ASOCIÁCIA ČISTIARENSKÝCH EXPERTOV SR

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Report

Monitoring of Psychoactive Compounds in Wastewater in Slovakia for 2024

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1. Theoretical Part

Information on the occurrence and consumption of legal and illegal drugs, including new psychoactive substances (NPS), is traditionally collected in cities and regions mainly through questionnaires, medical statistics (numbers of treated individuals and deaths), and police or customs records. Such administrative and survey-based data provide a certain picture of the demand, presence, and consumption of these substances across European regions. However, this picture may not always accurately reflect reality, as it can be affected by delays in evaluation, various types of bias, errors in data collection and processing, as well as their subsequent interpretation (Bodík, 2019; Bodík et al., 2021; Brandeburová et al., 2020; Castiglioni et al., 2021; Mackuľak et al., 2014; Klupczynska et al., 2016).

The 2024 report focuses in detail on the potential of monitoring the occurrence and consumption of selected types of illegal and legal drugs and their metabolites in wastewater from several Slovak cities. Modern analytical approaches enable tracking the occurrence and use of psychoactive substances over different time scales — from short-term daily or weekend variations to long-term trend analyses across years. The results can be normalized per 1,000 inhabitants to allow comparability between cities, facilitating both national and international assessments. The key platform for these approaches is wastewater-based epidemiology (WBE). This method is based on measuring the concentrations of selected markers or parent drugs in wastewater. A typical monitoring process includes a sampling plan, laboratory analysis, and subsequent back-calculations using selected correction factors. Samples are usually collected as 24-hour composite samples, typically by automatic samplers, which minimize the influence of short-term variability and random fluctuations. Analytical methods are dominated by LC-MS/MS, and the main targets include both parent compounds and their more stable metabolites. Commonly monitored substances include benzoylecgonine (a cocaine metabolite), amphetamine, methamphetamine, MDMA, and THC-COOH (a secondary metabolite of THC). In recent years, the analytical panel has expanded to include markers of new psychoactive substances (NPS). The outputs are expressed as daily mass loads normalized to 1,000 inhabitants (mg/day/1000 inhabitants). These calculated indicators allow for comparison of cities with different population sizes and infrastructures. At the same time, several uncertainties that may influence the results are taken into account, including compound degradation in the sewer network, water temperature, and hydraulic retention time. Rainfall, infiltration, and industrial inflows can cause dilution or temporary flow variations. To improve accuracy, population biomarkers and flow rate data are often incorporated. Even after corrections, significant regional differences in drug-use patterns remain evident. Across the EU and globally, different types of illicit substances are preferred, which is reflected in wastewater marker profiles (Bodík et al., 2021; Castiglioni et al., 2021; Mackuľak et al., 2019; González Mariño et al., 2019). Some cities exhibit higher stimulant levels, while others show greater proportions of cannabinoid metabolites. Weekend peaks of MDMA are typical indicators of short-term behavioral patterns. These signals often complement and help explain information from questionnaires, medical statistics, and police records. Integrated evaluations thus provide a more comprehensive and faster overview of drug consumption compared to traditional data sources alone. Wastewater monitoring is now systematically implemented in several EU countries (Brandeburová et al., 2020; Castiglioni et al., 2021; González Mariño et al., 2019; Ort et al., 2014). The broad network of participating cities allows for comparability and trend tracking over time. Interlaboratory comparisons and harmonized protocols further enhance reliability across studies. The resulting databases serve as valuable tools for public policy, prevention, and targeted interventions (Bodík et al., 2021; Castiglioni et al., 2021; Mackuľak et al., 2019; González Mariño et al., 2019; Brandeburová et al., 2020; Ort et al., 2014).

In 2013, under the European COST Action ES1307, an initiative was established focusing on the epidemiology and analysis of drugs and pharmaceuticals in wastewater. The COST program, funded under the EU Framework Programmes, continued through Horizon 2020 (2014–2020). This initiative gradually brought together scientists, analysts, public health experts, and wastewater treatment plant (WWTP) operators from multiple countries. Its main goal was to coordinate research and establish a common language between laboratories and data users. Emphasis was placed on the harmonization of sampling protocols, sample preparation, and analytical procedures. Equally important was unified statistical interpretation and transparent communication of uncertainties.

The network promoted experience sharing through workshops and thematic working groups. It included training schools for young researchers and short-term scientific missions. Interlaboratory comparison tests were organized to ensure analytical quality. The outcomes of the initiative often included recommendations, methodological guidelines, and shared databases to improve comparability of results. Today, the COST Association unites over 40 member countries and connects multiple scientific disciplines, aiming to foster international collaboration and accelerate knowledge transfer into practice. COST Action ES1307 established a platform for Europe-wide wastewater monitoring campaigns. Thanks to common standards, cities can now be compared at both national and international levels. The network significantly contributed to mapping regional differences in drug occurrence and consumption. The collected information helps identify emerging trends and local specificities more rapidly. Another important aspect of the cooperation is coordination with the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). EMCDDA uses processed outputs for assessing illegal drug trafficking and consumption in Europe. Joint initiatives have strengthened links between research, policy-making, and prevention programs. Overall, COST ES1307 laid the foundation for a modern, interconnected European network for wastewater-based epidemiology, which also proved invaluable between 2020 and 2023 for monitoring the spread of the COVID-19 pandemic in various populations.

1.1 *Examined Locations in Slovakia Regarding the Occurrence of Legal and Illegal Drugs*

In the 2024 report, the monitoring focused on specific legal and illegal drugs, NPS (new psychoactive substances), and their primary and secondary metabolites (a total of 57 compounds described in Table 1 – Appendix) detected in wastewater from selected Slovak cities (Table 2). Based on available data on potential drug-related issues in various Slovak regions, the 2024 monitoring campaign targeted eight Slovak cities, encompassing eight municipal wastewater treatment plants (WWTPs). One of these WWTPs is located in the Slovak capital: the Bratislava Wastewater Treatment Plant, situated in the Vrakuňa district, collects wastewater from the broader city center (including Lamač, Dúbravka, Ružinov, and Podunajské Biskupice, among others). In the following text, it is referred to as **WWTP Bratislava**. The WWTPs in Košice, Žilina, Prešov, Trenčín, Nové Mesto nad Váhom, Trnava, and Piešťany treat wastewater from their respective municipalities, collectively covering approximately one million inhabitants (Table 2).

Bratislava is the capital of the Slovak Republic. With a population exceeding 450,000, it is also the wealthiest Slovak region, characterized by the lowest unemployment rate. It serves as the country's main administrative, economic, and cultural center. Several universities and colleges operate in Bratislava, hosting over 100,000 students. Every day, an estimated 200,000 additional people commute to the city for work, education, and services, significantly increasing the daily population load. This dynamic places pressure on transportation, technical infrastructure, and urban services. Wastewater generated mainly in the city center is collected through an interconnected sewer network and subsequently treated at the Vrakuňa WWTP. The sewer system forms a connected structure with a main branch leading to this plant. The length of individual sewer sections varies depending on the area served, ranging from approximately 2 to 30 km.

Košice is the largest city in eastern Slovakia and the second-largest city in the country, with around 240,000 inhabitants. It comprises 22 city districts and is home to three universities, over 170 cafés, nightclubs, and dance venues. Along with Bratislava, Košice ranks among Slovak cities with the highest crime rates. Wastewater from the city is treated at the Košice WWTP, which is the second-largest plant in Slovakia in terms of population served, following Bratislava. The sewer network extends from 2 to 15 km. Substance use in Košice, as in other major cities, poses a long-term public health and safety challenge. Alcohol and tobacco are the most commonly mentioned substances, but cannabis and synthetic stimulants also appear in entertainment venues and festivals. Vulnerable groups are concentrated mainly near the city center, transport hubs, and socially disadvantaged neighborhoods, where the risk of problematic substance use is higher.

The city, in cooperation with the regional government, schools, and NGOs, implements preventive programs for pupils and students that focus on developing life skills rather than merely enforcing prohibitions. Healthcare and counseling services in the region provide addiction treatment, crisis intervention, and long-term therapy. The issue of discarded syringes in public spaces such as streets and parks is continuously addressed by municipal services and outreach workers who ensure safe collection and community education. For both the state and municipal police, drug-related crime remains a priority, emphasizing a balance between law enforcement and social support.

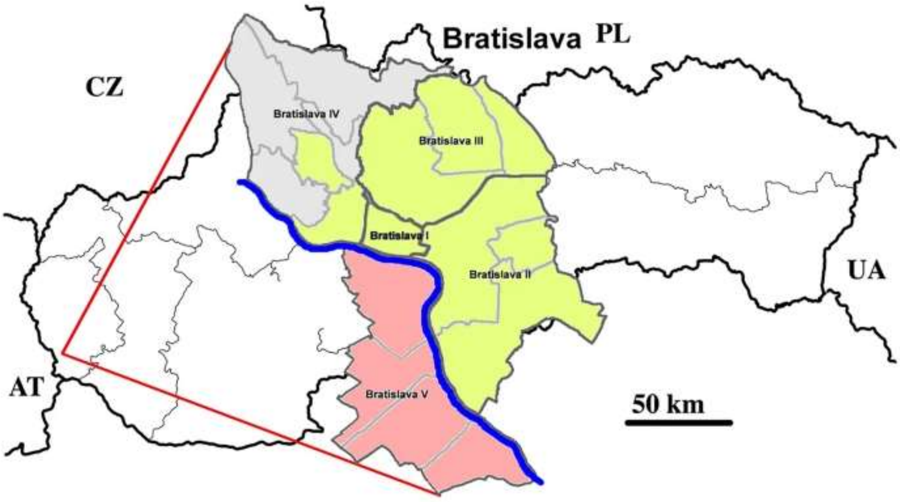

Figure 1a. Map of Slovakia and Bratislava with Districts. The green areas represent regions where wastewater is collected and treated at the Bratislava–Central Wastewater Treatment Plant (Vrakuňa), which serves approximately 450,000 inhabitants.

