Clinical Chemistry - accepted/uncorrected - 27 April 2017

Impact of Novel Psychoactive Substances on Clinical and Forensic Toxicology and Global Public Health

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Novel psychoactive substances (NPS) have been a part of the landscape of clinical and forensic toxicology for over a century, beginning with the introduction of a few new drugs like heroin, lysergic acid diethylamide (LSD), 3,4-methylenedioxymethamphetamine (MDMA) and gamma-hydroxybutyric acid (GHB). However, after the appearance of synthetic cannabinoids in the early 2000's there was a rapid emergence of hundreds of synthetic cathinones, benzodiazepines and opioids. Toxicology laboratories previously focused on a rather narrow range of compounds including amphetamines, cannabinoids, cocaine, opioids, antidepressants, salicylate and acetaminophen. Now potent fentanyl derivatives are mixed with heroin or substituted entirely, killing unsuspecting drug users at an alarming rate. Toxicology laboratories are challenged with detecting potent drug analogs that are only present in blood for a short period of time, urinary metabolites whose chemical formula and structures are initially unknown, and no available reference standards. Here four international experts discuss what fueled the global NPS market, how toxicology laboratories can best address this challenge, and how public health and law enforcement agencies can help reduce the morbidity and mortality associated with NPS.

What factors contributed to the explosion of NPS onto the recreational drug market?

Simon D. Brandt: There are important factors that contributed to this phenomenon but advances in information technology and communication and globalized trade were crucial. Entrepreneurs are now able to instruct contract synthesis laboratories overseas (often in China) to produce any compound of choice on a large scale and in high purity at low cost. From the perspective of a service provider, for example one specialized in the manufacturing of chemicals, the nature of synthesized compounds does not necessarily make any difference and some laboratories are well equipped to supply high quality pharmaceutical drugs and NPS alike. Once the demand is identified, product diversification and expansion of product catalogues follow, which are then available to prospective clients. Easy access to specialist and research literature from the comfort of one's living room facilitates the process by paving the way for new ideas. Electronic commerce is a powerful force that permeates our societies and impacts on how we deal with goods and services, but also on information exchange. As with any other commodity, drugs may be purchased *via* the 'visible' Internet or the 'dark web', where the latter also includes the trade of controlled substances. Legislative ambiguity in how to deal with the NPS phenomenon and

the supply of these substances is also an important consideration. In addition, trading within a globalized world allows for highly effective ways to supply and distribute the goods so they can reach any corner of the world relatively easily. In cases where organized crime groups are involved in distribution to end users, a wider penetration to distinct sub-populations may also be achieved. In previous decades, information and supply of then non-controlled alternatives to some of the 'classic' drugs, including natural products, originated from specialized mail order catalogues, flyers or magazine advertisements, which also limited access to specialized communities. Today, your mobile phone assists you in accessing this information, placing an order and sharing your experiences on a user forum.

Suman Rana: Multiple factors contributed to the explosion of NPS into the recreational drug market. First and foremost, in my opinion, was the widespread availability of Internet shopping. Internet shops made NPS just a "click" away from the experimental drug users and fueled the NPS explosion. Other factors included the ease of synthesis and alteration of molecular structures of existing drugs to circumvent regulations. Lack of effective and timely regulation made it easy for clandestine drug manufacturers to stay ahead of regulation as the drug culture started shifting. The ineffective control over NPS, not only in the United States but also in nations like China and India where the vast majority of synthesis for these chemicals occurs, played a huge role in their increasing prevalence. Effective control over their manufacturing could not be implemented, which, coupled with easy Internet channels for sale, resulted in a huge explosion. Because NPS were not effectively regulated, they became attractive to adolescents and young adults, particularly those seeking to avoid a positive drug screen. Other factors included similarity in their effects to some of the existing drugs. For example, the first generation synthetic cannabinoids mimicked the effects of marijuana and quickly gained popularity as "Legal Pot". However, as the chemical composition of NPS continued to evolve, so did their physiological effects.

Michael H. Baumann: Information freely available on the Internet is a major contributor to the explosion of NPS. Electronic biomedical databases provide information about the central nervous system (CNS) targets for drugs of abuse, such as neuroreceptors and transporters. Online chemistry journals and patent literature give detailed recipes for synthesizing compounds that

interact at these targets with high potency and efficacy. Commercial websites provide easy marketing and sales of anonymous purchases, facilitating global availability of NPS. Finally, online user forums provide a platform for information sharing among those users who seek to fine tune their drug-taking experiences.

