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# Self-reported Subjective Effects of Analytically Confirmed New Psychoactive Substances Consumed by e-Psychonauts: A longitudinal study with an innovative internet-based methodology

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Doctoral dissertation

# Self-reported Subjective Effects of Analytically Confirmed New Psychoactive Substances Consumed by e-Psychonauts:

## A longitudinal study with an innovative internet-based methodology

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# Summary:

**Background:** Current scientific methodologies have failed to provide enough reliable data regarding the use and effects of New Psychoactive Substances (NPS) in the timeframe when critical decisions must be made. Thus, this study aims to fill the current evidence gap using a new methodology to empower e-psychonauts to produce more reliable data than the obtained using online surveys, but with far less resources and time than data from randomized controlled trials.

**Methods:** To do so, a longitudinal and observational study design has been adopted, recruiting e-psychonauts and following their NPS self-use for 14 months, allowing them to send samples of the NPS they intend to use to an international drug checking service providing reliable information regarding the sample real composition. The drug checking services were provided for free as compensation for their dedication in reporting how they use these substances and their subjective effects, using a series of structured questionnaires provided by the research team. A systematic literature review was performed for each identified substance.

**Results**: From 184 screened candidates, 17 participants reported at least once on an analytically confirmed substance sample. A total of 64 confirmed reports were collected, identifying 40 different NPS. From those, 13 (32.5%) had not been previously reported in the scientific literature, and 12 (30.0%) had been reported but without descriptions of their subjective effects. Most of the analyzed substances (n=90; 93.8%) contained what the user expected, with only 8 samples (8.3%) containing the expected substance and others and only 6 samples (6.3%) containing only other substances than the ones expected. Finally, the chosen measurements to assess the substances' subjective effects were consistent with previous literature when assessed by substance class. No individual analysis was made due to the small sample size for each substance.

**Conclusion**: The feasibility of this methodology has been proven, despite some limitations that could be apparently addressed when replicated. The capacity to identify so many previously unreported NPS and to collect previously unreported data about subjective effects of significant amount of other NPS positions this methodology with the potential to fill an important evidence gap. While the current means of responding to emerging substances are widely seen as unable to respond to the challenges posed by NPS, this new methodology could be an effective response.

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# Introduction

## Importance of New Psychoactive Substances

To date, New Psychoactive Substances (NPS) still represent a very important challenge to legislate, monitor, study and develop health interventions. A Eurobarometer (2014) survey showed that, on average, 8% of youth in Europe had experience of NPS, which differed considerably from the 65.8% among a targeted population of nightclub visitors in the United Kingdom (Wood, Hunter, Measham, & Dargan, 2012). Understanding of usage patterns and effects remains poor, with most information being based on populations and settings where problems have already occurred (Higgins et al., 2019).

The ever-increasing number of psychoactive substances used nowadays represents a new challenge for medicine and psychiatry. Thepharmacodynamics,pharmacokinetics, public health impact and psychopharmacology of many NPS are not yet thoroughly understood (Schifano et al., 2019). In addition, NPS consumption rarely occurs in isolation from other behaviors but, on the contrary, is placed within a kaleidoscopic range of poly drug use trajectories. There seems to be no differential risk for NPS use compared with the use of traditional psychoactive substances such as alcohol, cannabis, or cocaine (Higgins et al., 2019). This new phenomenon represents an unprecedented challenge in the field of drug use as well as a fast-growing problem from social, cultural, legal, and political perspectives (Ornella Corazza et al., 2013).

## NPS: Definition and prevalence

NPS are substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat. It is important to note that different authors have previously referred to them as designer drugs, legal highs, herbal highs, bath salts and research chemicals. Moreover, the term new does not necessarily refer to new inventions but to substances that have recently emerged on the market (UNDOC, 2017). Hence, new can include a failed pharmaceutical or an old patent that has been rediscovered for recreational substance (Schifano et al., 2019).

Another distinction being made is between NPS and emerging psychoactive substances, where the latter term captures all NPS as well as drugs that may not be newly invented but have recently

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experienced a resurgence of, or increase in, use (Schifano et al., 2019). However, to simplify this work, only the term NPS will be used, also including all emerging psychoactive substances. Most NPS are the result of minor changes to the molecular structure of well-known legal or illegal drugs, such as opioids, ecstasy, or stimulants (Meader, Mdege, & McCambridge, 2018).

Between 2009 and 2017, 803 NPS were reported in 111 countries or territories. Additionally, by the end of 2020, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) was monitoring around 830 new psychoactive substances, 46 of which were first reported in Europe in 2020. (EMCDDA, 2021; Schifano et al., 2019; UNDOC, 2017). In the European Union, by the end of 2017, the number of NPS was over 670, of which 632 were notified after 2004 (EMCDDA, 2018; Schifano et al., 2019). However, evidence suggests that the NPS scenario could be much larger than that formally identified by international agencies. In a recent publication, Schifano et al (Schifano et al., 2019) used a web search engine to identify NPS discussed online by NPS enthusiasts. Using this methodology, they identified a few thousand NPS, a number which is about 4-fold higher than the figures suggested by European and international drug agencies.