Žilina is a regional capital in northern Slovakia. With a population exceeding 84,000, it is the fourth-largest city in the country. It serves as the administrative, economic, transport, and cultural center of northwestern Slovakia. Wastewater from the city is treated at the Žilina WWTP, which ranks among the largest in Slovakia in terms of connected population. The sewer network ranges in length from 1 to 10 km. Due to its location at the intersection of major transport routes, Žilina has strong regional and international connectivity. Industry, services, and education are well-developed, resulting in stable employment and good urban infrastructure. The WWTP is designed to handle fluctuating inflows and employs mechanical, biological, and tertiary treatment stages to meet effluent quality requirements. The sewer system is branched; in historical parts, a combined system is present, while newer districts have separate sanitary and stormwater networks. The city also supports rainwater retention and load-reduction measures to protect the aquatic environment.

Prešov is a regional capital located in eastern Slovakia, with a population of approximately 92,000. It is the third-largest city in the country and the capital of the largest Slovak region. In terms of crime, Prešov is slightly above average. Wastewater from the city is treated at the Prešov WWTP, and the sewer system ranges from 1 to 15 km in length. The historical center, rich in monuments and cultural institutions, gives the city a distinctive character. Its economy is based on a combination of industry, services, and education, offering a diverse range of employment opportunities. Public transport and regional trains enhance accessibility for daily commuters from surrounding municipalities. In the area of public safety, the city focuses on prevention, community programs, and youth engagement to reduce risky behavior.

Trenčín is one of the oldest cities in Slovakia, located in the western part of the country. With around 57,000 inhabitants, it ranks ninth in population and is among the Slovak cities with the lowest crime rates. Several major music festivals are held near the city. Wastewater is treated in two WWTPs (left-bank and right-bank systems); the Trenčín Left Bank WWTP serves approximately 47,000 residents. The sewer network length ranges from 1 to 15 km. Trenčín serves as a regional hub for services, education, and sports. Large events attract thousands of visitors annually, requiring temporary reinforcement of transport and municipal services. The division of the sewer system into left-bank and right-bank branches minimizes the need for pumping across the river, improving operational reliability.

Piešťany, located northeast of Bratislava, is Slovakia's most prominent spa town, with a population of about 30,000. Wastewater is treated at the Piešťany WWTP, and the sewer network extends from 1 to 10 km. The spa industry fundamentally shapes the city's economy and identity, offering year-round therapeutic and wellness services. Urban design prioritizes pedestrian accessibility, green spaces, and promenades for residents and visitors alike. Protection of thermal and healing water sources is a long-standing priority reflected in designated protection zones and monitoring programs. The operation of the WWTP takes into account its proximity to spa areas, emphasizing low noise and odor emissions. The city also promotes green and blue infrastructure to retain rainwater and improve the urban microclimate.

Trnava is a regional capital in western Slovakia and the seventh-largest city in the country, with approximately 66,000 inhabitants. It has relatively high levels of drug-related crime, particularly involving amphetamine and methamphetamine. Wastewater is treated at the Trnava municipal WWTP, with a sewer network length of 2 to 15 km. Trnava is an important industrial and academic center, attracting commuters and students from across the region. The city's drug policy combines school-based prevention, outreach social work, and accessible addiction treatment. Wastewater analysis provides complementary data on substance use trends, enabling local authorities to respond more effectively. The municipal police and community programs focus on higher-risk areas, prioritizing safety and public order. In cooperation with the WWTP operator, the city emphasizes modernization to manage fluctuating inflows with greater energy efficiency and lower environmental impact.

Nové Mesto nad Váhom is a district town in the Trenčín Region, situated along the Váh River at the foothills of the White Carpathians. As of December 31, 2024, it had 19,337 inhabitants and covers an area of 32.58 km². The first written record of the town dates back to 1253. It is an important transport hub with access to the D1 motorway and a railway station on the main Bratislava–Žilina line. A popular recreational area, Zelená Voda, hosts events and festivals during the summer. The city has wastewater treatment infrastructure suitable for modern drug monitoring. The municipal WWTP provides mechanical and biological treatment and collects wastewater from most of the urban area, making it possible to apply wastewater-based epidemiology (WBE) methods.

The total monitored population in this 2024 study was approximately 1 million inhabitants, of which over 150,000 people were monitored during five selected music festivals (Pohoda, Grape, Lovestream, Uprising, and Lodenica) (Table 2 – Appendix).

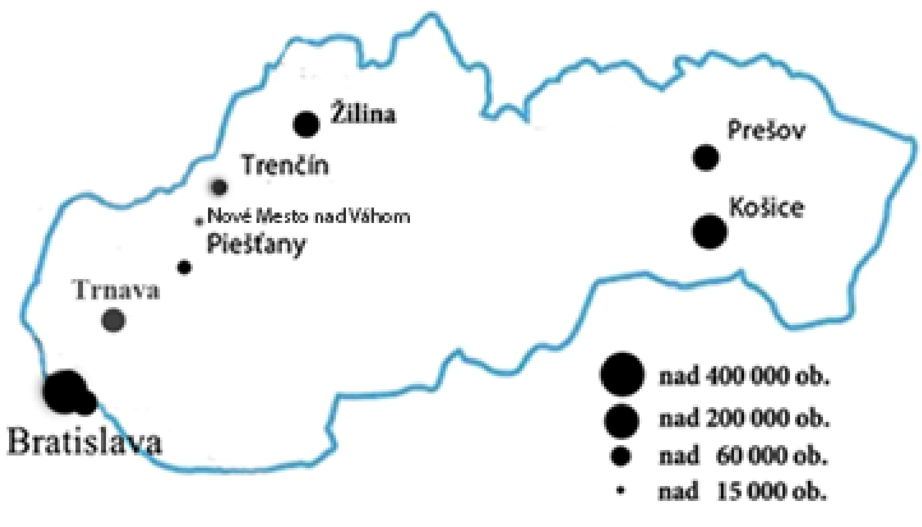

Figure 1b. Map Showing Selected Monitored Locations in Slovakia During 2024

Wastewater and Monitoring of Selected Festivals

Wastewater analyses that detect the presence of drugs and their metabolites can be used to monitor consumption at both continuous point sources (e.g., regular nightclubs and dance venues) and temporally limited events (e.g., music festivals). For a specific festival to be methodologically relevant in such monitoring, the event must either be connected to a public sewer system or ensure the transport of collected wastewater to a monitored wastewater treatment plant (WWTP). At music festivals, portable toilets are commonly used; their contents, once delivered to the WWTP, are typically diluted with raw influent wastewater. This dilution is necessary because of the high concentration of biocidal agents in such waste, which at elevated levels could inhibit the biological treatment processes at the WWTP. Studies focusing on wastewater analysis during music events represent a valuable and unique complementary method for monitoring the use of legal and illegal psychoactive substances within specific population groups (Mackuľak et al., 2019).

Figure 2a. Portable Toilets Used at Music Festivals

In the 2024 report, wastewater analyses conducted during music festivals were used to monitor the occurrence and consumption of more than 50 selected psychoactive compounds and their metabolites, including several new psychoactive substances (NPS) listed in Appendix – Tables 1 and 5.

Monitored Festivals:

Grape Festival – a multi-genre music festival with a strong focus on electronic and dance music. The 2024 edition took place on August 9–10, 2024, at the Trenčín Airport. The event attracted nearly 30,000 visitors, matching the declared capacity of the venue.

Uprising – a festival primarily dedicated to reggae music. In 2024, it was held on August 30–31, 2024, at Zlaté Piesky in Bratislava, featuring multiple stages and nearly one hundred performances. From the perspective of wastewater-based epidemiology (WBE), it is significant that wastewater from this location is discharged into the public sewer system leading to the Bratislava–Vrakuňa WWTP.

Country Lodenica – a traditional country/folk/bluegrass festival. The 2024 edition was held on August 22–24, 2024, at the Zelená Voda recreation area near Nové Mesto nad Váhom. The lineup and format featured three stages and dozens of performers. Attendance ranged between 10,000 and 15,000 people.

LOVESTREAM (Bratislava – Vajnory) – a mainstream pop/rock/dance festival. In 2024, it took place on August 16–18, 2024, at the old Vajnory Airport in Bratislava. The venue is part of the right-bank Bratislava sewer system connected to the Bratislava–Vrakuňa WWTP. The festival attracted approximately 20,000 visitors per day.

Pohoda 2024 (Trenčín – Airport) – a multi-genre cultural festival scheduled for July 11–13, 2024, at the Trenčín Airport. However, the 2024 edition was prematurely terminated after a severe storm on July 12 caused the collapse of a large tent. The organizers subsequently canceled the remainder of the event on July 13, 2024. Attendance was around 30,000 people.

