SOFT 2022
Abstract Submission Form

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*****Do not exceed 600 words including tables and charts.*****

TITLE: Evaluating Cross Reactivity of New Psychoactive Substances (NPS) on Immunoassay in Whole Blood

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ABSTRACT:

Background/Introduction: The use of new psychoactive substances (NPS) has been increasing since 2010 when they were first identified. In 2020, the United Nations Office of Drugs and Crime reported a total of 950 NPS which increased to over 1,100 less than a year later in November of 2021. Because of the rapid emergence, there is often little knowledge about the adverse effects or published methods on detecting NPS in biological matrices. Traditional screening in toxicology laboratories is often performed using enzyme linked immunosorbent assays (ELISA). While commercial kits provide information on cross-reactivity on other drugs within a class, many have limited or no information on emerging NPS.

Objectives: The objective of this presentation is to describe the cross reactivity of NPS opioids, NPS stimulants, NPS benzodiazepines and NPS hallucinogens on commercially available immunoassay kits for the purpose of toxicological screening.

Methods: Neogen fentanyl, opiate, amphetamine, benzodiazepine, and PCP ELISA kits were purchased for the project. Fifty different drugs across the various NPS subclasses were analyzed. Blank human whole blood was fortified with the target drug initially at concentrations below the cutoff of the ELISA plate. If no cross reactivity was observed, concentrations above the cutoff were evaluated. Cutoff concentrations were as follows: fentanyl 0.2 ng/mL, morphine 50 ng/mL, amphetamine 20 ng/mL, oxazepam 40 ng/mL and PCP 10 ng/mL. All fortified blood samples, controls and blanks were analyzed on the ELISA plates in duplicate. Following the addition of the fortified samples and drug conjugate, samples were run according to the manufacturer's instructions using Titertek-Berthold Crocodile Mini Workstation. Optical densities were collected at 450 nm. The average optical density was plotted relative to concentrations to assess cross-reactivity and percent cross-reactivity was calculated.

Results: Novel synthetic opioids were tested at concentration ranges of 1-80 ng/mL and 50-2000 ng/mL and had no cross-reactivity to the morphine ELISA plate at either concentration range. Fentanyl analogs were tested at concentrations ranging from 0.01-1 ng/mL on the fentanyl plate and had cross-reactivities ranging from 8.3%-178%. Para-chlorofentanyl, acryl fentanyl, furanyl fentanyl, para-bromofentanyl, and ortho-fluorofentanyl all had cross-reactivities greater than 100%. Para-fluorofentanyl had a cross-reactivity of 84%. NPS stimulants were tested at concentration ranges of 0.5-40 ng/mL and 20-2000 ng/mL on the amphetamine plate. 4-fluoroamphetamine was the only drug with cross-reactivity (3,354%). Prevalent NPS stimulants such as eutylone showed no cross-reactivity. NPS benzodiazepines were tested on the oxazepam plate at concentration ranges of 1-40 ng/mL and 40-500 ng/mL. Cross-reactivities were 35.4-263% and 134-20,804%, respectively. Desalkylflurazepam, flubromazolam, and metizolam all had cross-reactivities greater than 100%. NPS hallucinogens were evaluated on the PCP kit at concentration ranges of 0.5-10 ng/mL, which showed no cross-reactivity, and 10-1000 ng/mL, where some cross reactivity was observed. Hydroxy-PCP and chloro-PCP both had cross-reactivities greater than 100%.

Conclusion/Discussion: Following the cross-reactivity assessment, the structures of the NPS evaluated were compared to the target drug for each kit, which, in general, indicated that the more closely the structure aligned with the target drug, the better the cross reactivity. NPS benzodiazepines and fentanyl analogs, which are seen with limited frequency in cases since core structure scheduling, showed the greatest cross-reactivity across the class demonstrating the utility of using ELISA-based screening for these NPS subclasses. However, limited cross-reactivity was observed for the other NPS subclasses. Therefore, the utility of ELISA-based screening NPS subclasses is limited, and there is the risk of false negative results due to the low or nonexistent cross-reactivities.