There is an ongoing debate on the scale of challenges posed by NPS, as the evidence on the prevalence of NPS use is scarce. For example, general population surveys suggest that the prevalence of NPS use is relatively low, with the best estimates found in the scientific literature being between 1% and 2% in United Kingdom. However, the speed of technological innovation and the ease of synthesizing NPS present substantial challenges to regulatory authorities, researchers, and clinicians (Mdege, Meader, Lloyd, Parrott, & McCambridge, 2017; Meader et al., 2018).

For all these reasons, it is critical to gather data on the subjective effects of the exponentially growing number of NPS, as this might allow the early identification of the ones that will become popular among larger groups (Matthews et al., 2017).

## Challenges posed by the emergence of NPS

NPS may now pose a big challenge due to several factors:

First, the NPS consist of several different classes of substances, which vary in their psychological and physiological effects. Treatment is often difficult because of the young age of most users and

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the possibility of concurrent polysubstance use. The pattern of use is often intermittent in social settings, so it may be perceived as less of a problem (Weaver, Hopper, & Gunderson, 2015).

Second, NPS appear into—and sometimes disappear from—the market very quickly, and as such, they are not significantly impacted by regulatory efforts. Currently, new substances are identified in Europe at a rate of one or more per week (Wood et al., 2014). Several key studies have shown the continued use and popularity of mephedrone, a popular NPS, among specific drug-using populations after it was brought under control. The scheduling of new substances could even increase the speed at which manufacturers innovate, to bypass the law (O'Brien, Chatwin, Jenkins, & Measham, 2014).

Third, NPS are mainly distributed through the internet in a transnational market without any solid information about their effects and risks (O'Brien et al., 2014). During recent years, the widespread availability of internet access has led to a gradual, although only partial, shift from a street to a web market (Corkery, Orsolini, Papanti, & Schifano, 2017). The increased web-based distribution has been seen in both the surface web and dark net (Orsolini, Papanti, Francesconi, & Schifano, 2015).

Fourth, NPS can substitute traditional drugs in times when their availability is restricted (Mdege et al., 2017). This could be problematic, as this substitution happens both by introducing new substances in the market as well as by selling NPS as traditional drugs, exposing large populations, unknowingly, to the effects of a new unstudied substance without previous experiences. This is especially dangerous, combined with the rapid turnover of NPS, as they change before we can obtain research data using conventional methodologies (Meader et al., 2018).

Fifth, there is a concerted effort to grapple with the challenges of researching NPS, as traditional methodologies are too slow and expensive to generate relevant and timely data on the effects of NPS (Meader et al., 2018).

Sixth, Clinicians are not usually able to identify a potential NPS user, and NPS usually produce negative results to traditional drug tests, which are designed to assess a very limited number of traditional substances (UNDOC, 2017). On the other hand, NPS users rarely search for professional help linked exclusively to this problem, and clinicians are not trained to screen or identify NPS use.

## Mapping the evidence on the field of NPS research

Despite the high number of publications about NPS during the last 20 years, especially after a sharp increase in 2010, there are still concerning *gaps in our understanding* of the phenomena. From the evidence map about NPS research performed by Mdege et al. three key points should be highlighted (Mdege et al., 2017):

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First, most of the studies have been performed in general hospital population (n=294, 40.1%) or specialist settings (n=134, 18.2%), mostly reporting severe intoxications or other acute NPS-related problems.

Second, the most frequent study design reported in the indexed peer-reviewed literature was case series and/or reports (n=367), followed by the literature review (n=243), the survey (n=130) and the secondary quantitative data analysis (n=99), with only 13 existing randomized controlled trial (RCT), 6 prospective cohort studies and one case-control study (Mdege et al., 2017; Meader et al., 2018).

Third, virtually all the studies that used questionnaires, surveys, or interviews that assess the effects of NPSs are based on self-reported data without analytical confirmation of the substance that was truly ingested. This makes the data difficult to interpret, as the participants might have been told they are ingesting one substance when they are ingesting another, or a combination of substances.

Other authors focus on the limitations of the outcomes assessed by the existing literature, as only a handful of studies have moved beyond prevalence to explore subjective user experiences and motivations (Chatwin, Measham, O'Brien, & Sumnall, 2017; O'Brien et al., 2014; Soussan & Kjellgren, 2016).

Self-reports and surveys are mostly based on self-reported use rather than the analytical confirmation of the substance(s) used. In contrast, case reports are usually generated from hospital settings in the context of an intoxication or overdose with multiple substances involved, so there is analytical confirmation of the substance but no self-reported effects. Unsurprisingly, the literature is dominated by studies investigating the problems associated with NPS (773/995 records). Therefore, caution is required when interpreting these data because of the following two limitations:

First, users will report what they believe they have used, rather than whatever substance is really taken (Wood et al., 2014).