Figure 2b. Pohoda Festival

1.2 Possibilities for Monitoring Psychoactive Substances from Wastewater in Slovakia

Wastewater, in addition to general chemical (fats, sugars, proteins) and biological contamination (e.g., RNA/DNA fragments and pathogens) and solids, also contains various specific organic compounds whose concentrations are often below the mg/l range. These comprise a broad group of micropollutants and their metabolites or degradation fragments. Micropollutants found in wastewater include contrast agents and biocidal substances, pesticides, hormones, artificial sweeteners, antibiotics and other pharmaceuticals and their primary or secondary metabolites, as well as legal and illegal drugs (Buerge et al., 2003; Bodík et al., 2021; Karolak et al., 2010; Mackuľak et al., 2016; Pal et al., 2013; Zuccato et al., 2005). The primary sources of NPS, legal and illegal drugs, and their primary or secondary metabolites in Slovak wastewater are principally the users themselves, and to a lesser extent possible illicit manufacturers. Drugs and their metabolites enter the sewer mainly in excreta (primarily in urine, in feces and, in some cases, in sweat) and via showering, washing and laundering of clothing (Mackuľak et al., 2020). In the sewer, these compounds may undergo various biotic and abiotic degradation processes or sorb onto suspended solids and sewer surfaces. Their degradation is influenced by multiple chemical and physico-chemical factors such as: hydraulic retention time in the sewer, wastewater pH, oxygen or sulfide content, temperature, the microbial community, and, to a lesser extent, light (possible photodegradation), and—crucially—the chemical structure of the compound itself (Mackuľak et al., 2014; Mackuľak et al., 2019; Fedorova et al., 2013; Krizman-Matasic et al., 2019). On the basis of these findings, from 2005 onwards extensive national studies began to be published from a number of countries, highlighting regionally differing dominances of various NPS types or illegal drugs around the world (Castiglioni et al., 2011; Castiglioni et al., 2021; Irvine et al., 2011; Mackuľak et al., 2020; Mercan et al., 2019; Reid et al., 2011; Salgueiro-Gonzalez et al., 2024).

The possibility of monitoring illegal drug consumption or certain pharmaceuticals using wastewater analysis was first proposed by Daughton and Ternes in 1999 at a conference in New York. The development of new analytical methods led to the gradual establishment of the term “sewage epidemiology,” or wastewater-based epidemiology (WBE), within the scientific community. From 2005 it became evident that analysis of a given drug or its dominant metabolite can be used to estimate the number of doses consumed within a sewered area. Numerous studies subsequently published the potential for monitoring various classes of legal and illegal drugs and NPS via wastewater (Brandeburová et al., 2020; Mackuľak et al., 2015; Mackuľak et al., 2016; Ort et al., 2014; Salgueiro-Gonzalez et al., 2024). It was further recognized that not only the concentration of the parent drug in wastewater can inform on consumption in a city or region, but also the occurrence of a dominant metabolite (Castiglioni et al., 2006; Castiglioni et al., 2011; Brandeburová et al., 2020). For back-calculation of illegal drug use, a more stable metabolite is often used instead of the parent compound—for example, benzoylecgonine for cocaine (Ort et al., 2014; Thomas et al., 2012; Irvine et al., 2011; Van Nuijs et al., 2012).

Twenty years after the first studies, it is clear that this scientific field continues to develop rapidly—particularly in biochemistry, genetics, analytical chemistry and the search for new biomarker groups. This is also evidenced by the most recent works from 2024/2025, which describe monitoring possibilities for various groups of endogenous human metabolism biomarkers and new types of drugs and psychoactive substances (NPS) (Salgueiro-Gonzalez et al., 2024). Based on quantitative measurement of specific biomarkers in wastewater from different regions and cities, it is also possible to evaluate the population's lifestyle in a given city or region, the incidence and spread of certain disease types, and the negative or positive impacts of the environment on public health (Van Nuijs et al., 2012; Bodík et al., 2019; Mackuľak et al., 2020). Table 1 of the report presents the proposed coefficients used to back-calculate consumption of, for example, illegal drugs and selected psychoactive compounds. If the parent compound in wastewater is stable over the sampling period, it can be used directly for consumption back-calculation (for example, MDMA). If the drug is structurally unstable in the sewer (e.g., nicotine or cocaine) and rapidly subject to various degradation processes, it is necessary to use a stable metabolite and calculate consumption based on its metabolic coefficient (Mackuľak et al., 2020; Ort et al., 2014; Thomas et al., 2012).

Table 1. The first column lists the analyzed compounds detected in wastewater, which are subsequently converted—using the corresponding coefficient—into estimated consumption values of their associated parent drugs.

Analyzed Compound in Wastewater	Conversion Coefficient	Drug
MDMA	1,5	MDMA
THC-COOH	152	Marijuana/THC
Benzoylecgonine (BE)	2,77	Cocaine
Methamphetamine	3,3	Methamphetamine
Amphetamine	3,3	Amphetamine
EDDP	6,3	Metadon

Data acquisition through wastewater analysis using LC-MS/MS methods and subsequent evaluation of the monitored compounds in units of mg/day/1000 inhabitants is now comparable to values reported in other studies within this scientific field. To calculate the amount expressed in mg/day/1000 inhabitants, it is necessary to know the number of people connected to the monitored sewer network and the daily wastewater flow entering the treatment plant. From the measured daily concentration and corresponding daily flow, the total daily mass of the monitored compound entering the sewer system (mg/day) can be determined. Based on the known (or estimated) number of connected or present inhabitants in the monitored area on that day, the specific daily drug load per 1,000 inhabitants can be calculated (mg/day/1000 inhabitants) (Bodík et al., 2021; Mackuľak et al., 2015; Fedorova et al., 2013; Škubák et al., 2014).

The described method also has certain disadvantages and limitations. For example, the current methodology cannot yet identify the gender or age groups of psychoactive substance users. The analysis is also limited to areas with centralized sewer systems, which poses a challenge since only about 70% of Slovakia’s population is connected to public sewers. However, research in this field is rapidly advancing, and ongoing developments are addressing the limitations of this analytically demanding method for monitoring drug use (Bodík et al., 2014; Salgueiro-Gonzalez et al., 2024).

Figure 3. Wastewater Treatment Plant (Poprad – Matejovce)

Description of Analyzed Psychoactive Substances in Wastewater in the Slovak Republic

The aim of the study conducted in 2024 was to perform a detailed analysis of 57 selected psychoactive compounds and their metabolites (Table 1 – Appendix) in wastewater from selected cities across Slovakia. In Bratislava, wastewater samples were analyzed from the Bratislava–Vrakuňa Wastewater Treatment Plant (WWTP).

1.3 Detailed Description of Selected Psychoactive Substances

Cathinone (Benzoylethanamine) is chemically similar to ephedrine, cathine, and other amphetamines. Its effects are often described as somewhere between those of ecstasy (MDMA) and methamphetamine. Compared to methamphetamine, the effects are less intense but more euphoric. The substance is not physically addictive, though it can cause strong psychological dependence. Side effects are generally similar to those of amphetamine and MDMA (ecstasy).

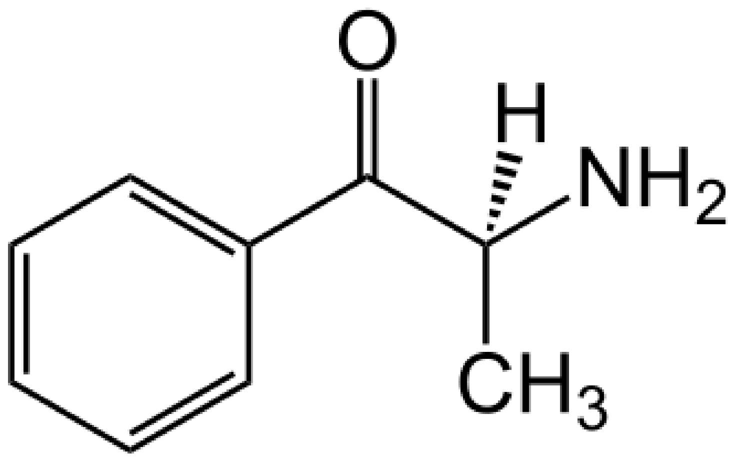

Figure 4. Cathinone

Oxycodone is a narcotic medication used for pain relief. In the 1990s, it was also widely prescribed as an analgesic. In terms of potency, its effect lies between morphine and codeine. Due to its euphoric effects on the brain, oxycodone has become one of the most commonly abused drugs, particularly in the United States. Its symptoms are similar to those of heroin use. Oxycodone is highly addictive, and because of its pharmacological properties, it carries a high risk of overdose.

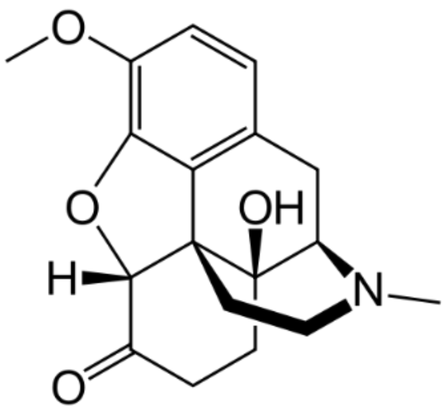

Figure 5. Oxycodone

Methamphetamine is a synthetic stimulant drug that is produced in Slovakia mainly from ephedrine or, more recently, from pseudoephedrine through reduction with hydroiodic acid in the presence of phosphorus. Although it was once used in medicine, today it is primarily abused as an illegal drug. Methamphetamine induces strong euphoria and causes severe psychological dependence.

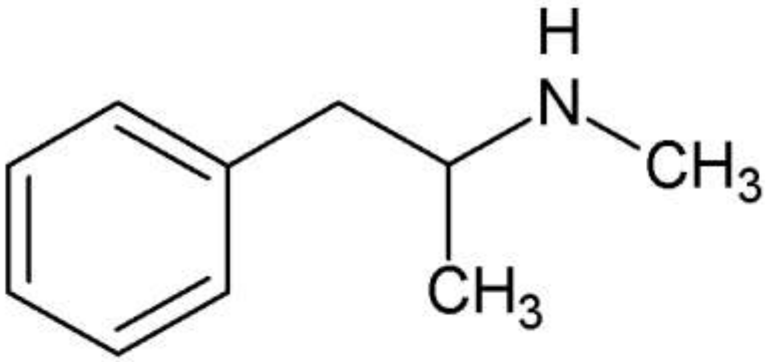

Figure 6. Methamphetamine

Ketamine is a pharmaceutical substance used in both human and veterinary medicine as an anesthetic, marketed under trade names such as *Ketanest*, *Ketaset*, *Ketalar*, *Narkamon*, and *Calypsol*. In addition to its anesthetic effects, it can cause hallucinations and hypertension. Ketamine is often sold online as a mixture of both isomers and is commonly abused as a recreational drug. It can be administered by injection, insufflation (snorting), or smoking. In the liver, ketamine is metabolized by the cytochrome P450 enzyme system into hydroxynorketamine and norketamine, both pharmacologically active metabolites that contribute to prolonging the drug's effects.

