min.
- Neutral Red Uptake: AM TOX-002, based on ISO 10993:2009 and OECD Guideline 129 (2010); tested in BALB/c 3T3 (mouse fibroblast) and A549 (human lung epithelial) cell lines.
- Bacterial Reverse Mutation Assay (Ames): AM TOX-003, based on OECD Guideline 471 (2020); tested in TA98, TA100, TA102, TA1535, and TA1537 strains of *S. Typhimurium*.
- Micronucleus Test: AM TOX-020, based on OECD Guideline 487 (2023); tested in human lymphoblast TK6 cells; scored using flow cytometry (MicroFlow *in vitro* 250/50 Kit, Litron).

## Chemical Characterization

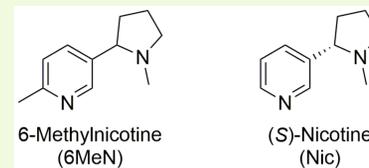


Figure 1. Molecular structures of 6MeN and Nic.

- Predicted physicochemical properties<sup>4</sup> for 6MeN and Nic were found to be comparable (Table 1).
- GC-MS analysis (Figure 2) confirmed nicotine methylation, with <sup>1</sup>H NMR indicating methyl group was in the pyridyl 6-position (Figure 3). Latter also showed 6MeN purity to be >98%
- Chiral analysis showed the received 6MeN was racemic (i.e. 1:1 (R)/(S)-enantiomers) and Nic was >99% (S)-nicotine.
- Aerosol transfer efficiency from 4.8% e-liquid formulations (freebase and benzoate salts) was assessed by GC-FID, showing comparable behavior between 6MeN and Nic for both forms (Table 2).

Table 1. Predicted physicochemical properties comparison.

Physicochemical Property	Predicted Average Values	
	6-Methylnicotine <sup>4</sup>	(S)-Nicotine <sup>5</sup>
Physical State	Liquid	Liquid
Melting Point (°C)	20.9	18.1
Boiling Point (°C)	259	246
Density (g/cm <sup>3</sup> )	1.01	1.03
Vapor pressure (mm Hg)	7.30 × 10 <sup>-3</sup>	2.39 × 10 <sup>-2</sup>
Partition coefficient, log K <sub>ow</sub>	1.42	0.928
Solubility in water (mol/L)	0.849	2.18
Surface tension (dyn/cm)	37.4	38
Flash point (°C)	112	98.6
Viscosity (cP)	11.8	7.28

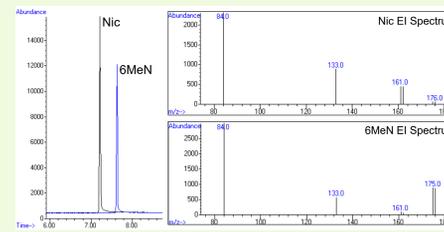


Figure 2. GC-MS analysis of 6MeN and Nic: (left) overlapped SIM traces (m/z 84); (right) individual EI mass spectra.

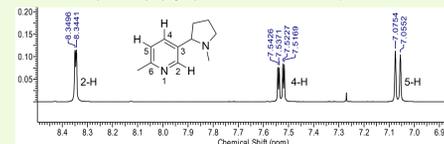


Figure 3. Aromatic proton region of the 6MeN <sup>1</sup>H NMR spectrum.