Volker Auwärter: A prerequisite for the phenomenon was the introduction of online retail markets for drugs and the resulting high availability, combined with transfer of information on new drugs via social media and, in particular, online drug forums. Relatively high prices and often low quality of classical illegal drugs, particularly in rural regions, may also have contributed. Finally, the degree of repression on drug users (comprising extensive 'work place drug testing' programs and driving license issues) and the legal status of NPS certainly influenced the dynamics.

What are the advantages and disadvantages to drug users of taking NPS rather than classical drugs?

Suman Rana: The obvious advantage to the users is their easy availability and avoidance of detection during a routine drug test since most drug testing programs do not include NPS in their testing profile. Even if programs mandated testing for NPS, there are a limited number of laboratories that have the capability to develop testing to detect the ever-changing chemicals. In addition, the legal status of many of these drugs makes NPS a favorable choice over classical drugs since there are no legal repercussions for using something that is not controlled.

Disadvantages of using NPS are mainly the toxicity associated with their use, and the lack of toxicity studies prior to their introduction in the market. In the absence of such data, users are literally playing with their life since the harmful effects are not well known and/or understood. Another disadvantage is the lack of quality control in the manufacturing process of products containing NPS. NPS-laced products are prepared in clandestine laboratories where product consistency is not maintained, resulting in "hot spots" within the product where drug concentration may be much higher than other parts of the same preparation. As a result, the user may experience acute toxic effects due to an increased dose.

Michael H. Baumann: NPS are cheap, easy to obtain, and not detected by traditional drug screening methods, which rely on antibody-based technologies that recognize only a few specific drugs and their metabolites. A major driving force for the misuse of NPS is their lack of detection, especially in those populations that are subjected to routine toxicology screening (e.g., military personnel, parolees, probationers). A major disadvantage of using NPS is the risk for adverse effects since the active ingredients in the products are often unknown, and the biological effects of most NPS have not been evaluated in animal experiments. To date, no controlled clinical trials have examined the effects of NPS in human subjects.

Volker Auwärter: The advantages include high availability, relatively low prices, low risk of being prosecuted, wide variety of drugs for experimental drug use, online shopping rating systems, exchange in online forums (e.g. on quality of drugs and reliability of suppliers), and no personal contact with potentially dangerous drug dealers.

The disadvantages include a greater risk of new compounds due to lack of experience and knowledge on specific toxicity (resulting in relatively high numbers of intoxications/deaths), greater difficulties in judging identity and/or purity of the drugs, and greater problems regarding drug dosing.

Simon D. Brandt: NPS are often easier to obtain, and the context in which a particular NPS is taken might also be relevant. Although access to classical controlled drugs does not appear to be a problem in many countries, some users may not wish to engage with the friendly street dealer around the corner and are, therefore, looking for non-controlled substances and/or other sources of supply. Others might wish to obtain alternatives to CNS-active medicines that are available on prescription only. This can be a challenge for patients who received prescription drugs for a limited amount of time, but who might wish to continue with the drug treatment without medical supervision. Individuals who contemplate use of alternatives to prescription drugs without consulting a healthcare professional have access to a range of NPS classes that might serve their needs, either for recreational or self-medication purposes. There is a fraction of users who are solely interested in the exploration of new chemical entities and desire NPS primarily for their

novelty. The fast rate at which NPS arrive on the drug market can place significant challenges on the ability to detect them within the clinical and forensic setting, and users who might wish to avoid drug detection stand a better chance of being successful. Overall, it is still a challenge to obtain accurate information about the scale of NPS use in the general population and how this compares with traditional drugs that appear to dominate the scene. However, there is a segment of society that appears to be more prone to NPS exposure compared to the general population, including those with a history of problematic drug use, homelessness or mental health problems, or prison service users. The difficulty encountered in drug detection may be an advantage for some of these users but ultimately drug consumption under these conditions is essentially driven by which drugs are available rather than choice.

Synthetic cannabinoids initially led the introduction of NPS, but currently NPS are available for almost all drug classes. Do you think we will continue to see the introduction of new NPS in all drug classes and why?