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And second, intoxications with multiple substances in hospital settings do not target the information on psychopharmacological effects of a particular NPS (Mdege et al., 2017; Meader et al., 2018).

In their empirical and conceptual review to produce research recommendations, Mdege et al provide the following advice for research, among others (Mdege et al., 2017):

1. The need to be aware of innovation opportunities, such as testing emerging NPS brands online as they become available.
2. Using cohort study designs to better understand the determinants of NPS use and related physical and mental health, psychosocial problems, and how patterns of involvement and consequences change over time.
3. Use a systems-based prevention approach that develops existing responses and emphasizes commonalities between NPS and other legal and illegal drug use. They also suggest focusing on the following research questions:
    a. What are the prevalence and patterns of NPS use in the general UK population and do they differ between subgroups of the population?
    b. Are there sentinel populations capable of being monitored to provide early warnings of new trends?
    c. Which acute intoxication problems are associated with NPS use?
    d. Which promising approaches are currently available or can be made available in the United Kingdom for intervening with NPS use?

Additionally, they concluded that there is a need for a major research effort to be directed at NPS, which should address NPS together with other forms of licit and illicit drug use (Mdege et al., 2017; Meader et al., 2018).

In summary, both public institutions and leading scientists recognize the importance of conducting research beyond the expert-driven discourse, empowering the individuals previously regarded as subjects like participants, collaborators, scientific citizens or lay experts (Barratt & Lenton, 2010; Mdege et al., 2017; Meader et al., 2018; Söderberg, 2016).

The considerations stated above lead to the necessity of conducting a longitudinal study in a sentinel population to assess the effects of recently emerged NPSs, along with the attitudes and perceptions produced in the mentioned population. Conducting this study in a population of

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people who have extensive experience and high curiosity for consuming emerging NPS might be able to predict future harms and challenges to more extensive populations (Barratt, 2011; Schifano et al., 2019).

## The e-psychonaut population

Both the limitations and recommendations stated above lead to the necessity of conducting a longitudinal study in a specific and potential sentinel population, such as internet NPS-consuming communities. This would allow for early assessments of the effects of recently emerged drugs and to study the patterns of consumption, harm reduction strategies, and long-term drug-related problems.

People with expertise in consuming emerging NPS often refer to themselves as e-psychonauts. The term psychonaut was first described by Newcombe (Ornella; Corazza et al., 2013) as an adult user of psychoactive drugs who takes these substances in normal, everyday settings with the intention of subjectively exploring their effects.

Some years later, O'Brien et al (O'Brien et al., 2014) coined the term cyber-psychonauts to refer to their sample composed predominantly of NPS consumers. Cyber-psychonauts are further defined by their commitment to harm reduction, to using NPS safely and responsibly, and to purchasing chemicals online (Mdege et al., 2017). This population is clearly differentiate from other known populations such as partygoers or archetypically addicted patients, despite its probable minor intersections.

Tackett-Gibson (Tackett-Gibson, 2008) also documents the existence of online communities populated by self-defined experts in using NPS, providing a contrasting narrative around drug use and risk to that established by the scientific community. A brief perusal of relevant websites confirms the existence of a great number of NPS-related discussion threads, suggesting the existence of an online community of more discerning NPS users.

Orsolini et al. also refer to this population in their more recent study, identifying educated and informed users within online drug forum communities, who can provide reliable information on psychoactive compounds (Orsolini et al., 2015). They refer to these users as e-psychonauts, providing the best characterization of the population to date. The e-psychonauts appear to be

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mainly young and unmarried white males, presenting good or excellent employment conditions and with a set of key skills, such as high standards of knowledge about drugs' chemical and pharmacological issues; and high levels of both technology-related skills. They are meant to have a wide vocabulary to define their own on drug experiences in the most subtle and precise way possible.

Among this population, the frequency of NPS use is high, with one-third of the participants reporting its use in the last week. They view themselves as knowledgeable consumers who use the internet to accumulate information about NPS and share their own experiences, informing fellow users of potential harms. However, other studies (Winstock et al., 2011) reported possible stimulant dependence (3 or more dependence symptoms) in 30% of mephedrone users. Mdege et al (Mdege et al., 2017) also found that NPS users often report substance use disorder symptoms, especially craving.

This community may have some other distinct characteristics. A total of 19% of the sample reported that an NPS was the first drug that they had ever taken. Of those who ceased using NPS, 91% found it either easy or very easy to stop. Most commonly, cessation was due to the side effects of NPS (Fletcher, Tasker, Easton, & Denvir, 2016). They also perceived internet forums as an important channel through which to communicate information on new drugs, and retailers reported monitoring forums to determine which drugs to stock in their store (Mdege et al., 2017).

These users also tend to post online warnings based on first-hand experiences about the potential harms of the substances consumed, willing to avoid harm to their peers. Orsolini et al even stated that posting online the on drug experience report is arguably the trait d'union of all e-psychonauts, considering the intention behind using a substance the most significant difference between a psychonaut and a typical drug user (Orsolini et al., 2015). O'Brien et al also identified the role of e-psychonauts in disseminating emerging information about NPS-related harm and considered them well equipped to make a valuable contribution to NPS policy debates in general, and e-psychonauts are ideally placed to report on the effects of recent policy changes on NPS-related harms in particular (O'Brien et al., 2014).