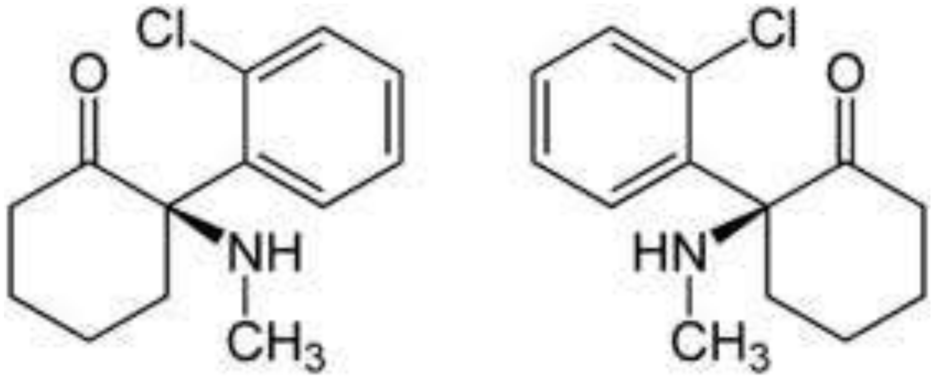

Figure 7. Ketamine

2-oxy-3-hydroxy-LSD is one of the metabolites of LSD used as evidence of LSD consumption in wastewater analysis.

LSD (Lysergic Acid Diethylamide) is a crystalline, colorless compound used as a hallucinogen. LSD does not induce physical dependence and has low toxicity, although its use may cause adverse psychological effects. At present, there are few scientific studies examining the effects of LSD on the human body. Some studies suggest that LSD may have therapeutic potential, particularly in the treatment of migraines. LSD is typically consumed orally, in the form of blotter papers soaked with the substance, or as gelatin, tablets, or crystals.

Amphetamine, along with methamphetamine, belongs to a class of stimulants that increase levels of the neurotransmitters norepinephrine, serotonin, and dopamine in the brain. It is used medically in the treatment of attention deficit hyperactivity disorder (ADHD) and chronic fatigue syndrome. However, it is frequently abused as a recreational drug, commonly known as "Speed." In the past, it was also used as a doping agent. Its effects are similar to those of other amphetamine-based stimulants.

Cocaine is a narcotic substance chemically known as benzoylmethylecgonine. It is a stimulant derived from the South American plant *Erythroxylum coca*. Pure cocaine appears as a crystalline white powder with a bitter taste. On the black market, cocaine is often adulterated—sometimes mixed with other stimulants (e.g., amphetamine) or local anesthetics—and may contain various impurities. By additional thermal processing, cocaine can be converted into "crack," a smokable form of the drug with more intense effects. Cocaine is typically administered by snorting or injection.

Benzoylecgonine is one of the dominant metabolites of cocaine. Approximately 1–9% of cocaine is excreted in urine unchanged, 35–55% as benzoylecgonine, and 32–49% as ecgonine methyl ester. Various studies based on direct monitoring of cocaine in wastewater assume that around 10% of the drug is excreted from the body and that 60–80% of cocaine degrades in wastewater at pH 6 and 20 °C. Calculations based on direct cocaine measurement tend to yield higher values; therefore, the indirect method using its metabolite benzoylecgonine is more commonly applied.

THC – Tetrahydrocannabinol (Δ9-THC) is one of over 100 cannabinoids and the main psychoactive compound found in *Cannabis sativa* and its variants (*Cannabis sativa var. indica*). THC is present in the dried plant material; in living plants, it can be found mainly on the surface. In pure form, THC appears as translucent crystals that become sticky when heated. It is poorly soluble in water but highly soluble in organic solvents (ethanol, hexane) and fats. According to the 1961 Single Convention on Narcotic Drugs, "cannabis" refers to the flowering or fruiting tops of the plant (excluding seeds and leaves not attached to the tops), from which the resin has not been extracted. In common terminology, however, "cannabis" includes both marijuana and hashish.

In wastewater, the monitored compound is THC-COOH (11-nor-9-carboxy-Δ9-tetrahydrocannabinol), one of the main metabolites of THC. Only the free form of THC-COOH is monitored. The expected concentration of THC-COOH in Slovak wastewater ranges between 10 and 200 ng/l.

Buprenorphine is a derivative of thebaine, an opioid used as an analgesic. Buprenorphine exhibits effects similar to morphine but has a longer duration of action. It is usually administered orally in tablet form. The compound is used in the treatment of opioid addiction; however, non-medical use by healthy individuals can also lead to dependence.

Mephedrone is a synthetic stimulant belonging to the classes of amphetamines and cathinones. According to available information, it is present in certain stimulant preparations. Mephedrone can appear in the form of capsules, tablets, or white powder and can be snorted, injected, or taken orally. The usual dose ranges from 50 to 150 mg.

MDMA (Ecstasy) is a synthetic drug patented in 1913 by the German company *Merck*. Initially, it was marketed as a weight-loss medication. Users of MDMA often experience feelings of happiness, relaxation, and empathy toward others, with enhanced sensory perception. In the liver, MDMA is metabolized into MDA (3,4-methylenedioxyamphetamine), a structurally similar but more toxic compound. Approximately two-thirds of MDMA is excreted unchanged in urine, with 7% excreted as MDA. The biological half-life of MDMA in the human body is about six hours.

MDA (3,4-Methylenedioxyamphetamine) is a drug very similar to ecstasy, but less expensive. It is sold as tablets, powder, or crystals. MDA is considerably toxic, with effects lasting up to 10 hours. It is also excreted as a metabolite when MDMA is consumed.

MDEA (3,4-Methylenedioxy-N-ethylamphetamine) is a hallucinogenic drug and a synthetic derivative of amphetamine closely related to ecstasy. Its subjective effects are similar to MDMA. MDEA is produced in illicit laboratories worldwide and is sold in tablet form, either alone or mixed with other substances.

MBDB (N-Methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine) is a phenylethylamine chemically related to MDMA. It has been used experimentally in psychotherapy and as a recreational drug. In Europe, it is sold as tablets or powder, often misrepresented as ecstasy and sometimes combined with LSD or ketamine. The drug's effects last 4–6 hours. MBDB produces empathogenic effects similar to ecstasy but milder.

Methylphenidate (MPH) is a compound similar to amphetamines but with weaker stimulant effects. It is prescribed to treat attention deficit hyperactivity disorder (ADHD) in children and adults, improving concentration in affected individuals. It is also used for narcolepsy and certain types of brain injury. Chemically, it is a fine, white, odorless crystalline powder sold under trade names such as *Ritalin*, *Rilatine*, *Rubifen*, *Focalin*, and *Centedrin*. Methylphenidate increases dopamine levels in the brain and is commonly abused by students, especially during examination periods.

Risperidone is an antipsychotic medication with a balanced effect on neurotransmitter systems in the brain. It is used to treat symptoms of schizophrenia and depression and has fewer side effects than classical neuroleptics.

Midazolam (INN) is a short-acting benzodiazepine. In English-speaking countries, it is marketed under brand names such as *Dormicum*, *Hypnovel*, and *Versed*. In the Czech Republic and Slovakia, several medical products containing midazolam are registered. It has strong anxiolytic, hypnotic, anticonvulsant, muscle relaxant, and sedative effects. Like other benzodiazepines, midazolam has a rapid onset of action, high efficacy, and low toxicity.

Methadone (synthetic opioid) is used in medicine for the treatment of narcotic addiction (e.g., as a substitute for heroin). Methadone is metabolized slowly and is highly lipid-soluble, which allows it to act longer in the body than morphine-based drugs. Its average duration of effect is 25 hours, much longer than that of heroin (4–6 hours). It can be taken orally, eliminating complications associated with intravenous use. Methadone, like other opioids, is addictive. When injected, it produces effects similar to morphine, including euphoria. EDDP is one of the main metabolites of methadone. In wastewater, it is a highly stable compound, resistant to both biological and chemical degradation processes, and is used to calculate methadone consumption.

Heroin (Diacetylmorphine) is a semi-synthetic opioid. In pure form, it is a fine white powder with a bitter taste. Heroin is one of the most dangerous drugs due to the rapid development of physical and psychological dependence, as well as severe health and social consequences of abuse. It is mainly administered intravenously into veins, muscles, or under the skin, though it may also be smoked or inhaled as vapor from foil or spoons. Less commonly, it is snorted or ingested orally. The effects occur within 15–30 seconds and last 6–8 hours. The primary risk of heroin use lies in its high potential for addiction and dependency.

Nicotine is present in all tobacco products, including cigarettes, cigars, and nicotine gum. Many young people use tobacco products regularly before the age of 15. Nicotine is metabolized in the human body into several metabolites, primarily cotinine, which is the dominant one. About 10–15% of nicotine is excreted unchanged, while approximately 70% is converted into cotinine. Other tobacco alkaloids such as anatabine and anabasine are also excreted in urine. In wastewater, nicotine use is typically confirmed through the detection of its more stable metabolite, cotinine.

