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Letter

A Taste for New Psychoactive Substances: Wastewater Analysis Study of 10 Countries

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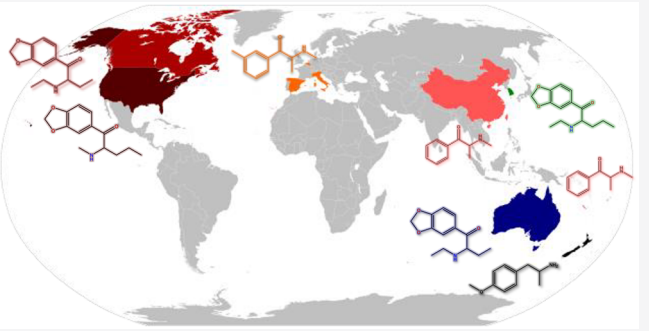
ABSTRACT: New psychoactive substances (NPS) are compounds designed to mimic both licit and illicit drugs, and these substances are being discovered each year through forensic toxicology, drug enforcement agencies, and health authorities. However, there is limited information surrounding their international popularity. In this work, influent wastewater samples (n = 144) were collected from 25 sites in 10 countries: Australia, Belgium, Canada, China, Fiji, Italy, New Zealand, Republic of Korea, Spain, and the United States over the 2020−2021 New Year period. All samples were extracted in the country of origin then shipped and analyzed centrally at the University of South Australia using validated liquid chromatography−mass spectrometry methods. This study focused on 28 NPS stimulants, with 11 detected. The emerging substances eutylone and 3-methylmethcathinone (3-MMC) were detected most frequently and with the highest mass loads, indicating international popularity. Interestingly, the "older" generation stimulants, *para*-methoxyamphetamine (PMA), methylone, and mephedrone, were also detected. From the sites monitored in this work, areas in New Zealand had the highest loads of NPS stimulant consumption. Results here show that wastewater analysis can elucidate the dynamic nature of the NPS market, providing near real-time information on changing consumption patterns whose information can be used to minimize public risk.



INTRODUCTION

In the last two decades, new psychoactive substances (NPS) have emerged as a global threat to public health, and their continuous evolution has posed a challenge to authorities.[1] The United Nations Office of Drugs and Crime Early Warning Advisory (UNODC EWA) is the first point-of-call for any global information on NPS, collecting data from international forensic, border security, health, and drug enforcement agencies.[2] While it does provide an insight into international prevalence, information is not necessarily timely or publicly available. Hence, early warning advisories have been established on local and national levels, for example, in Australia,[3] New Zealand,[4] the United States,[5] and Europe wide,[6] to provide critical information to the public in a timely manner.

The continuing presence of NPS on the international drug market shows no signs of abating. According to the UNODC EWA, more than 1000 NPS have been reported worldwide, from 126 countries, as of January 2021.[7] Europe accounts for the majority of detected NPS, with approximately 830 being monitored by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), and 400 previously reported substances are found on the European market every year.[8] The most common classes reported to the UNODC and EMCDDA are synthetic cannabinoids and synthetic cathinones. These trends are reflected in most other countries, with the exception of the United States, where designer benzodiazepines are the most common NPS class, according to the latest data from the U.S. Drug Enforcement Agency.[9]

Monitoring NPS use is challenging due to differences in type, timing, and availability of data from various sources such as law enforcement drug seizures, drug checking services, healthcare reports and data, the dark web, population surveys,

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Table 1. Results of All NPS Stimulants Found in This Study, Mean Mass Load per Site (mg/day/1000 People), and Frequency of Detection (Shown in Parentheses)[a]

