K33 Novel Stimulants N-Ethyl Pentylone and Dibutylone: Case Reports, Quantitative Confirmation, and Metabolic Profile Determination

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After attending this presentation, attendees will be able to evaluate N-ethyl pentylone and dibutylone concentrations in postmortem and Driving Under the Influence of Drugs (DUID) cases. In addition, attendees will be able to describe the metabolic biotransformation of N-ethyl pentylone and dibutylone and identify the metabolites in toxicological casework.

This presentation will impact the forensic science community by characterizing biomarkers of two emerging stimulants and providing analytical data for use in qualitative and quantitative interpretation.

Novel stimulants, like other Novel Psychoactive Substances (NPS), have been subject to various chemical modifications resulting in the appearance of a rapid succession of novel substances. Information related to the metabolism of these substances is often limited due to the lack of *in vitro* and/or *in vivo* studies, or unreported identification in authentic human specimens. In addition, uncharacterized chromatographic retention times, lack of identified target ions, and undetermined recreational or toxic concentration ranges create challenges for analytical detection and toxicological interpretation.

N-ethyl pentylone and dibutylone have been identified as emerging stimulants in impaired driving and death investigation casework, as well as in recreational drug users. The metabolic pathways of neither have been previously characterized.

Separate *in vitro* incubations of N-ethyl pentylone and dibutylone were performed with pooled human liver microsomes in duplicate over three days. Biotransformations identified for N-ethyl pentylone included demethylenation, ketone reduction, and hydroxylation. Biotransformations identified for dibutylone included demethylenation, ketone reduction, hydroxylation, and N-demethylation, forming butylone. After characterization of *in vitro* metabolic pathways, *in vivo* verification of these metabolites was accomplished using authentic specimens from toxicological casework or drug user studies.

Blood specimens (*n*=20) were quantitatively analyzed for N-ethyl pentylone by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) from postmortem cases (*n*=12), DUID cases (*n*=5), and cases with unspecified history (*n*=3). The mean (± Standard Deviation (SD)), median, and range for N-ethyl pentylone concentrations are shown in Table 1. One postmortem blood specimen was positive for pentylone (200ng/mL), a species not identified as a metabolite during microsomal incubations. In total, five metabolites of N-ethyl pentylone were confirmed in these specimens.

	Postmortem Cases (*n*=12)	**DUID Cases (*n*=5)**	**Unspecified Cases (*n*=3)**	**Overall (*n*=20)**
Mean	247 (±259)	41 (±26)	813 (±618)	280 (±374)
Median	155	34	1,140	95
Range	12-833	21-87	100-1,200	12-1,200

Table 1: *N-ethyl pentylone concentrations (ng/mL).*

Blood (*n*=4), urine (*n*=3), and vitreous (*n*=1) specimens were quantitatively analyzed for dibutylone and butylone by LC/MS/MS. All specimens were analyzed from postmortem cases (*n*=4), with overlap in specimens collected from the same individual. Specific case concentrations are shown in Table 2. In total, five metabolites of dibutylone were confirmed in these specimens.

	Blood (*n*=4)		**Urine (*n*=3)**		**Vitreous (*n*=1)**	
	Dibutylone	**Butylone**	**Dibutylone**	**Butylone**	**Dibutylone**	Butylone
Case 1	383	130	3100	69	250	108
Case 2	<10	385	16500	3060	-	-
Case 3	61	<10	2140	149	-	-
Case 4	1400	600	-	-	-	-

Table 2*: Dibutylone and butylone concentrations (ng/mL).*

N-ethyl pentylone and dibutylone were detected in combination in blood specimens (*n*=5) from death investigation cases. In four cases, mean (±SD), median, and range for N-ethyl pentylone concentrations were 479 (±316), 545, and 38-790ng/mL, respectively, and for dibutylone were 18 (±14), 12, and 10-40ng/mL, respectively. One additional blood specimen was positive for N-ethyl pentylone at 50,000ng/mL and dibutylone at 14ng/mL. Butylone was quantitatively confirmed in only two cases, above the analytical threshold.

Additional NPS identified in these cases included methylone, dimethylone, ethylone, 4-fluoroamphetamine, 4-chloro-alpha-PVP, acryl fentanyl, tetrahydrofuranyl fentanyl, carfentanil, para-fluoroisobutyryl fentanyl, U-47700, and U-49900. Causes of death included drug overdose, homicide, suicide, and vehicular crash. Reports of suspected "Molly" and "bath salt" use were noted in two cases. Specimens originated from Pennsylvania, New Jersey, New York, Florida, Texas, Utah, Vermont, Illinois, Missouri, and the District of Columbia. The majority of individuals were male (86%).

A comprehensive analytical approach is necessary to confirm novel stimulants and NPS in biological specimens, as drugs are often found in combination. Specimen concentrations for novel stimulants can vary, as high as µg/mL; therefore, appropriate dynamic range, detection limits, and dilution capabilities should be assessed. Rapid identification of biomarkers can be useful in the determination of unique and/or common metabolites between related substances, possibly providing additional information about ingestion and prolonging detection windows.

N-Ethyl Pentylone, Dibutylone, Postmortem

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