Available data on sentinel populations are growing. For example, several studies of attendees of gay-friendly night clubs suggest that the trend in reduction of mephedrone witnessed nationally

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may also occur in this subgroup. However, the study of this sentinel population has failed to predict future harms and trends in the global NPS market (Meader et al., 2018).

Conversely, data on another sentinel population, namely, e-psychonauts, have been able to predict future NPS-related harms occurring in more general settings (Schifano et al., 2019). In fact, the sentinel population of e-psychonauts has been considered by several authors as potentially useful in identifying NPS availability, market, and diffusion in advance.
This population is believed to be responsible for shaping and influencing the drug scenarios of the future (Orsolini et al., 2015). In addition, Corazza et al provided evidence supporting the claim that the online NPS scenario predicts the real-life NPS scenario (Ornella; Corazza et al., 2013).

Different authors believe that these internet communities are a huge opportunity for researchers. The qualitative analysis of how different groups interact with online communities may help to systematize and codify needs, values, and preferences that are relevant to the group (Hewson, 2007). In internet communities, researchers can simply recruit participants or even go further and engage drug users more fully in dialog (Illingworth, 2001). Some authors even state that the lack of physical presence and separate physical settings all reduce researcher control and power, thereby potentially leading to a more balanced relationship between researchers and participants (Cuschieri, 2019; Seddon, 2014). In any case, e-psychonauts are a hidden, hard-to-reach population that may have a significant influence on future drug trends.

Some authors even consider cyber-psychonauts to be ideally placed to become involved in the actual implementation of innovative responses to the increasing prominence of NPS markets, as it is difficult to imagine a more efficient method for the rapid dissemination of new information about things such as the adverse effects of new products to consumers (Barratt, 2017; Barratt, Ferris, & Lenton, 2015; Maddox, Barratt, Allen, & Lenton, 2016; Palamar, Barratt, Ferris, & Winstock, 2016).

The recent alarm related to the growth of the NPS market and the gradual shift from the street to the cyber-drug market may call for the implementation of preventive tools and practices tailored to these new drug users' characteristics.
Finally, in their empirical and conceptual revision of the NPS research field, Mdege et al. concluded that there was a clear need to move beyond an expert-driven discourse on NPS and

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involve people who use NPS as active and valuable research collaborators and stakeholders instead of passive research participants (Mdege et al., 2017; Meader et al., 2018).

A great advantage of recruiting e-psychonauts is that they self-organize in forums in which they collectively reflect on and give meaning to their own practices. They also tend to be highly educated and with a highly articulated and assertive discourse (Söderberg, 2016). In some sense, psychonaut communities contest the authority claimed by government agencies and medical doctors, paralleling, to some extent, the phenomena of patient group activism (Akrich & Rabeharisoa, 2012; Madeleine & Vololona, 2012)..

Working with e-psychonauts, however, poses several challenges, as they are a hidden and hard-to reach population. Several authors have tried to engage cyber-psychonauts as research participants. Mdege et al. found difficulties in involving NPS users throughout the project due to a lack of willingness on the part of NPS users to be contacted in ways other than email. In addition, working with this population has inherent sampling problems: internet research participants are, by definition, a nonrandom and self-selecting sample, and it is very difficult to know the characteristics of the overall pool from which the sample is drawn (Barratt, 2011; Barratt & Lenton, 2010; Chiauzzi, Dasmahapatra, Lobo, & Barratt, 2013; Mdege et al., 2017).

Collected data usually suffer from apparently intrinsic limitations: The recruited sample must be non-randomized, self-selected and with idiosyncratic characteristics that limit the capacity to assess its representativeness from the populations from which is drawn. Additionally, e-psychonauts might be hard to reach for a variety of reasons, some of which can be addressed following guidelines developed by themselves (Andrews, Nonnecke, & Preece, 2003; Barratt, 2017; Barratt et al., 2015; “Bluelight research Standards,” n.d.; “Forum Guidelines to post recruitment announcements,” n.d.; Chiauzzi et al., 2013; Eysenbach & Wyatt, 2002).

## Summary

In summary, traditional methodologies repeatedly fail at trying to produce timely and balanced scientific evidence about the skyrocketing number of different NPSs, but collaboration with e-psychonaut communities with innovative methodologies can provide the evidence that is currently lacking to the field.

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## How this study will contribute to tackle these challenges:

Thus, this study aims to fill the current evidence gap using a new methodology to empower e-psychonauts to produce more reliable data than the obtained using online surveys, but with far less resources and time than data from randomized controlled trials. To do so, a longitudinal and observational study design has been adopted, recruiting e-psychonauts and following their NPS use for 14 months.