Compound	Site (mean mass load, detection frequency)
3-Methylmethcathinone (3-MMC)	BL (2.2, 7/7); ES (0.17, 8/8); IT (1.4, 9/9); NZ1 (0.07, 8/8); NZ2 (0.02, 1/8); NZ3 (0.61, 8/10)
Ethylone	AU1 (0.06, 3/8); CA (0.46, 2/7); NZ2 (0.01, 3/8); US2 (<LOQ, 1/8)
Eutylone	AU1 (0.25, 8/8); AU2 (0.46, 8/8); AU3 (0.36, 7/7); AU4 (0.62, 8/8); CA (0.09, 7/7); NZ1 (1.4, 8/8); NZ2 (4.6, 8/8); NZ3 (17.6, 10/10); US1 (0.17, 7/7); US2 (0.03, 8/8); US3 (0.13, 3/3); US5 (0.01, 1/3); US6 (0.47, 2/3); US7 (0.26, 1/3); US8 (0.11, 2/3); US9 (0.69, 1/3); US10 (0.02, 3/3)
Mephedrone	AU2 (0.05, 5/8); CA (0.1, 1/7); NZ1 (2.7, 8/8); NZ3 (0.25, 10/10); US1 (<LOQ, 7/7)
Methcathinone	AU1 (0.65, 8/8); AU2 (1.02, 8/8); AU3 (0.63, 7/7); AU4 (0.64, 8/8); BL (0.23, 7/7); CA (0.41, 7/7); CN (0.18, 8/8); ES (0.38, 7/8); FJ1 (0.01, 3/4); IT (0.38, 9/9); KR (1.5, 1/1); NZ1 (0.028, 8/8); NZ2 (0.023, 8/8); NZ3 (0.05, 10/10); US1 (2.9, 6/7); US2 (0.75, 8/8); US3 (0.69, 3/3); US4 (0.69, 3/3); US5 (0.78, 3/3); US6 (0.66, 3/3); US7 (0.55, 3/3); US8 (3.9, 3/3); US9 (1.3, 3/3); US10 (0.83, 3/3)
Methiopropamine	AU2 (0.04, 2/8)
Methoxetamine	AU1 (0.03, 2/8)
Methylone	AU1 (0.02, 2/8); AU2 (0.04, 3/8); AU3 (0.04, 2/7); NZ1 (0.02, 8/8); NZ2 (0.02, 5/8); NZ3 (0.07, 10/10)
N-Ethylpentylone	AU1 (0.005, 1/8); AU2 (0.002, 1/8); CA (0.04, 2/7); KR (0.01, 1/1); NZ2 (0.005, 6/8); NZ3 (0.007, 2/10)
Pentylone	US1 (0.38, 7/7); US2 (0.02, 5/8)
Para-methoxyamphetamine (PMA)	NZ1 (0.30, 8/8)

[a]Abbreviations: AU, Australia; US, United States; CA, Canada; KR, Republic of Korea; NZ, New Zealand; FJ, Fiji; BE, Belgium; IT, Italy; ES, Spain; CN, China.

and analysis of human biological samples.[1,10] Analysis of influent wastewater is challenging but recognized as a complementary tool to traditional data sources and can provide community-wide information on the consumption of NPS.[11] This involves the analysis of influent wastewater collected prior to any treatment process at a wastewater treatment plant to provide population-scale estimates of human consumption within the catchment area.[12] So far, both qualitative screening approaches and quantitative target methods have been successfully applied for the detection of NPS,[13] including in Australia,[14−16] China,[17] and Europe.[18−20] In addition, wastewater analysis has provided evidence on the use of NPS in special events (e.g., music festivals, sporting events, and holiday periods).[21−24] Monitoring sites over time can provide insight into the changing drug scene, which is particularly necessary for the ever-changing NPS market. This potentially allows health agencies working with drug users to focus their interventions and resources to minimize the risk of harm.

Due to their popularity among consumers and concern from public health authorities, this study focused on the prevalence of NPS in wastewater samples collected from 25 sites in 10 countries across Asia, Oceania, Europe, and North America over the 2020−2021 New Year period. This expands upon our previous international study by including more countries and sites, adding new compounds to the targeted method, and investigating the impact of international COVID-19-pandemic enforced international lockdowns.