Caffeine is an odorless compound that affects the human body even at low concentrations. Symptoms include increased heart rate and blood pressure. Caffeine provides a sensation of increased energy and can cause hyperactivity. In some cases, dependence may develop. After ingestion, caffeine enters the bloodstream within 30–45 minutes. Its half-life is approximately 4 hours (ranging from 2 to 10 hours depending on metabolism). In the body, caffeine blocks specific adenosine receptors in neural tissue, increasing alertness and wakefulness. Caffeine is also a component of many analgesics due to its ability to constrict blood vessels and relieve migraine symptoms.

1.4 Detailed Description of New Types of Selected Psychoactive Substances (NPS)

New Psychoactive Substances (NPS) can be defined as compounds that produce effects similar to traditional illicit drugs and are designed to replace them. NPS are often not regulated by international conventions. Although many NPS are synthesized through minor modifications of existing chemical structures, the term “new” does not necessarily refer to “newly developed” compounds but rather to “newly misused” ones. The illicit NPS market is therefore highly dynamic, with new alternative substances being rapidly developed—sometimes with the aid of artificial intelligence. According to EMCDDA data, more than 800 different NPS were detected between 2005 and 2024

NPS can be classified in various ways, not only according to their chemical structure. Cathinones and synthetic cannabinoids are among the most frequently detected groups of NPS, but benzodiazepines, arylcyclohexylamines, phenethylamines, and synthetic opioids have also been reported. These new substances are becoming increasingly accessible to the general public, primarily through online markets. EMCDDA considers them an emerging problem in many EU countries, as they have been linked to numerous fatal intoxications.

In the 2024 report, in addition to classical legal and illegal drugs and their metabolites, we also analyzed compounds that may represent a significant threat in the near future. As the prevalence of traditional drugs tends to stagnate, users are turning increasingly to new types of psychoactive substances, often referred to as “designer drugs.” Their danger lies primarily in the unpredictability of their effects and toxicity (EMCDDA, 2019, 2021, 2024; Salgueiro-Gonzalez et al., 2024).

Information on many of these substances remains limited. In numerous cases, little is known about their behavior in sewer environments—whether they undergo biodegradation or persist to reach wastewater treatment plants—which makes it difficult to monitor their consumption. Consequently, their detection in wastewater is considered evidence only of presence (e.g., at music festivals or within specific regions), not of quantitative consumption (Bade et al., 2017; Castiglioni et al., 2016; Castiglioni et al., 2021; Salgueiro-Gonzalez et al., 2024).

Description of New Analyzed Compounds

α-Pyrrolidinopentiophenone (α-PVP) – This compound is often found in powder form, allowing it to be easily mixed with other substances. α-PVP is a synthetic stimulant belonging to the cathinone family, first developed in the 1960s and marketed as a "designer drug."

3-Methylmethcathinone (3-MMC) – Similar to 4-Methylethcathinone (4-MEC), 3-MMC was classified as a psychoactive substance in 2014 as a replacement for mephedrone. It reaches Slovakia primarily from neighboring countries such as Hungary, Poland, and the Czech Republic. 3-MMC, first detected in Sweden in 2012, belongs to the cathinone family and is currently listed under Schedule I of controlled narcotic and psychotropic substances in Slovakia (https://www.zakonypreludi.sk/zz/1998-139).

Methylenedioxypyrovalerone (MDPV) – First synthesized in 1969 as a stimulant. It can act for more than seven hours, producing feelings of excessive energy and euphoria. It is often combined with other psychoactive substances, such as mephedrone. Compared to mephedrone, MDPV causes a more abrupt decline in effects.

Methoxetamine (MXE) – An arylcyclohexylamine compound chemically related to ketamine and the internationally controlled substance phencyclidine (PCP). Over six EU member states have reported around 20 fatalities linked to MXE use.

25I-NBOMe – A potent synthetic derivative of 2,5-dimethoxy-4-iodophenethylamine (2C-I), a classic serotonergic hallucinogen. This compound underwent risk assessment and control measures and has been subject to criminal sanctions at the EU level since 2003. The specific effects of 25I-NBOMe on humans are difficult to predict. It is often sold in blotter-paper form, similar to LSD, and is frequently mixed with other psychoactive substances such as 25C-NBOMe or 25H-NBOMe.

Dextromethorphan – Although widely used as an effective cough suppressant, in excessive doses it acts as a dissociative hallucinogen. It is an opioid derivative with a rapid onset, metabolized in the liver and excreted in urine. While not physically addictive, its easy availability and misuse pose serious risks of severe adverse effects and potentially fatal overdose.

Fentanyl – A highly potent synthetic opioid analgesic similar to morphine, commonly used in hospitals for pain management. Fentanyl is frequently abused as a substitute for morphine or heroin and is approximately eighty times more potent than morphine. In Slovakia, there have been cases where fentanyl, like methamphetamine, is illegally produced in "home laboratories." It is currently classified under Schedule II of controlled narcotic and psychotropic substances (https://www.zakonypreludi.sk/zz/1998-139).

2,5-Dimethoxy-4-Methylamphetamine (DOM) – A psychedelic compound structurally related to amphetamine, developed by the chemist Alexander Shulgin. On the street, it is also known as "STP." A typical dose ranges from 3 to 10 mg, although early tablets contained up to 20 mg. Its effects are similar to LSD.

1-(3-Chlorophenyl)piperazine (mCPP) – A new psychoactive substance first detected in 2005 in France and Sweden. It is sold in tablet form and is often mixed with MDMA. mCPP has a long history, as it was even tested by the U.S. Army as a potential incapacitating agent capable of inducing migraines.

Ethylphenidate – A psychoactive stimulant often sold at concerts and music festivals. Users purchase ethylphenidate as a cheaper alternative to cocaine or methylphenidate.

Synthetic Cannabinoids – These compounds act as agonists of cannabinoid receptors, producing effects similar to delta-9-tetrahydrocannabinol (THC). They are commonly added to herbal mixtures and sold under names such as *Spice* or *K2*. In Slovakia, substances such as AB-CHMINACA, along with several others mentioned in this report, are classified under Schedule I or II of controlled narcotic and psychotropic substances (https://www.zakonypreludi.sk/zz/1998-139). MDMB-CHMINACA (also known as MDMB(N)-CHM) is a similar compound to MDMB-FUBINACA, which was responsible for over 1,000 hospitalizations and around 50 deaths in Russia.

4,4'-Dimethylaminorex (4,4'-DMAR) – A psychoactive stimulant first reported in the Netherlands in 2012. It later spread across Europe (Denmark, Hungary, the UK, Finland). 4,4'-DMAR is often combined with other substances, which can lead to fatal outcomes.

Para-Methoxy-N-Methylamphetamine (PMMA) – A stimulant related to amphetamine-type drugs. PMMA is still poorly studied in terms of toxicity.

Methcathinone – Commonly known as “cat” or “Jeff,” this psychoactive compound induces euphoria in users. It is addictive and can be consumed by smoking, injection, or oral ingestion. It is controlled under the Convention on Psychotropic Substances.

N,N-Dimethylcathinone (Hydrochloride) – A compound that can enter the sewage system primarily through excreta, as it is a potential metabolite of various cathinone derivatives.

Ethcathinone – Also known as ethylpropion or ETH-CAT, this stimulant is one of the metabolites of diethylcathinone and is frequently added to tablets sold as ecstasy. Several seizures have been reported, for example, in Australia.

4-Fluoromethcathinone (4-FMC, Flephedrone) – A stimulant drug belonging to the cathinone group. It has been banned since 2010 in Poland, Sweden, Australia, and Lithuania and was classified as a controlled substance in China in 2015.

3,4-Dimethylmethcathinone (3,4-DMMC) – A designer drug now widespread worldwide. First detected in 2010, it is believed to have emerged following the prohibition of mephedrone. Numerous seizures have been reported in Australia.

4-Methylethcathinone (4-MEC) – A compound chemically similar to mephedrone. It is often mixed with other cathinone-type psychoactive substances and used as an adulterant in ecstasy tablets. Such cases have been documented, for instance, in New Zealand.

Bufedrone (MABP) – First synthesized in 1928, this cathinone stimulant remains legal in several countries when used for scientific purposes.

Pentedrone – Also known as α-Methylamino-valerophenone, this cathinone stimulant has occasionally been added to bath salts. The first reports of its appearance date to 2010.

Methylone – Also known as 3,4-Methylenedioxy-N-Methylcathinone (MDMC, βk-MDMA, or M1). Chemically similar to MDMA (ecstasy), methylone was first synthesized in 1996 by chemists Peyton Jacob III and Alexander Shulgin and was initially studied as a potential antidepressant. Reports of its recreational use began emerging around 2004.

Ethylone – Known as 3,4-Methylenedioxy-N-Ethylcathinone (MDEC, βk-MDEA), this designer drug is an analog of MDEA. Research on its toxicity, metabolism, and other properties remains limited. Several deaths associated with ethylone use have been reported in the United States.

Butylone – Also known as β-Keto-N-Methyl-Benzodioxolylbutanamine (βk-MBDB), it was first synthesized in 1967 by chemists Koeppe, Ludwig, and Zeile. It is a psychoactive phenethylamine and an analog of MBDB.

Pentylone – Known as β-Keto-Methylbenzodioxolylpentanamine (βk-MBDP), this compound is frequently mixed with other cathinone-type drugs.

Naphyrone – Also known as O-2482 or naphthylpyrovalerone, naphyrone was introduced to the black market as "NRG-1," though few samples actually contained it. Like pentylone and other psychoactive substances, it is often found in mixed forms.

1-Naphyrone – A derivative of naphyrone. Very little is known about its pharmacological effects or overall toxicity.