To be able to analytically confirm the substances ingested and reported, a collaboration with the international drug checking service of Energy Control-Associació Benestar i Desenvolupament (EC-ABD) provides a critical opportunity, both to analyze the collected samples and to provide free drug-checking opportunities to reward participation. This service is already being paid for by a significant proportion of e-psychonauts, and it has a well-regarded institutional presence in most of their communities (Brunt et al., 2016; Caudevilla et al., 2016; Giné et al., 2017; González, Ventura, Caudevilla, Torrens, & Farre, 2013; Measham, 2018).

The study has been designed to answer the following questions:

1) Is it possible to collect reliable data using this innovative and methodology with the collaboration of the e-psychonaut communities?
2) What are the characteristics of the recruited participants?
3) What substances will they intend to use during the data collection period, and will those be what they expect?
4) What are the subjective effects of the analytically confirmed substances they ingest and report on during the study duration?

The general hypothesis of the study is that it is possible to recruit this specific population and that they will provide complete the study protocol, providing data on previously unknown NPS and their subjective effects. The specific hypothesis can be found in the methodology section.

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# Methodology

## Specific goals and hypothesis:

The already stated general hypothesis can be summarized in the following bullet-points:

A. It is possible to recruit this specific population
B. These participants will complete the study protocol
C. This will result in the collection of data on previously unknown NPS and their subjective effects.

The specific hypothesis can be then unpacked from these three main points.

### A) It is possible to recruit this specific population

1. We will be able to engage the key stakeholders of the internet communities of e-psychonauts to establish rapport and agree on a recruitment strategy that is consistent with the approved protocol.
2. Our recruitment strategy will provide at least 80 study participation applications from candidates.
3. At least 40 participants will be recruited from these candidates.

### B) These participants will complete the study protocol

1. The accepted participants will show high commitment to the study and their sociodemographic data will match the consistently reported characteristics of the e-psychonaut population: White, middle-aged males with medium or high education with wide previous drug experiences
2. About 50% of the accepted participants will complete the study protocol until the one-year follow-up.
3. The completion of the study protocol by these 40 participants will provide approximately 400 reports on self-administration trials.
4. We also predict that participants will be able to predict drug purity and if the drug has been adulterated.

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C) Data on previously unknown NPS and their subjective effects will be collected.

1. The NPS samples analyzed will be less adulterated than the illicit drugs commonly sold in the street level
2. Most of these substances will be cathinones (stimulants) and psychedelics, and the most common rout of administration will be orally.
3. Previously unreported NPS will be detected. Also, we predict the collection of data on subjective effects from NPS with no previous reports on these effects.
4. The collected data on NPS' subjective effects will be reliable, with acceptable validity, comparable with previous databases and consistent with the narrative reports and type of NPS.

## Overview

The study is aimed to discover the characteristics of the e-psychonaut population and the effects of the NPS they use with a longitudinal design and no control group. The study is conducted online, recruiting participants using an innovative and specifically developed platform as part of the study project: Global research and analysis of new substances project [GRASP]. The platform allowed controlled interaction between participants and also between participants and researchers, and was used to promote a sense of community and to promote participant engagement (Ip, Barnett, Tenerowicz, & Perry, 2010).

The study has been designed and will be reported using the Checklist for Reporting Results of Internet E-Surveys (CHERRIES) (Eysenbach, 2004) and the Strengthening The Reporting of Observational Studies in Epidemiology (STROBE) statement checklist for observational studies (Cuschieri, 2019), with the support of the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement checklist of 2013 (Chan et al., 2013).

The protocol of the study was published before the data analysis (Grifell et al., 2021).

## Ethical considerations

The study protocol was submitted and approved by the Clinical Research Ethics Committee (CEIC Parc de Salut Mar, Barcelona, Spain, ref. 2018/8283/I). The 4$^{th}$ and last protocol version were approved on May 27$^{th}$, 2019. The study was conducted according to the Declaration of Helsinki recommendations as well as the emerging recommendations on online research on sensible topics (Barratt, 2011; Barratt & Lenton, 2010). All the data collected online on the participants

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were encrypted according to the European and Spanish data protection regulations (2016/679 European Parliament and 27/4/16 General Data Protection Regulation, Spanish Royal Decree).

All participants will receive the participant information sheet and will be required to read and fill the IC form, which will be sent to admin@grasp.pw. The principal investigator will be responsible for reviewing all candidates' IC forms and screening questionnaires for inclusion and exclusion criteria. All candidates and participants will have the opportunity to ask as many questions as needed before proceeding to any part of the study, both through the forum and through contacting the leading researcher email (admin@grasp.pw).

Participants will not be required to sign IC forms with their real identities to protect their anonymity and avoid sharing data that could be used to track their physical identity. The identity that will be protected by the researchers will be the online one, as no other information relatable to the real identities will be given. This procedure is consistent with the methodology of previous studies (Barratt, 2011; Barratt et al., 2015; Barratt & Maddox, 2016; Maddox et al., 2016; Ross, Potter, Barratt, & Aldridge, 2020). Also, participants could only send samples from their own substances, obtained by themselves from sources unknown to the researchers. This observational and non-interventionist approach was based on previously published research developed in our institution (Papaseit et al., 2018b, 2020, 2021; Poyatos et al., 2021).