MATERIALS AND METHODS

Sample Collection. Influent wastewater samples (flow or time proportional 24 h composite (Table S1)) were collected for up to 9 days over the 2020−2021 New Year period from 25 sites in 10 countries: Australia (AU; four sites), the United States (US; 10 sites), Canada (CA; one site), Republic of Korea (KR; one site), New Zealand (NZ; three sites), Fiji (FJ; two sites), Belgium (BE; one site), Italy (IT; one site), Spain (ES; one site), and China (CN; one site). All sites provided at least one sample corresponding to 31 December 2020, with all collection dates listed in the Supporting Information (Table S1). Due to confidentiality agreements with the wastewater treatment plants or participating municipalities, further identifying information cannot be disclosed. All participants received instructions to follow for sample collection, to reduce variability, with specific information found in the Supporting Information (Section S1).

Sample Treatment. A validated sample treatment protocol was used by all participants.[25] Briefly, following filtration (GF/A 1.6 μm, Whatman, Kent, UK), the pH was adjusted to 4.5−5 using aqueous ammonia (28%) prior to solid phase extraction (UCT XtracT DAU, 500 mg/6 mL; UCT Inc., Bristol, PA, USA). The samples (100 mL) were loaded under gravity and the cartridges washed with a sodium acetate buffer (20 mM, 6 mL), acetic acid (0.1 M, 2 mL), and methanol (6 mL) before being air dried for 15 min. The dried cartridges were stored at −20 °C prior to shipping to UniSA for analysis.

On arrival at UniSA, cartridges were again stored at −20 °C for no longer than 48 h before elution. A mixture of dichloromethane:isopropanol:aqueous ammonia (80:16:4 v/v/v, 6 mL) was used to elute the analytes from the cartridges and evaporated to approximately 200 μL under nitrogen at 40 °C. A solution of 1% HCl in methanol (20 μL) was then added, before being evaporated to dryness. The dry residue was reconstituted with 0.1% formic acid in methanol (20 μL) and 0.1% formic acid in ultrapure water (80 μL) to give a final volume of 100 μL and a concentration factor of 1000 times.

Instrumentation and Data Analysis. Validated liquid chromatography−mass spectrometry methods were used for this work, which incorporated a Sciex ExionLC coupled to a Sciex 6500 + QTrap (Toronto, Canada), fitted with a TurboSpray IonDrive source and a biphenyl analytical column (150 mm × 2.1 mm × 1.7 μm) at a flow rate of 0.3 mL/min and an injection volume of 2 μL.[25,26] Limits of detection and quantification are shown in Table S2. Precision (repeatability) was assessed by analyzing two concentration levels across all batches, with acceptable values less than RSD 20% (Table S3).

For each compound, a 10-point calibration curve was constructed from 0.04 to 100 ng/L. For positive identification, retention time compatibility with the reference standard in solvent (±2%), as well as two matching transitions, were necessary. Concentrations were calculated using the isotope dilution method. As many participants used different internal standards in their laboratories, the chosen internal standards