Ethyl Sulfate (Ethyl Hydrogen Sulfate) – In recent years, this compound has become an indicator of alcohol consumption. Analyses are based on wastewater samples. Ethanol itself cannot be quantified in wastewater because it is rapidly biodegradable. Ethyl sulfate, however, is resistant to degradation in sewer systems, and its concentration (ng/l or µg/l), combined with back-calculation methods, the number of inhabitants connected to the analyzed WWTP, and the daily influent volume, can be used to estimate alcohol (ethanol) consumption. In our 2020 report, this compound was used to calculate alcohol consumption in Slovakia, including during the COVID-19 lockdown.

25iP-NBOMe – Members of the NBOMe series are primarily N-methoxybenzyl analogs from the 2C-X group of phenethylamines and act as 5-HT2A (serotonin 2A) receptor agonists. Some are also amphetamine analogs. Except for a few cases (e.g., mescaline-NBOMe), these compounds are highly potent, with effective doses below a milligram. They appeared on the market around 2010 and produce LSD-like effects. Reports describe their effects as unpredictable—even for the same user taking the same dose at different times—causing confusion, delirium, recursive and uncontrolled thinking, disorganized communication, nausea, insomnia, paranoia, fear, panic, and other adverse reactions.

PMA (4-Methoxyamphetamine) – In pure form, PMA is a white powder, though street samples may appear beige, pink, or yellowish. It has been found in pressed tablet form sold as MDMA (ecstasy). There is little information about its safe recreational dosage. Doses below 60 mg can raise blood pressure, body temperature, and heart rate. Because PMA acts more slowly than MDMA, users may consume multiple tablets, significantly increasing the risk of overdose.

NEDPA (Ephenidine) – A little-known dissociative compound from the diarylethylamine group. Ephenidine is an NMDA receptor antagonist, structurally related to other diarylethylamines. Known effects include hallucinations, anesthesia, and dissociative experiences characterized by an "out-of-body" sensation. Onset occurs within 10–30 minutes, lasting 5–7 hours. Effective doses range from 30–70 mg (mild), 70–100 mg (moderate), and 100–150 mg (strong). As with other NMDA antagonists, chronic use can lead to psychological dependence and tolerance, requiring progressively higher doses to achieve the same effects.

2.3 Sampling and Analysis of Wastewater Samples

Sampling for the monitoring of the occurrence and consumption of selected psychoactive substances was carried out at wastewater treatment plants (WWTPs) located in different regions of Slovakia (Figure 1). Samples were collected at the influent of each WWTP using an automatic sampling device (24-hour composite samples collected from 7:00 a.m. to 7:00 a.m.). Sampling and subsequent analyses were conducted throughout the entire year 2024. The collected samples were first frozen at -4 °C and then transported to the laboratory. In the next step, the samples were pre-treated and analyzed using LC-MS/MS and Orbitrap instruments (Fedorova et al., 2013). In total, more than 60 samples were collected and analyzed (Table 2 – Appendix). The detection of NPS in wastewater was performed according to the study by Brandeburová et al. (2020).

Description of sample analysis: To 10 ml of homogenized and filtered (GFC, 0.45 μm) wastewater sample, isotopically labeled internal standards were added. The pre-treated samples were then analyzed using an SPE-HPLC system coupled with a hybrid quadrupole (Orbitrap) ultra-sensitive mass spectrometer. This method enables the quantitative analysis of selected psychoactive substances and/or their metabolites in wastewater at concentrations in the range of nanograms per liter (ng/l) (Brandeburová et al., 2020; Fedorova et al., 2013; Mackuľak et al., 2014).

2 Occurrence of Selected Psychoactive Compounds in Wastewater from Selected Cities in Slovakia

Slovakia belongs among the Central European countries (similarly to the Czech Republic) where, according to the results of several monitoring studies and European reports (EMCDDA 2017–2024), the most dominant illicit drugs among the population are methamphetamine and marijuana. In the 2024 report, out of 57 analyzed compounds, 20 psychoactive pharmaceuticals, illegal and legal drugs, and their metabolites were detected in wastewater at concentrations higher than 25 ng/l. The concentrations of NPS were monitored from a detection limit of 0.4 ng/l. The measured results of selected compounds were processed into several tables in units of nanograms per liter (ng/l) (see Appendix). The measured concentrations of selected drugs, pharmaceuticals, and their primary or secondary metabolites were then recalculated into daily loads (mg/day/1000 inhabitants) based on the actual daily wastewater flow at each WWTP and the number of inhabitants connected to the treatment plant. These normalized values (mg/day/1000 inhabitants) enable direct comparison of cities with different population sizes. The exception are the results for new psychoactive substances (NPS) (Table 4), which are presented only as concentration values in ng/l—indicating their presence in wastewater rather than quantified consumption. Table 2 in the Appendix describes the sampling locations across eight wastewater treatment plants in eight Slovak cities, along with the corresponding population served and sampling dates. The same table also indicates the dates of sampling that coincided with major music festivals. During 2024, several music festivals were held in Slovakia. In this report, five festivals were included in the monitoring campaign (LOVESTREAM, Pohoda, Lodenica, Uprising, and Grape). In Bratislava, a single but major wastewater treatment plant—WWTP Bratislava—was analyzed. The total monitored population in the 2024 study was approximately **1.05 million inhabitants**, of which over **150,000 individuals** were included during the monitoring of the five selected festivals. Tables 3 and 4 in the Appendix present the quantities of selected compounds (amphetamine, methamphetamine, cocaine, cocaine metabolite benzoylecgonine used to calculate cocaine consumption, MDMA – the active ingredient in ecstasy, THC secondary metabolite THC-COOH, EDDP – a methadone metabolite, and methadone itself) detected in wastewater from eight Slovak cities, normalized to a population of 1,000 inhabitants.

In addition to intercity comparisons, Tables 6a–6d in the Appendix illustrate changes in the quantities of illicit drugs during the strict lockdown period (March–May 2020) and over the years 2013–2024 for selected Slovak cities (no wastewater monitoring was conducted in 2023).

2.1 Amphetamine

Amphetamine is among the less preferred drugs in Slovakia. In wastewater from monitored Slovak cities during 2024, it was detected at concentrations up to 320 ng/l (Trenčín, August 8, 2024 – the start of the Grape Festival; see Table 3, Appendix). When comparing cities (both during and outside festival periods), the highest average loads per 1,000 inhabitants were found in Trenčín (56 mg/day/1000 inhabitants), followed by Trnava (34 mg/day/1000 inhabitants) and Bratislava (24 mg/day/1000 inhabitants). In contrast, the lowest levels were observed in Prešov and Nové Mesto nad Váhom, both below 9 mg/day/1000 inhabitants (Table 3, Appendix).

Temporal variations in amphetamine occurrence were observed in all analyzed cities (Table 3, Appendix). In several locations, fluctuations in drug levels were recorded over specific periods. The highest non-festival values occurred between May and August in Trnava, Trenčín, and Bratislava, while the lowest loads per 1,000 inhabitants were recorded between February and April. Weekly monitoring in Bratislava and Piešťany also revealed significant fluctuations in amphetamine levels (Table 3, Appendix). For example, in Bratislava, sewer system loads between April 8–15, 2022 ranged from 7 to 25 mg/day/1000 inhabitants.

During the monitored festivals, the highest average value was recorded for the Grape Festival (104 mg/day/1000 inhabitants), representing a notable increase compared to the long-term average in Trenčín (56 mg/day/1000 inhabitants) (Table 3, Appendix). It should be noted that a significant portion of the amphetamine detected in Slovak wastewater may originate from users of another drug—methamphetamine—since amphetamine is one of its primary metabolites. Data in Table 3 (Appendix) show that the occurrence of amphetamine in wastewater often mirrors that of methamphetamine. Currently, in Slovakia, amphetamine remains secondary compared to methamphetamine (Table 3, Appendix; González-Mariño et al., 2019).

2.2 Methamphetamine

The analysis results obtained in selected Slovak cities for the psychoactive drug methamphetamine in 2024 indicate that it remains one of the most widely used illicit drugs in Slovakia, with consumption levels continuing to increase (Table 3, Table 6a – Appendix). The long-term dominance of this drug in Slovakia has also been consistently confirmed by previous reports covering the period 2013–2022 (Table 6a – Appendix) as well as by several EMCDDA reports from 2020–2024. During the 2024 study, methamphetamine was detected in wastewater samples from all monitored cities, with concentrations ranging from 200 ng/l (Nové Mesto nad Váhom, August 20, 2024) to 1600 ng/l (Bratislava and Trenčín; Table 3 – Appendix). The highest average daily loads per 1,000 inhabitants were recorded in Trenčín (342 mg/day/1000 inhabitants), Bratislava (292 mg/day/1000 inhabitants), and Trnava (268 mg/day/1000 inhabitants). The lowest average values were observed in Nové Mesto nad Váhom (33 mg/day/1000 inhabitants) (Table 3 – Appendix).

The highest concentrations recorded during festivals were observed at Pohoda 2024 and Grape—490 mg/day/1000 inhabitants and 418 mg/day/1000 inhabitants, respectively—compared to the Trenčín non-festival average of 342 mg/day/1000 inhabitants. Elevated values were also observed during the LOVESTREAM Festival (379 mg/day/1000 inhabitants), exceeding the Bratislava non-festival average of 292 mg/day/1000 inhabitants. Weekly monitoring in Bratislava and Piešťany revealed fluctuations in methamphetamine levels, similar to those observed for amphetamine. For instance, in Piešťany, methamphetamine loads ranged from 184 mg/day/1000 inhabitants to 311 mg/day/1000 inhabitants (Table 3 – Appendix).

The most pronounced variations in methamphetamine concentrations were observed in Trenčín and Trnava, while Nové Mesto nad Váhom showed a more stable pattern. A stable occurrence of the drug was also noted in Prešov and Košice. Studies by González-Mariño et al. (2019) and Mackuľak et al. (2020) highlight similar findings regarding the regional dominance of methamphetamine use across Europe, with the Central European region—including Slovakia—showing particularly high prevalence. The findings from the 2024 report, consistent with those from 2021/2022, align closely with EMCDDA statistics from 2020–2024, which identify Slovaks, together with Czechs and Nordic populations, as among the most significant consumers of methamphetamine in Europe (EMCDDA 2020–2024).