Michael H. Baumann: It seems certain that NPS that mimic the effects of all major classes of abused drugs will continue to be available. In this regard, the most alarming new trend is the appearance of various novel synthetic opioids (NSO) in the street drug marketplace. NSO include high-potency analogs of fentanyl such as acetylfentanyl, butyrylfentanyl, and furanylfentanyl, as well as non-fentanyl analogs like U-47700 and MT-45. These substances are contributing to the current spike in opioid overdose deaths. NSO are synthesized in Asian laboratories and marketed via the Internet, similar to the pathway for manufacture and sale of cathinone and cannabinoid NPS. NSO are now present in the street drug marketplace as stand-alone products, adulterants in illicit heroin, and counterfeit opioid medications. Most users are completely unaware of their exposure to NSO. The continued spread of synthetic opioids represents a significant public health threat.

Volker Auwärter: Yes, I do think so, basically because there will be an ongoing demand for new compounds due to legislative regulations, and there is a lot of money to be made in this market. In different countries, there will certainly be different drug preferences depending on

cultural circumstances, as well as availability, quality, prices, and law enforcement pressure on classical illegal drugs.

Simon D. Brandt: The introduction of NPS is largely driven by what is manufactured in contract synthesis laboratories that treat these compounds like any other saleable chemical and where diversification of the product portfolio increases further business opportunities. The development of NPS that are emerging onto the market reflects modifications of known, or classical chemical structures with psychoactivity. The plethora of analogs and derivatives that can theoretically arise from endless permutations has not yet been exhausted and one can envisage many more candidates that could still be released. Another approach to exploring potential NPS ideas may be linked to the identification of primary pharmacological targets that are correlated with CNS activity. This might well be an important area for further investigation. In pharmaceutical drug development, structurally diverse compounds are investigated for their ability to produce desired CNS effects through agonism or antagonism at specific biological targets. Clandestine NPS producers appear to be adopting this approach. It seems likely that the number of potentially available NPS will increase if large-scale manufacturing facilities continue to exist. At the same time, one must realize that not every developed compound leads to the desired psychoactive effects in humans, which might explain in part why the existence of many compounds on the market has been short-lived.

Suman Rana: Yes, I do think that we will continue to see the introduction of new NPS in all drug classes. The scientific communities are trying to do a better job of disseminating information about NPS, and the regulatory agencies are constantly trying to make regulations more effective, so it is imperative that clandestine drug manufacturers continue to market more NPS across all drug classes to stay one step ahead of the regulations. Manufacturing NPS is a lucrative business for drug-making laboratories, so they will not quit easily.

What tools do toxicology laboratories need to best identify NPS intake in biological specimens? Should all laboratories offer these services or is it a better allocation of resources for reference or centralized laboratories to offer NPS testing?

Volker Auwärter: At the moment, I believe that the most efficient strategy consists of having regularly updated and targeted LC-MS/MS methods. To keep the methods up to date, a continuous market monitoring combined with tools for identification of the main metabolites is required. Incubation of NPS with prepared human liver microsomes and identification human urinary metabolites by mass spectrometry is the most cost effective technique of several available approaches. Given the relatively low general prevalence of NPS in most countries, the time-consuming and expensive efforts required to keep pace with market developments, and the extensive analytical expertise required, I believe that centralized laboratories will be the more effective solution.

Simon D. Brandt: Implementation of gas and liquid chromatography-based techniques coupled to various mass analyzers (including single stage, MS/MS and/or MS^n) with low and high resolution appear to be essential tools. The key challenge is to unambiguously identify NPS in a practical timeframe. Many laboratories struggle to do this task, particularly in the absence of reference material that may be included in the corresponding screening procedures. Lack of specific instrumentation, however, might also contribute to this challenge, especially when dealing with low NPS concentrations. The idea of centralized laboratories that specifically deal with NPS cases is an interesting option, which could alleviate the burden faced by some forensic providers provided this is carried out in a timely fashion. Centralized collection of data might also help to minimize the problem of underreporting. In some countries, publicly-funded and centralized laboratories that deal with forensic and clinical casework exist, which has the advantage that a more complete picture can be painted regarding acute toxicity induced by NPS currently circulating on the market. At the same time, the reliance on a limited number of laboratories that dominate the market place might be a cause for concern to others. Toxicology laboratories could perhaps also benefit from small- or micro-scale organic synthesis facilities to improve analysis capability by supplying reference materials for NPS and their metabolites.

Suman Rana: Laboratories need analytically sensitive instrumentation for detection, parent drug and metabolite reference standards for identification, and qualified staff to develop methods for analysis of NPS in biological matrices and provide interpretation of these data. Unfortunately, most public sector laboratories do not have funds to acquire expensive instrumentation or

research staff. Laboratories that have such resources should offer these services. For others, there needs to be reference or centralized laboratories where testing could be outsourced.

