K51 Disposition of Nicotine and Metabolites in Human Meconium Following In Utero Tobacco Exposure

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After attending this presentation, attendees will learn the disposition of nicotine, cotinine, 3'-trans-hydroxycotinine, nornicotine and norcotinine in human meconium after in utero tobacco exposure, including relative analyte concentrations, metabolite ratios, and degree of glucuronidation.

This presentation will impact the forensic community, as this is the first quantification of nicotine and metabolites in meconium, the first data on the percentages of total and free nicotine and metabolites in meconium and the first report of the importance of nicotine as a biomarker of in utero tobacco exposure in meconium. These data will aid in the identification of prenatally tobacco exposed infants.

Approximately one-quarter of pregnant women smoke tobacco despite nicotine's known effects on fetal growth, lung and nervous system development, and increased risk of nicotine dependence in adulthood. Detection of cotinine in meconium by immunoassay is the primary means of monitoring *in utero* nicotine exposure. Recently, the first chromatographic assay for nicotine and metabolites in human meconium was developed and validated in our laboratory. Liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry in positive ion mode was employed to simultaneously quantify nicotine and metabolites, cotinine, OH-cotinine, norcotinine and nornicotine in meconium from 125 neonates.

Meconium (0.5 g) was homogenized with 3 mL of acidified methanol. After sonication, centrifugation, reconstitution in buffer and *E. coli* β-glucuronidase hydrolysis for 18 h at 37°C, solid phase extraction using a mixed-mode cation exchange column was performed. Limits of quantification (LOQ) were 1.25 ng/g for OH-cotinine, cotinine, and norcotinine and 5 ng/g for nicotine and nornicotine. Specimens were analyzed with and without β-glucuronidase enzymatic hydrolysis to determine total and free concentrations.

Fifty-nine specimens (47.0%) were positive for at least one free drug, with nicotine being the primary analyte detected (43.2%), followed by OH-cotinine (37.6%), cotinine (34.4%), nornicotine (12.0%) and norcotinine (9.6%). The highest percentage of specimens (20.0%) contained nicotine, cotinine and OH-cotinine, 8.8% were positive for all five analytes, and 8.8%, 0.8% and 2.4% for nicotine, cotinine and OH-cotinine only, respectively. No specimen was positive for only nornicotine or norcotinine. Average free drug concentrations of positive specimens (±SD) were 72.6 (±92.2) ng/g OH- cotinine, 69.9 (±70.3) ng/g cotinine, 59.6 (±76.3) ng/g nicotine, 4.5 (±3.7) ng/g norcotinine, and 11.8 (±6.2) ng/g nornicotine. Average total drug concentrations of positive specimens (±SD) were 99.2 (±118.2) ng/g OH-cotinine, 80.3 (±78.5) ng/g cotinine, 60.0 (±73.3) ng/g nicotine, 4.2 (±3.9) ng/g norcotinine, and 11.8 (±5.1) ng/g nornicotine. Two specimens had OH-cotinine concentrations greater than the 500 ng/g upper limit of quantification, but could not be reanalyzed due to lack of additional specimen. Statistically significant differences were shown between total and free OH-cotinine and cotinine using a paired t-test ($P<0.05$). Amongst positive specimens, the average percentage (±SD, range) of total OH- cotinine, cotinine, and nicotine present as glucuronide conjugates were 29.4 (±21.1, -19.4-73.3), 15.7 (±16.2, -18.6-54.5), and 4.2 (±12.4, -36.2-30.6), respectively. Free drug concentrations greater than total drug concentrations can be attributed to analytical imprecision and lack of homogeneity in the matrix despite extensive mixing prior to sampling. OH-cotinine total drug and glucuronidation results should be interpreted with caution, as hydrolysis efficiency of authenticated OH-cotinine-O-glucuronide was determined to be 15% during method validation. Possibly, the high degree of OH-cotinine glucuronidation observed could be due to the presence of di-glucuronide or N-glucuronide species in addition to the O-glucuronide control that was tested for hydrolysis efficiency. Hydrolysis efficiencies for nicotine- and cotinine-N-glucuronide were greater than 80%. Average free drug metabo- lite ratios (±SD) were: OH-cotinine/nicotine 1.75 (±1.74) [median (range), 1.37 (0.055-8.67)], cotinine/nicotine 1.44 (±1.28) [1.00 (0.31-5.90)], norcotinine/nicotine 0.058 (±0.080) [0.028 (0.009-0.288)], nornicotine/nicotine 0.11 (±0.11) [0.08 (0.04-0.46)], OH-cotinine/cotinine 1.41 (±0.98) [1.18 (0.15-3.94)], and norcotinine/cotinine 0.08 (±0.03) [0.08 (.04-0.13)].

Meconium, Nicotine, Cotinine, In Utero

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