K36 Measuring Inhibition of Neurotransmitter Transport *In Vitro* to Predict Effects and Abuse Potential of Novel Cathinone-Type Stimulants

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Learning Overview: After attending this presentation, attendees will have a better understanding of *in vitro* assays for stimulants, including what is measured, applicability, and limitations of this type of assay.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing attendees with the tools to understand this type of *in vitro* data and correctly implement it in casework.

The pharmacodynamics of stimulant drugs are complex and responsible for the different effect profiles of drugs such as cocaine, amphetamine, and fluoxetine. An important mechanism is the inhibition of Dopamine (DAT), Serotonin (SERT), and Norepinephrine (NET) transporters in the nervous system. The relative preference for inhibiting the different transporters is important for drug effects, and drugs with similar profiles can be expected to have similar effects. Similarly, the relative preference for DAT and SERT has been linked to abuse potential.

A novel assay was developed to measure transport inhibition and characterized seven novel cathinone type stimulants. Stimulants were incubated at 15 different concentrations between 50µM and 3nM for 3h (NET), 6h (SERT) or 10h (DAT) with three different cell lines, each expressing either the DAT, SERT, or NET transporter, and a proprietary dye mix. During incubation, the concentration of fluorescent dye inside the cells increased, while the fluorescence outside the cells was silenced by a quencher unable to enter the cells. The resulting increase in fluorescence was measured and the obtained dose-response curves were used to calculate Inhibitory Concentration 50% (IC_{50}) concentrations. The results from eight well-characterized stimulants were compared to literature values. In addition, inhibition profiles of 3F-alpha-PVP, 4Cl-alpha-PVP, alpha-PiHP, MPHP, 4-methylpentedrone, N-ethylnorhexedrone, and N-ethylpentylone (ephylone) were obtained.

All the characterized novel stimulants were most potent at inhibiting DAT and had inhibition profiles similar to that of alpha-PVP. The IC_{50} in nM for DAT were 160 (cocaine), 12 (alpha-PVP), 13 (3F-alpha-PVP), 8.0 (4Cl-alpha-PVP), 260 (4-methylpentedrone), 13 (alpha-PiHP), 17 (N-ethylpentylone), 4.5 (MPHP), and 47 (N-ethylnorhexedrone). The IC_{50} in nM for SERT were 200 (cocaine), >10,000 (alpha-PVP), >10,000 (3F-alpha-PVP), 1,100 (4Cl-alpha-PVP), 1,300 (4-methylpentedrone), >10,000 (alpha-PiHP), 510 (N-ethylpentylone), 1,900 (MPHP), and 9,000 (N-ethylnorhexedrone). The IC_{50} in nM for NET were 560 (cocaine), 66 (alpha-PVP), 33 (3F-alpha-PVP), 70 (4Cl-alpha-PVP), 1,000 (4-methylpentedrone), 150 (alpha-PiHP), 270 (N-ethylpentylone), 86 (MPHP), and 140 (N-ethylnorhexedrone). All stimulants appeared to be full inhibitors except 4-methylpentedrone, which only partially (75%) inhibited SERT transport.

Based on the data from inhibition of DAT, SERT, and NET, it can be expected that the toxicity of most of these novel stimulants is similar to that observed for alpha-PVP. They were most potent in inhibiting DAT, while higher concentrations were needed for NET (2.5–19x higher IC_{50} than for DAT) and SERT (30->830x higher than DAT) inhibition. The exception was 4-methylpentedrone for which a more balanced profile, more similar to that of cocaine, was observed. Also, 4-methylpentedrone was the only partial inhibitor observed in this study. As all stimulants were selective toward inhibiting DAT over SERT, the abuse potential of these novel drugs is expected to be high.

Potency, NPS, Cathinones