Michael H. Baumann: High volume standard toxicology screening methods for the detection of abused drugs have relied on antibody-based technologies (e.g., immunoassays), which can identify a limited number of drugs and their metabolites. Such tests do not detect the presence of most NPS. Given the rapid appearance and disappearance of NPS in the marketplace, the expensive and cumbersome process of developing new immunoassays cannot keep pace. Thus, more sophisticated analytical methods such as liquid chromatography with mass spectrometry or high-resolution mass spectrometry are required to detect newly emerging drugs.

Human hepatocyte incubations and high resolution mass spectrometric identification of urinary NPS metabolites, and verification of identified markers in authentic urine specimens is necessary to identify NPS intake and to provide data to commercial standard manufacturers for the targeted synthesis of reference standard metabolites. Is this a feasible approach to keep pace with the rapidity of NPS introduction?

Simon D. Brandt: The evaluation of various cellular preparations is helpful to identify urinary markers and how verification can be obtained from authentic urine samples. It is therefore an important source of information to guide the targeted synthesis of metabolites. An alternative approach to the identification of human urinary markers, although admittedly less common, may involve self-experiments. For the purpose of metabolism studies, an ingested dose might fall below what might be considered an active dose, thus, reducing or avoiding psychoactive effects. Generally speaking, important key metabolites may be predictable, either with or without support from *in silico* studies, so commercial suppliers may not be limited anymore by the unavailability of these data. Cost implications may also be relevant. There are cases where distinct NPS can result in the formation of identical transformation products, so the search for potentially distinctive candidates could also be a fruitful area of investigation. The search for biological markers arising from potential synthesis impurities present in NPS might also deserve some attention.

Suman Rana: In my opinion, this is not the most effective approach to keep pace with the rapidity of NPS introduction. While it is a necessary approach for urine drug testing, the use of alternate matrices like oral fluid is a much better approach for effective monitoring and testing for NPS. The time and resources involved in identifying the urinary metabolites, synthesizing the identified metabolites to be used as a reference standard in routine testing, and developing complex methods of testing capable of identifying multiple metabolites is futile, because new NPS are introduced so rapidly into the market that by the time the method is developed and implemented it is no longer effective. This whole process is detrimental to any laboratory. Since parent drugs are detected in oral fluid, there is no need to spend time and resources in identifying the metabolites. Drug reference standards are more rapidly available for parent drugs so the methods for detection can be developed relatively easily. The interpretation of a urine test could also be challenging. Since the NPS are produced by slight modifications of the existing drug structures, many of them produce metabolites common to multiple drugs, thus complicating interpretation since it is challenging to identify the parent drug consumed. In contrast, oral fluid testing allows identification of the parent compound. It is much easier for laboratories to stay current with the drug market by using oral fluid testing.

Michael H. Baumann: Urinary detection of NPS and their metabolites will continue to be a main source of information, but human specimens are not readily available in many research settings. Additional strategies for investigating the metabolism of NPS are available. *In vitro* metabolism studies using prepared human liver microsomes or hepatocytes can provide useful data about the metabolism of NPS. Likewise, *in vivo* experiments in animal models can also be important because only a few of the many potential metabolites generated *in vitro* are present after NPS administration *in vivo*. As an example, the synthetic cannabinoid AM-2201 generated 13 different metabolites when examined *in vitro*, but only three of these metabolites were detected after *in vivo* administration of AM-2201 to rodents. Therefore, combined *in vitro* and *in vivo* approaches can be useful for predicting metabolic profiles for NPS as they emerge.

Volker Auwärter: I would slightly disagree, because marker identification can also be performed effectively by using prepared human liver microsome assays, which are less expensive and time consuming. In addition, pure reference standards of metabolites are

useful for quantitative analysis but not necessarily needed for an unambiguous proof of drug intake. Nevertheless, quick identification of valid consumption markers is essential and has proved to be feasible.

High-resolution mass spectrometry (HRMS) is an important tool to combat the problem of NPS. How do you use HRMS in your laboratory, and do you think that HRMS will be an essential tool for all laboratories within 5 years?