The researchers did their best to limit the influence of their interactions on the participants' behavior, especially the ones targeted in the study. However, as recommended by previous research, the participants will be involved in discussing the study design and incentivized to share their opinions on how the study could be improved (Barratt & Lenton, 2010; Chiauzzi et al., 2013; Keijsers, Bossong, & Waarlo, 2008)**.**

Protocol changes will be communicated to the participants through the online platform once they are approved.

A more extensive ethical analysis by principles can be found in **Annex 6**

## Study Setting

The study is conducted mainly though internet, using 3 main tools:

1. The specifically designed GRASP platform

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2. The Qualtrics survey service licensed though Columbia University
3. The Google Suite platform as an email service to contact candidates and attend to the private questions and concerns of the study participants.

In addition, the samples were received through traditional mail in the Energy Control Headquarters in Barcelona, where they were they followed the usual procedures and protocol of analysis of the drug checking service organization.. The research team worked at both the EC headquarters and the IMIM laboratories. During the COVID-related lockdown that was established in Spain in March 2020, the laboratory analysis was interrupted for 3 months.

The usability of the platform and the multiple automated processes, such as sending an email with a specific link to a questionnaire, and the logic pathways (adaptive questioning) and validation requirements used in the Qualtrics questionnaires were systematically tested by the research team. A checklist of all possible scenarios was devised, and they were all executed by a blind research team member and the principal investigator. Once errors were identified, they were corrected, and the process was repeated from the beginning. In addition, participants were encouraged to report any problems or ideas to improve the procedures, so changes could be implemented when needed during the study.

## Recruitment and participants:

All the participants were correctly and fully informed by writing (refer to the participant information sheet in annex 1) and prompted to ask any questions by email. In that case, answers were provided until the candidate confirmed that they had no more questions and were satisfied with the information received. All participants indicated their agreement to participate and signed an informed consent (IC) form (annex 2) that was sent to the project email address and checked by the principal investigator before inclusion. It was not possible for candidates or participants to answer any online questionnaires without previously receiving the specific link, which was sent by the research team only when the participant met the criteria to fill the questionnaire.

The operational definition for online communities of people who use NPS has been adapted from the study by Barratt (Barratt et al., 2015; Barratt & Maddox, 2016; Chiauzzi et al., 2013). Participant recruitment ads were sent during July 2019 to the moderators of the 4 online communities of people who use NPSs that met the study inclusion criteria in October of 2018:

1. Surface websites with at least 5 years of existence.

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2. Presence of participation forums dedicated to discussing the use of NPS in general, without focusing on a particular substance or group of substances.
3. At least weekly activity on the community forum.
4. The use of pseudo-anonymity by community members to identify themselves.
5. The presence of official and analytical drug checking services in the community.

Recruitment ads were sent to the moderators of the selected online communities after establishing bilateral communication with them, mainly to ask permission and explain the goals of the project. Following research guidelines from the communities, community leaders were approached first, to establish rapport and ask permission and collaboration(Barratt & Lenton, 2010; Chiauzzi et al., 2013). Each community moderator posted the research ad in the most appropriate way in their community after discussing it with the research team. Research ads were posted on all communities during the summer of 2019 (the generic ad given to moderators can be found in annex 3). The recruitment was designed to be sequential until the designed sample size was reached, or the study reached its duration limit.

When the study design was completed (October 2018), there were 4 communities meeting the previously stated criteria:

1. Bluelight: Established in 1997, bluelight is probably the most prominent community of people who take illicit drugs, with approximately 250,000 members. Within the community, there is a subdivision in which the use of NPS is exclusively discussed. The community is known for its commitment to promoting risk management and harm reduction strategies among its participants as well as its formidable contributions to similar research projects [28]. Registration is required to access the content.
2. Reddit: Established in 2010, this subreddit community allows almost any type of discussion regarding NPS. The community has approximately 90,000 members, but it is part of a broader community of people who use illicit drugs (not only NPS), with over 700,000 members. Both of these are part of the global reddit community, where all types of topics are discussed. The platform does not require registration to access the content.
3. Drugs-forum: Established in 2003, this community also seems to have approximately 250,000 members. Registration is required to access the content.

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DNstarsVIP: Established after discussing about NPS sources was banned on the reddit community, DNstars is a strongly emerging community with approximately 2000 users. Registration is required to access the content.

The main communities that were assessed and excluded were as follows:

1. Legal-highs forum: excluded because of the lack of weekly interactions, technical website problems, and impossibility to contact community managers.
2. Erowid: excluded because of the lack of an active forum.
3. Psychonaut wiki: excluded because of the lack of an active forum.
4. Tripsit: excluded because of the lack of an active forum, although there was an IRC-supported (Internet Relay Chat) chatroom.
5. Dimethyltriptamine-nexus: excluded because of the lack of a specific NPS subsection.
6. Ecstasy data: excluded because of the lack of an active forum and the lack of a specific NPS subsection.
7. Shroomery: Excluded because of the lack of a specific NPS subsection.