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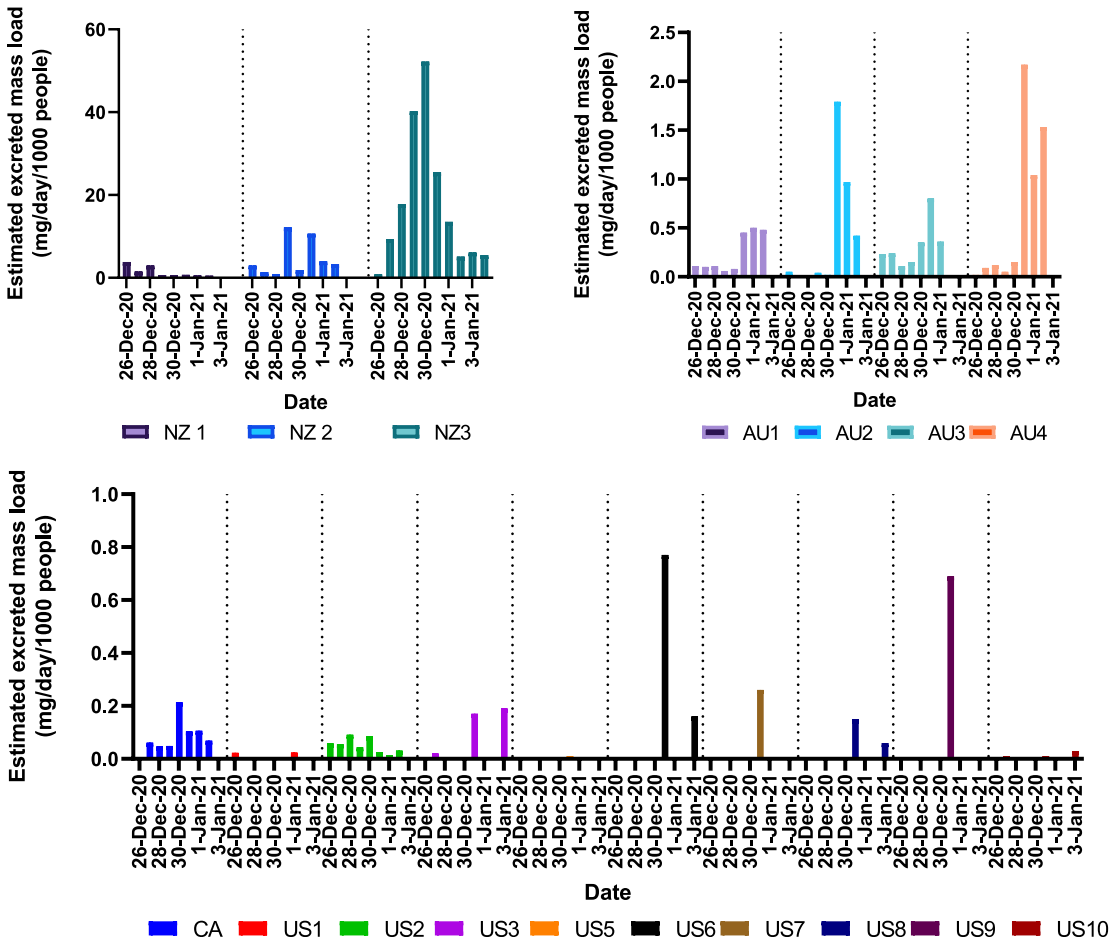

Figure 1. Estimated excreted mass loads of eutylone found in this study. Only sites where the compound was found are shown.

were utilized for quantification (Tables S4−14). A new calibration curve was made for the same drug, matched to the internal standard used by respective laboratories. To ensure accuracy, at least seven of the calibration points had to fall within 80%−120% of the expected value, following guidelines of the European Medicines Agency.[27] Concentrations were then transformed to mass loads using flow rate and population data provided by the respective participating laboratories (Table S1).

All data were acquired with Analyst 1.7 (Sciex) and processed using MultiQuant 3.0.2.

RESULTS AND DISCUSSION

Of the 27 substances investigated, a total of 11 NPS were detected (Table 1, with more detailed information in Supporting Information, Tables S4−S14), with methcathinone, eutylone, and 3-methylmethcathinone (3-MMC) the most common and present at some of the highest mass loads. Most of these compounds were cathinones including mephedrone, ethylone, methylone, N-ethylpentylone, and pentylone. Sixteen analytes were not detected, including four phenethylamine compounds, five synthetic opioids, and seven synthetic cathinones (Table S2).

Detections occurred at every site except for FJ2 and varied between locations with CN and FJ1 reporting a single compound,and sites in NZ and AU with as many as six. The only NPS found in KR were methcathinone and N-ethylpentylone; the latter previously seen in the US, AU, and NZ[15,23,28,29] before the emergence of eutylone. This result suggests eutylone emergence may be expected in KR in the future. N-Ethylpentylone was also found at low levels in AU, CA, and NZ (0.002−0.05 mg/d/1000 people), together with eutylone. One concerning finding was the detection of *para*-methoxyamphetamine (PMA), present in NZ1 across the entire sampling period. PMA is a drug that has been associated with fatalities.[30] It was seen at mass loads from 0.1−0.7 mg/d/1000 people, but the fact it was consistently seen across a week is a cause for concern and entreats ongoing monitoring.

Eutylone and 3-MMC are two of the synthetic cathinone classes of NPS that have recently gained in prominence and popularity. Eutylone was found with high loads in New Zealand and was also present in Australian and North American samples. In contrast, 3-MMC was mainly confined to Europe but also seen for the first time in New Zealand. It is therefore of interest that both these substances and the more established mephedrone, methcathinone, and PMA were found across the sites.