Suman Rana: HRMS systems are important but not essential to combating the NPS problem. While high-resolution systems provide important structural information about the NPS metabolites and help with initial method development, once that information is available the analysis could be performed using nominal mass analyzers. I think HRMS is an essential tool for understanding and identifying the urinary metabolites for new drugs and should be used at reference or centralized laboratories that are engaged in metabolite identification studies for the new NPS; however, having HRMS systems in every laboratory and having qualified staff to interpret the HRMS data at every laboratory in the next 5 years is not realistic or necessary. My laboratory is one of the leading laboratories in the United States that provides NPS testing but currently we do not have an HRMS system. We send our hepatocyte or microsome incubation samples out to other reference laboratories for structure elucidation/identification for initial method development.

Michael H. Baumann: Novel mass spectrometry methods such as sequential window acquisition of all theoretical mass spectra (SWATH) can be used to accurately measure many NPS and their metabolites in a single confiscated drug product or biological specimen. One of the chief advantages of SWATH is that the method can be continuously updated for the detection of a diverse panel of analytes to keep pace with the changing street drug landscape.

Volker Auwärter: We use HRMS mainly for confirmation of tentatively identified metabolites and to screen biological samples in specific cases where we suspect compounds not covered by our routine NPS methods. I believe that in 5 years most of the workload of clinical and forensic laboratories will still be processed without HRMS. Nevertheless, the technique offers great

added value and will become standard as soon as prices have come down to be comparable to those of high-end triple quadrupole instruments and the software tools have improved (e.g. reliable, semi-automated data analysis).

Simon D. Brandt: HRMS has indeed helped with the increase of information content, most notably when detecting potential drug candidates that have not been seen previously. In these situations, the combination of high-resolution single- and tandem-mass spectrometry facilitates the identification process or helps to narrow down potential candidates that are consistent with the molecular formulae obtained from the protonated molecule (in positive mode), but also from the resulting high-resolution product ions that support the formulation of dissociation pathways. Important information can be derived from using low-resolution approaches as well, but the high-resolution option provides more confidence in the interpretation. Whether HRMS should be considered an essential tool remains to be seen, but it seems likely that increasing popularity combined with lower pricing will contribute to a further expansion of this method of analysis. In part, this is also driven by specific marketing efforts by manufacturers of HRMS products. Implementation of new technologies, for example in the form of instrumentation and novel software applications, will always be needed and the expertise and experience gained by the analyst will maximize their impact.

What do you think is the best strategy for local, state, and federal laboratories to address the problem of NPS?

Michael H. Baumann: NPS often cause localized epidemics of toxic exposures, where many patients simultaneously present to emergency departments exhibiting life-threatening adverse medical symptoms. In many cases, the precise chemical compounds causing the medical emergency are not known because typical hospital settings do not have the sophisticated analytical technology available for identifying NPS, especially when new drugs first appear on the market. Creating a network of regional sentinel laboratories across the United States could provide rapid and accurate forensic analysis of confiscated products and biological specimens when epidemics of NPS first arise. These laboratories should have the infrastructure and staffing needed to perform a variety of mass spectrometry methods. The resulting analytical findings

could be shared in an open-access online format to allow instant access for all stakeholders of interest, including clinical toxicologists, research scientists, and law enforcement personnel.

Volker Auwärter: The worst strategy is to close one's eyes and neglect the problem, because this will inevitably lead to a considerable number of undetected NPS in case work. It is also not sufficient to implement methods targeting a range of compounds without being updated regularly according to the drug market scene. Therefore, a few specialized laboratories should thoroughly tackle the problem, provide comprehensive methods, and offer their service to other local laboratories. This applies in particular to forensic case work, where missing a compound or producing a 'false positive' may have serious consequences.

Simon D. Brandt: Toxicology laboratories play a crucial role in the assessment of identifying acute harms that might be associated with NPS use. The provision of real-time information can form part of NPS toxicovigilance that is needed to inform policymakers and other stakeholders faced with this phenomenon. Technological challenges aside, perhaps one of the most important strategies is to facilitate fast, effective, and unbiased information exchange and data dissemination so that effective response to this dynamic drug market can be formulated in conjunction with public healthcare and harm reduction professionals. A key emphasis here is the contribution to an early-warning system.

Suman Rana: This is a difficult question. The only way I see local and state laboratories managing the NPS problem is by sending out testing to reference or centralized facilities. Investment in centralized laboratories is necessary to establish testing protocols and to support ongoing research needed to keep up with the changing drug scene. Updating the testing procedures on an ongoing basis to include the most current drugs is essential for successful handling the NPS problem. Having the centralized laboratories capable of keeping the testing protocols updated will ensure successful detection of NPS cases.