The authors were not able to find evidence of important online communities were not considered. Their assesment was consistent with previous research (Soussan & Kjellgren, 2014).

The ads were posted along with a public PGP (Pretty Good Privacy) key to allow verification of the authenticity of the posts by the potential candidates.

The participants' inclusion criteria used are stated below:

1. Self-reported previous use of NPS.
2. Self-reported intentions of maintaining consumption of NPS for the following 6 months before knowing about the study.
3. Self-reported age above 18 years.

Participants received no monetary compensation for their participation, but instead were offered the possibility to get the NPS they reported on analytically tested for free in the EC-ABD laboratory, located in the Hospital del Mar Research Institute (IMIM), Barcelona. The cost of this service is around 90 EUR if contracted independently through the Energy Control international drug checking service. The participants could only send samples from their own substances, obtained by themselves from sources unknown to the researchers. This naturalistic approach was

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based on previous research developed in our institution (Papaseit et al., 2018a, 2020, 2021; Poyatos et al., 2021).

The GRASP platform, which allowed for interaction among participants themselves and with the research team, was the main tool to promote participant engagement and minimize dropout rates. The study recruitment began in August 2019 and ended in October 2020.

Note that there is no restriction on the geographical location of the participants, as the study will not collect such data to further protect the participants' physical identity. Therefore, participants from around the globe could participate in the study. Both inclusion and exclusion criteria have been mainly assessed by direct self-reporting in an initial screening questionnaire (Q0), except the following:

1. Previous participation in forums has been assessed by self-report and exclusive advertisement of the study on these forums.
2. Difficulties in using new technologies have been assessed by the steps required to complete the screening process, such as sending an IC form in a particular format, registering to the platform, and following the instructions there to introduce themselves to the research team and other participants.
3. Difficulties in communicating in English and the presence of potential psychopathological impairments have also been evaluated by the principal investigator, assessing the answers to long and elaborate open questions in the screening questionnaire (Q0) and in the written introductions to the online platform.
4. The potential of being pregnant was assessed by indirect questioning using the same screening questionnaire (Q0) questionnaire.

## Sample size:

In the most recent review consulted by the authors, the sample sizes reached with web-based questionnaires in people who use illicit drugs ranged from 80 to 9867 (Miller & Sønderlund, 2010). The expected losses while filling these types of questionnaires are about 50% of the sample, but the authors have not found other online longitudinal studies including questionnaires like this one. According to the review, the authors expected 80 candidates as the best possible estimate to achieve 40 final participants. These 40 participants were expected to produce around 400 self-administration trials (SATs). The SAT includes submitting a substance sample, sending the sample, receiving the result, filling baseline questionnaire (Q3a), ingesting the substance, and

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filling dug effects questionnaire (Q3b) within 24 hours of the ingestion of the substance (Miller & Sønderlund, 2010).

## Study procedures and timeline:

The study's internal timeline and workflow are graphically represented in annex 4. The first recruitment effort consisted of online discussions with forum moderators and posting the Institutional Review Board (IRB)–approved announcement for candidates (displayed in its entirety in annex 3) in those forums. In the announcement, potential candidates were instructed to send an email from a secure and non-identifiable email address to the research team (admin@grasp.pw). Then, candidates were informed more broadly about the study. Candidates were informed homogeneously by sending the IRB-approved information for candidates' sheet to their email (the sheet used in this study can be found in annex 1).

The principal investigator then offered the candidates to answer any questions that might have arisen after reading the participant information sheet. When the participants had read and discussed the given information about the study with the principal investigator, they were asked to register on the GRASP platform and send the IRB-approved IC form, completed with the registered username to the study email. In annex 2, the IC form is available for consultation.

Finally, the candidates were asked to complete the screening questionnaire (Q0) and introduce themselves on the platform without providing information that might reveal their real-world identities. When all these processes were complete, the principal investigator checked the IC form, the screening questionnaire, and the platform introduction to assess if the participant met the inclusion/exclusion criteria. Candidates were then informed by email about the results of the assessment, thus either being rejected or accepted as participants.

Once participants were accepted, they could interact with other participants on the online platform and received detailed instructions on how to conduct the study. However, NPS sourcing and the effects of the substances included in the study were not allowed. In case of a severe protocol violation such as this one, participants were immediately removed from the study and their information was deleted. In case of minor protocol violations, participants were notified and given the opportunity, if applicable, to amend their noncompliant behavior.

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The first mandatory step was to fill a sociodemographic and drug use history questionnaire (Q1). This questionnaire was available to each participant through a participant-specific link, which was sent by email once they were accepted.