All samples were collected with the backdrop of the COVID-19 pandemic. Many countries around the world were in lockdown, limiting social interaction and large get togethers, and thus, the distribution and consumption of certain drugs were expected to be affected. Recent studies have shown that the restrictions enforced by authorities have influenced the use of illicit drugs and NPS by the population.[31−33] The only sites involved in this study that

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were not subject to any restrictions at the time of collection were from New Zealand and sites US3−10.

Due to limited information on pharmacokinetics and metabolisms of the NPS investigated as well as the unavailability of potential metabolites in this study, only the parent compounds were monitored. Direct disposal could possibly lead to an overestimation of the calculated excreted mass loads. However, the data obtained in this study are not consistent with direct disposal events, where spikes on individual days might have been observed. Considering the manufacturing origins and natures of NPS use, appreciable quantities of drugs being disposed appear unlikely.

Methcathinone was found in every location except FJ2. Considering that the compound is also the oxidation product of ephedrine and pseudoephedrine, we were cautious to link its presence in wastewater solely to illicit drug consumption. The next most abundant NPS were eutylone and 3-MMC. These substances deserve specific consideration as they are relatively recent additions to the NPS market. Isolated instances of their use appeared in our previous study,[23] with the current work demonstrating that their presence has spread globally.

Eutylone. We have previously shown that eutylone was present in influent wastewater samples from Australia, The Netherlands, New Zealand, and the United States,[15,23] indicating its international prevalence. This compound has been of particular concern in New Zealand[34] and the United States,[35] with public health alerts released regarding the increasing prevalence of the substance. Eutylone has also been identified in postmortem and toxicological samples from 13 states in the United States[36] and Taiwan[37] between January 2019 and April 2020. In addition, recent work has shown that reports of eutylone on the social media platform Reddit have been increasing since early 2020.[38]

In this study, eutylone was found in all sites in AU, NZ, and CA and all but one site in the US, with no detections in the sites investigated in Europe or Asia (Figure 1). All sites showed increased consumption over New Year's Eve, except NZ 1, where elevated levels were between 27 and 29 December. Overall, the highest levels appeared in NZ, with mass loads of up to 52 mg/day/1000 people found in NZ3. These high levels are in agreement with media releases surrounding eutylone in New Zealand over the 2020−2021 New Year period.[34] In fact, more than half of ecstasy pills tested at New Zealand festivals and events over the New Year period were found not to contain MDMA but other cathinones. Eutylone was the most common substitute.[39] Eutylone was the NPS with the highest mass load in the Australian sites, while it was also seen in the US and CA with increases observed over New Year's Eve. This shows that despite the COVID-19 pandemic restrictions, people were still showing behavior consistent with a festival season.

3-Methylmethcathinone (3-MMC). 3-Methylmethcathinone (3-MMC) is a positional isomer of mephedrone (4-methylmethcathinone). As the popularity of mephedrone increased and international concerns surrounding its toxicity were raised, it was banned in many countries. However, several structurally similar NPS have emerged with 3-MMC becoming widespread among NPS consumers.[40] In a recent review on 3-MMC, both fatal and nonfatal intoxications have been linked to the substance; however, all cases were confined to Europe.[40] We have previously found this substance in wastewater samples from The Netherlands, Spain, and Italy, indicating the European propensity for this drug.[23] This has been corroborated by seizure data from Italy, with 3-MMC being the most detected NPS in drug seizures in May−October 2020,[41] while The Netherlands has restricted access to the drug from the autumn of 2021.[42]

In the current study, 3-MMC was again particularly evident in the European sites, being found on every day of the sampling week in BE and IT and on 7 of 8 days in ES (Figure 2). Increases in the mass loads were seen on New Year's Eve in