2.3 Cocaine

Cocaine is considered one of the more expensive illicit drugs in Slovakia. Its purity often fluctuates significantly, usually ranging from 5% to 33%. Results from the 2024 report indicate that cocaine use is predominantly concentrated in western Slovakia, particularly in central Bratislava (Table 3 – Appendix). Wastewater monitoring conducted in eight Slovak cities showed cocaine concentrations ranging from 4 ng/l (Košice, June 5, 2024; Prešov, August 26, 2024) to 837 ng/l (Bratislava, April 13, 2024; Table 3 – Appendix). When comparing cities, the highest average daily loads per 1,000 inhabitants outside festival periods were found in Bratislava (101 mg/day/1000 inhabitants), while other cities reported values below 50 mg/day/1000 inhabitants. The lowest levels (up to 3 mg/day/1000 inhabitants) were measured in Prešov and Košice (Table 3 – Appendix). Data from previous reports (2013–2022) confirm a long-term centralization of cocaine use in the Slovak capital and the western part of the country. Cocaine levels in wastewater can vary significantly over time, similar to methamphetamine. For instance, weekly wastewater analyses in Bratislava showed cocaine levels between 117 and 225 mg/day/1000 inhabitants, while in Piešťany, the range was narrower—between 17 and 45 mg/day/1000 inhabitants (Table 3 – Appendix).

As emphasized in several studies, the accurate estimation of cocaine consumption relies primarily on measuring its dominant metabolite – benzoylecgonine (Mackuľak et al., 2020; Ort et al., 2014). This compound is much more stable in wastewater and therefore provides a more reliable indicator for calculating cocaine consumption (Van Nuijs et al., 2012).

In this report, the following equation was used to calculate cocaine consumption:

$$\textit{Cocaine [g/day]} = \textit{c(benzoylecgonine) [ng/l]} \times \textit{flow [l/day]} \times 2.77$$

The results of this analysis revealed benzoylecgonine concentrations ranging from 12 to 2033 ng/l (Bratislava, April 14, 2024; Table 3 – Appendix).

Cocaine Consumption in Selected Slovak Cities Based on Benzoylecgonine (BE) Metabolite Calculations

Several studies have highlighted the significant degradation of cocaine in sewer systems (Ort et al., 2014; Thomas et al., 2012; Krizman-Matasic et al., 2019). To ensure accurate consumption estimates, the measured amount of benzoylecgonine must be multiplied by a correction factor of 2.77 during back-calculation (Castiglioni et al., 2011). Based on monitoring results from eight Slovak cities in 2024, the highest average cocaine consumption per 1,000 inhabitants (outside festival periods) was found in Bratislava (662 mg/day/1000 inhabitants), followed by Piešťany (195 mg/day/1000 inhabitants), Trenčín (165 mg/day/1000 inhabitants), Trnava (144 mg/day/1000 inhabitants), and Žilina (111 mg/day/1000 inhabitants). The results also suggest a possible distribution route along Slovakia's highway network, supported by earlier findings (2022 report) showing increased cocaine presence in Nitra and Sereď. The lowest average values were recorded in Prešov and Košice (up to 33 mg/day/1000 inhabitants) (Table 3 – Appendix). ***During festivals, elevated cocaine consumption was detected in Bratislava, with levels reaching 685 mg/day/1000 inhabitants during the Uprising Festival and 1036 mg/day/1000 inhabitants during LOVESTREAM. The most significant overall values were recorded at Grape (5235 mg/day/1000 inhabitants) and Pohoda 2024 (2857 mg/day/1000 inhabitants), while the lowest average value was found at Lodenica (164 mg/day/1000 inhabitants).***

Cocaine consumption levels can vary significantly over time. In Bratislava, weekly analyses showed values ranging from 663 to 1513 mg/day/1000 inhabitants, and in Piešťany, from 129 to 319 mg/day/1000 inhabitants (Table 3 – Appendix). Subsequently, the report summarized cocaine consumption trends from 2013 to 2024, including the lockdown period (March–May 2020) in several Slovak cities (Table 6b – Appendix). The results indicate a gradual increase in cocaine use in Bratislava between 2013 and 2020, despite restrictions on domestic and cross-border movement (especially toward Austria, a common source of illegally imported cocaine). Slovaks are also known to travel abroad to purchase the drug illegally. The long-term increase in cocaine use in Bratislava appears to be driven by improving economic conditions, proximity to Austria and Hungary, and greater market availability.

In 2022, another rise in cocaine consumption was recorded, surpassing 2021 levels—a trend that continued in 2024. In Košice, consumption stabilized between 2018 and 2022, with a slight increase in 2024. In Piešťany, a significant decline was observed in 2021 compared to 2018–2020, followed by recovery in 2022 and a substantial rise again in 2024. Žilina showed a long-term decline until 2022, followed by renewed growth in 2024, similar to Trenčín. In Prešov, consumption remained stable, while in Nové Mesto nad Váhom, a decline was recorded in 2024.***According to González-Mariño et al. (2019), higher cocaine prevalence is observed mainly in Western Europe (Spain, Italy, France, the UK, Belgium, etc.). Cocaine consumption in Slovakia remains lower than in Western Europe (Mackuľak et al., 2020; González-Mariño et al., 2019). However, the 2024 report indicates a post-2022 increase in cocaine use, particularly in Bratislava, Piešťany, Trenčín, Trnava, and Žilina.***

*2.4 **Ecstasy (MDMA – Active Ingredient of the Drug)***

MDMA (3,4-Methylenedioxymethamphetamine)—the active ingredient in the drug ecstasy—is among the substances in Slovakia that are primarily associated with weekend and club use. The consumption of MDMA is often linked to specific music genres or social groups, such as rap, pop, techno, or dance (Mackuľak et al., 2019; Mackuľak et al., 2020). In the 2024 report, wastewater from several music festivals (Lodenica, Pohoda 2024, Grape, LOVESTREAM, and Uprising) was analyzed to assess MDMA occurrence and consumption. During the monitoring of eight cities outside festival periods, MDMA concentrations in wastewater ranged from 8 to 230 ng/l (Bratislava, September 2, 2025—still potentially influenced by Uprising participants; Table 3 – Appendix). When comparing Slovak cities, the highest average MDMA loads per 1,000 inhabitants were found in Bratislava (16 mg/day/1000 inhabitants), Trenčín (15 mg/day/1000 inhabitants), and Žilina (10 mg/day/1000 inhabitants). The lowest values were observed in Prešov, Košice, and Nové Mesto nad Váhom, all below 5 mg/day/1000 inhabitants (Table 3 – Appendix). In terms of temporal variation, MDMA concentrations—similar to those of cocaine—fluctuated over time. These fluctuations are likely related to weekend nightlife and music events, such as Friday and Saturday parties. For instance, in Bratislava, weekly MDMA levels ranged from 9 to 35 mg/day/1000 inhabitants, while in Piešťany the range was 3 to 25 mg/day/1000 inhabitants.

During festivals, significantly elevated MDMA levels were measured. The Grape Festival recorded 1767 mg/day/1000 inhabitants, Pohoda 2024 reached 256 mg/day/1000 inhabitants, and Uprising recorded 55 mg/day/1000 inhabitants, while the Lodenica Festival showed only 6 mg/day/1000 inhabitants. The report also summarized MDMA occurrence in wastewater between 2013 and 2024, including the strict lockdown period from March to May 2020 across several Slovak cities (Table 6c – Appendix). The results show that MDMA levels tend to fluctuate depending on local events and weekends (Table 3 – Appendix). Over the long term (2013–2024), MDMA concentrations either decreased or stagnated in cities such as Bratislava, Piešťany, Banská Bystrica, Žilina, and Košice up to 2022, but increased again in 2024—except in Trenčín, where a sharper rise was observed.

Studies by González-Mariño et al. (2019) and Bodík et al. (2021) indicate that MDMA is still not a major drug in Slovakia, although its occurrence—especially during music festivals—shows a substantial upward trend.

2.5 Marijuana – Monitoring of the Secondary Metabolite THC-COOH

According to the EMCDDA, **marijuana** remains one of the most commonly used illicit drugs in Slovakia, as confirmed by multiple EMCDDA reports from 2017–2024. The majority of users belong to the **16–24 age group**, and their numbers continue to grow (EMCDDA 2020–2024). In the 2024 report, wastewater analysis was used to detect marijuana consumption through its **secondary metabolite THC-COOH**, which is relatively resistant to degradation processes in sewer systems. This stability allows for its detection in influent wastewater at treatment plants (Thomas et al., 2012; Mackuľak et al., 2020).

The concentration of this metabolite in Slovak cities' wastewater during 2024 ranged from **24 to 420 ng/l** (with the highest value observed in Bratislava on September 2, 2024, likely influenced by the Uprising Festival). When comparing cities, the highest average loads per 1,000 inhabitants were found in **Trenčín (69 mg/day/1000 inhabitants)**, **Bratislava (62 mg/day/1000 inhabitants)**, and **Piešťany (61 mg/day/1000 inhabitants)**. The lowest levels were recorded in **Košice (10 mg/day/1000 inhabitants)** and **Nové Mesto nad Váhom (11 mg/day/1000 inhabitants)** (Table 3 – Appendix).

Weekly monitoring of THC-COOH in Bratislava and Piešťany showed variable loading patterns: **Bratislava 53–160 mg/day/1000 inhabitants**, **Piešťany 54–70 mg/day/1000 inhabitants** (Table 3 – Appendix).