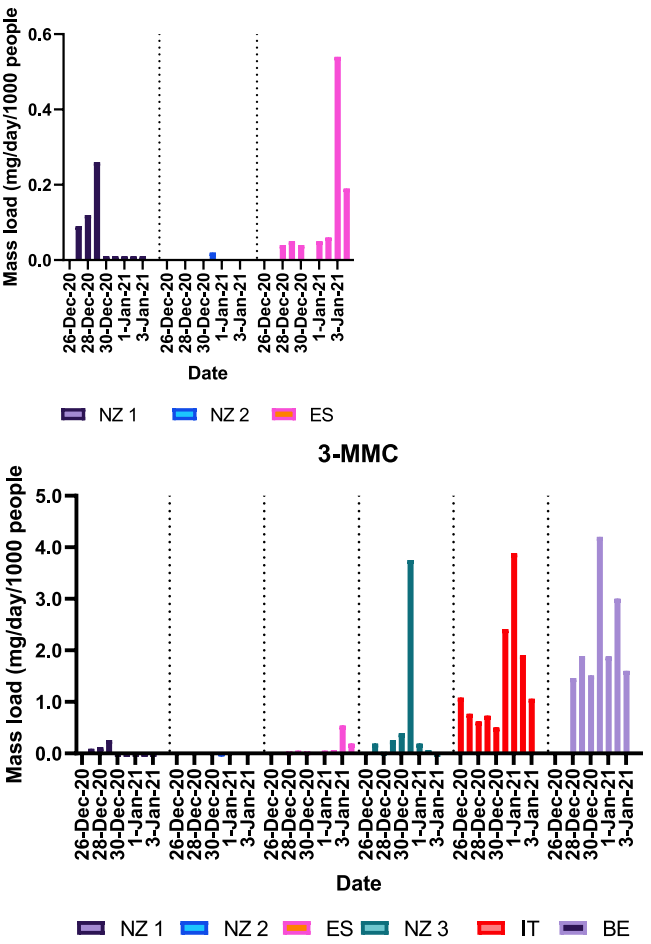

Figure 2. Estimated excreted mass loads of 3-MMC found in this study. Only sites where the compound were found are shown.

most sites as well as January 2 and 3 (a weekend) in BE and ES, indicative of party drug consumption. For the first time, 3-MMC was also seen in NZ. The site NZ3 had a sharp increase over New Year's Eve, more than 10 times higher than other days of the sampling week. Although the drug was also found at NZ1 and NZ2 at low levels, it was nevertheless present.

Interestingly, both 3-MMC and mephedrone were found in the three NZ sites (Figure S1) in this study. NZ1 had quite high levels of mephedrone, up to 8 mg/day/1000 people, between 20 and 100 times that of 3-MMC at the site. On the other hand, NZ3 showed the opposite, with 3-MMC up to 5 times higher than mephedrone. NZ2 showed similar levels of both drugs, and each were found only on one day. To the best of the authors' knowledge, 3-MMC has never before been reported in New Zealand, and the results from this work indicate that it is found in levels similar to that seen in sites in Europe.

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This study showed that NPS use was still consistent with party behavior over the New Year period despite widespread international lockdowns, outside of the sites in New Zealand and parts of the United States. By increasing the number of countries and sites investigated from our previous work, a more comprehensive overview of international NPS consumption can be gleaned. Some NPS, such as eutylone and 3-MMC, found in specific locations in our previous study over the same festive period, have now spread to other countries around the globe. Monitoring of these compounds in wastewater is thus a useful and complementary tool to understand the changing NPS landscape. This work has the potential act as a global early warning system and can help international public health agencies to better focus their efforts to reduce harm.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.1c00807.

Population, sampling mode, and flow rates for all sites in this study (Table S1). Additional information on sample collection (Section S1). Limits of detection and quantification and recovery for all substances in the method (Table S2). Analytical precision for all compounds in the method (Table S3). Estimated excreted mass loads for all NPS found in this study (Tables S4−S14). Figure of estimated excreted mass loads for mephedrone and 3-MMC in the NZ sites (Figure S1). (PDF)

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Notes

The authors declare the following competing financial interest(s): R.U.H. and E.M.D. are cofounders of AquaVitas, LLC, 9260 E. Raintree, Ste. 130, Scottsdale, Arizona 85260, United States, an Arizona State University startup company providing commercial services in wastewater-based epidemiology. R.U.H. also is the founder of OneWaterOneHealth, a nonprofit project of the Arizona State University Foundation.

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