From a long-term perspective (2013–2024), wastewater loads of THC-COOH in Slovak cities have been highly variable. For example, in Bratislava's Petržalka district, the metabolite levels ranged between **24–37 mg/day/1000 inhabitants** during 2013–2021, followed by a notable decline in 2022 to **10 mg/day/1000 inhabitants**. Fluctuations in THC-COOH concentrations between 2013 and 2022 were also observed in **Košice, Piešťany, Dunajská Streda, Prešov,** and **Žilina** (Table 6d – Appendix).

The 2024 results indicate **record-high THC-COOH concentrations** in **Trenčín, Piešťany,** and **Bratislava** for the entire monitoring period (2013–2024). Comparing festival data, the highest average THC metabolite values were recorded at **Grape (97 mg/day/1000 inhabitants)**, **Pohoda 2024 (93 mg/day/1000 inhabitants)**, and **LOVESTREAM (68 mg/day/1000 inhabitants)**. Conversely, the lowest values were found at **Uprising (55 mg/day/1000 inhabitants)** and **Lodenica (39 mg/day/1000 inhabitants)**.

3 Monitoring of Selected Psychoactive Pharmaceuticals in Wastewater from Eight Slovak Cities in 2024

Table 4 in the appendix presents the measured quantities of the psychoactive substance methadone and its dominant metabolite EDDP in wastewater from various Slovak cities, normalized per 1,000 inhabitants. In addition to intercity comparisons, Table 4 also illustrates the variations in the amounts of these psychoactive substances over the selected time periods and during specific festivals for the monitored cities (Table 4 – Appendix).

Methadone and Its Metabolite EDDP

Methadone is a synthetic opioid still used in the treatment of narcotic dependence, primarily as a substitution therapy for heroin addiction. Methadone is metabolized in the human body more slowly than heroin, but prolonged use can also lead to strong physical and psychological dependence (Mackuľak et al., 2015; Mackuľak et al., 2020).

In the 2024 report, wastewater from eight Slovak cities was analyzed for both methadone and its dominant metabolite EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine). This compound shows high stability in sewer systems and resistance to degradation processes, making it a reliable indicator for estimating methadone consumption (Table 4 – Report).

During the 2024 monitoring period, methadone concentrations in wastewater—particularly in Bratislava—ranged from 11 to 17 ng/l (April 11, 2024). In Trnava, methadone levels reached up to 2 ng/l (May 6, 2024). In Žilina, Košice, and Prešov, concentrations ranged between 2 and 11 ng/l (August 26, 2024 – Prešov; September 11, 2024 – Košice) (Table 4 – Appendix).

Methadone and EDDP were also detected in wastewater from Trenčín, with the highest measured value of 6 ng/l (June 16, 2024) (Table 4 – Report). It is assumed that the decrease in concentrations observed during festival periods was caused by dilution of wastewater due to increased inflow from festival participants.

4 Monitoring of New Psychoactive Substances (NPS) in Wastewater in Slovakia for 2024

During the wastewater monitoring campaign in Slovakia in 2024, several selected **new psychoactive substances (NPS)** were analyzed (Table 5 – Appendix). Some of these compounds are already classified as high-risk substances under current EU legislation, while others have been banned by law (Act No. 139/1998 Coll.). Compared to common illicit drugs, NPS are not yet widespread in the Central European region—on the Slovak market, only a few detections per year have been reported, although their occurrence continues to rise (EMCDDA 2017–2024). During the 2024 monitoring, these compounds were analyzed in the same manner as illicit drugs, using wastewater samples collected from several Slovak cities and during music festivals. As in previous years (2019–2022), it was confirmed that their concentrations are frequently below the detection limits of the applied analytical method (LC-MS/MS).

In 2024, **none of the selected NPS** were detected in Slovak wastewater samples. The obtained results from both cities and festivals indicate that NPS currently do not reach the popularity of traditional illicit drugs. Scientific publications from 2020 to 2024 still provide limited information on the environmental behavior of these substances within sewer systems (Castiglioni et al., 2021; Mackuľak et al., 2020; Salgueiro-Gonzalez et al., 2024). Similar to the period 2018–2024, it is assumed that NPS remain relatively rare in Slovakia and have not displaced conventional drugs such as methamphetamine (Brandeburová et al., 2020; Castiglioni et al., 2021; Salgueiro-Gonzalez et al., 2024). However, it must be emphasized that **systematic NPS monitoring in Slovak wastewater is still lacking**, even in 2025, compared to other EU countries.

At present, only a **limited number of scientific studies** describe the successful detection and quantification of NPS in wastewater (Castiglioni et al., 2021; Brandeburová et al., 2020; Salgueiro-Gonzalez et al., 2024; Baz-Lomba et al., 2025).

5 Conclusion

In the 2024 report, the monitoring focused on the occurrence and consumption of **57 psychoactive compounds** (illegal and legal drugs, new psychoactive substances (NPS), psychoactive pharmaceuticals, and their metabolites) in wastewater from **eight Slovak cities**. Monitoring was conducted throughout the year at eight municipal wastewater treatment plants and also during five music festivals. In total, approximately **1.05 million inhabitants** were monitored in 2024, with over **150,000 participants** included during the festival periods. Regarding new psychoactive substances, monitoring covered around **1 million inhabitants**.

- **Amphetamine – Low Prevalence and Relation to Methamphetamine** Amphetamine is among the less preferred drugs in Slovakia, with low concentrations detected in wastewater. The highest values appeared in Trenčín during the Grape Festival, indicating a link to music events. The occurrence of the drug often mirrors methamphetamine, as amphetamine is one of its main metabolites. Overall, amphetamine has a secondary role compared to other substances in Slovakia.
- **Methamphetamine – Together with THC, the Most Widespread Illicit Drug** Methamphetamine remains one of the most widespread illicit drugs in Slovakia, as confirmed by reports from 2013–2024. The highest concentrations in 2024 were recorded in Trenčín, Bratislava, and Trnava, with values increasing significantly during the Pohoda and Grape festivals. Data show a growing consumption trend and stable presence even outside the festival season. This corresponds with EMCDDA findings, which rank Slovakia among the countries with the highest methamphetamine use.
- **Regional Differences in Methamphetamine Consumption** While the western part of Slovakia (Bratislava, Trnava, Trenčín) shows pronounced fluctuations in concentrations, the situation in Košice and Prešov is more stable. Nové Mesto nad Váhom exhibits consistently low levels of occurrence. These differences reflect regional drug market characteristics and substance availability. Methamphetamine remains the dominant drug in the Central European region, as confirmed by international studies.
- **Cocaine – Concentrated Use in the Western Region** Cocaine is still considered a luxury drug in Slovakia, with the highest consumption in Bratislava and its surroundings. Monitoring results show a sharp contrast between western and eastern cities, where consumption is significantly lower. The highest

concentrations were recorded during the Uprising, LOVESTREAM, and especially Grape festivals, with several-fold increases compared to regular days. This concentration pattern is associated with economic and geographical factors. Cocaine distribution in Slovakia may also be linked to highway connections between major cities and the capital.

- **Significance of Benzoylecgonine (BE) in Cocaine Analysis**
  For accurate estimation of cocaine consumption, monitoring the stable metabolite benzoylecgonine is essential. In 2024, concentrations ranged from 12 to 2033 ng/l, with the highest values measured in Bratislava. After applying the standard correction factor of 2.77, the highest consumption was again found in the capital. This approach allows a more precise estimation of real cocaine use within the population.
- **Long-Term Trend of Rising Cocaine Consumption**
  Between 2013 and 2024, a steady increase in cocaine use was observed, particularly in Bratislava and Trenčín. Despite COVID-19 restrictions in 2020, consumption did not significantly decrease. After 2022, another increase was recorded, likely linked to improved economic conditions and higher drug availability. In eastern Slovakia, cocaine occurrence remains relatively stable and low.
- **MDMA – The Weekend and Festival Drug**
  MDMA (ecstasy) is typically associated with weekend and dance events. The highest concentrations appeared in Bratislava and Trenčín, with sharp increases during the Grape and Pohoda festivals. On regular days, MDMA levels remained low, but rose significantly during musical events. In Slovakia, MDMA is therefore a drug characterized by episodic but intensive use.
- **Temporal Dynamics and Variability of MDMA Occurrence**
  Data from 2013–2024 indicate that MDMA concentrations fluctuate with seasons and specific events. After a decline during 2019–2022, an increase was again recorded in 2024, especially in Trenčín. MDMA consumption is closely linked to cultural and social factors such as clubs and festivals. Outside these events, its presence remains marginal.
- **Marijuana – The Most Widespread Illicit Substance According to EMCDDA**
  Marijuana continues to be the most commonly used illicit drug in Slovakia, as confirmed by EMCDDA reports. Monitoring of its metabolite THC-COOH revealed the highest values in Trenčín, Bratislava, and Piešťany. Its occurrence corresponds with the festival season, with the highest concentrations recorded during Grape and Pohoda. Eastern Slovakia consistently shows lower values.

- **Long-Term Trends in THC-COOH Occurrence**
  Between 2013 and 2024, significant regional differences in THC-COOH concentrations were observed. While levels were lower in previous years, 2024 marked the highest recorded values in Trenčín, Piešťany, and Bratislava. This trend may be linked to increasing social tolerance toward marijuana and its greater availability.
- **Methadone** was found in the highest amounts in wastewater samples from Bratislava and Košice, where the largest numbers of users undergoing substitution therapy are expected.
- In the **2024 report**, wastewater analyses also included over **25 specific psychoactive compounds (NPS)** that may pose potential risks due to the lack of toxicological knowledge. None of the selected NPS were detected in Slovak wastewater in 2024; however, compounds such as 3-MMC, methcathinone, mephedrone, and N,N-DMT were not analyzed that year.

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