After that, participants were asked to fill the sample submission questionnaire (Q2), where information about the sample they intended to consume was asked. This questionnaire was then reviewed by the principal investigator and approved if the substance met the study criteria of being a new psychoactive substance. The samples that did not meet the inclusion criteria were not accepted, and the participant was notified by email.

The sample submission questionnaire (Q2) was available to all accepted participants as a link on the platform. All the questionnaire answers were reviewed weekly by a member of the research team to communicate to the participant the acceptance of the sample and to mark them as valid or invalid data for later analysis.

If the sample was approved, the participant received a specifically generated sample code with the instructions to send a small amount of the sample (approximately 30 mg, usually below the psychoactive threshold) via traditional mail to the laboratory at the IMIM. The sample was analyzed there, and the result was sent back to the user, along with harm reduction advice when appropriate.

Meanwhile, the users could consume the substance whenever they decided, as the study was intended to be observational. However, most of the participants waited until they had the result of the laboratory analysis to proceed with the self-administration trial. The self-administration trial started with the users filling the drug effect baseline questionnaire (Q3a), and then, they consumed the reported substance and filled the drug effect questionnaire (Q3b) 24 hours after filling the baseline questionnaire (Q3a). The links to these questionnaires were available for all participants in the forum, and the veracity of the information was ensured by asking information only available to each participant, such as the sample code of the reported sample.

Study recruitment began in August 2019 and ended in November 2020. In August 2020, the first participant concluded the 1-year follow-up.

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## IRB-approved protocol changes during the study:

The protocol has been subjected to amendments twice, both approved by the IRB of the institution (Clinical Research Ethics Committee-IMIM).

The first amendment, submitted in January 2019, reported the following changes in the protocol:

1. Minor changes in the study advertisement sheet, participant information sheet, and IC form
2. The assessment of inclusion criteria was no longer done by the community moderators and was entirely assessed by self-reporting on online questionnaires
3. An increase in the required sample quantity to be sent to the laboratory from 30 to 50 mg by default, accepting exceptions depending on the substance potency
4. Addition of a key measurement timepoint at baseline before ingesting the substance.

The second amendment, submitted in November 2019, reported the following changes in the protocol:

1. Unblinding of the research team to the participant behavior and participation
2. Addition of an optional timepoint for data collection in the reporting of the subjective effects of the reported substances
3. Extension of the duration of the study from 6 months to 1 year for each participant
4. Reduction of the required age for inclusion from 21 to 18 years.

## Main outcomes:

The domains and measurements used in the study have been based in previous laboratory studies to maximize consistency in methodology. Also, some of the outcomes to assess the subjective effects of the ingested substances were specifically designed to balance the positive and the negative effects assessed. The balance was desired to reduce the bias present in most of previous literature of asking mainly about the negative effects of psychoactive substances.

The measurements typically used in psychopharmacological studies conformed one measurement instrument, and the other measurements designed to balance the effects constituted the second measurement instrument. Instrument 1 had 51 items, with most of them suggesting a negative connotation (24 of the items, 47.1%). On the other side, Instrument 2 had 42 items, with only 9 (21.4%) suggesting negative connotations.

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Origin of the used traditional visual analog scales and codification into positive, negative, and neutral effects of both traditional and newly developed visual analogue scales (VAS). More information regarding the origin of Instrument 1 and the creation of Instrument 2 can be found in **annex 7**.

When comparing both instrument's connotations the new items have more positive connotations than the items with reference in literature. This is intentionally, to balance the quantity of items with negative connotations of the instrument 1.

## Data collection:

The study data was collected using a Columbia University Q*ualtrics*[1] license, a well-known questionnaire platform compliant with the guidelines to store sensible information about research participants. The questionnaires were adapted to be answered from both computers and smartphones.

### Questionnaires:

The screening questionnaire (Q0) and the sociodemographic questionnaire (Q1) collected self-reported information about the participants' medical and drug history, psychosocial situation, and beliefs and behaviors related to drugs.

The subjective effects of drugs were assessed via visual analog scales, using the same parameters used in most laboratory studies to determine the subjective effects of drugs. The main outcome was the difference in milimeters from the drug effect questionnaire (Q3b) at 24 hours (referring to the peak experience) and the baseline questionnaire (Q3a).

Each questionnaire also had at least one validity entry to be filled by the researcher directly using the Qualtrics database. There were no automated consistency or completeness checks before the questionnaire was submitted, other than the validation criteria for certain questions. For example, the question sample code reported could not be submitted if the answer was not a 5-digit number.

[1] https://www.qualtrics.com/es

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The participants could go back through the questionnaire, but once submitted, they could not change their answers. A summary of the answers was not displayed either before or after submission. Two options were available to change the participants' answers if they were incorrect according to the participant or not valid according to the researcher. If the change was small, the researcher could just edit the participant response in the Qualtrics database according to the correct response provided by the participant using email or the platform's private messaging system. If the changes were relevant, the researcher could mark the questionnaire as invalid and provide another link to the participant.

Public links to copies of the used questionnaires can be found in the references cited below:

1. Q0 screening questionnaire("Q0 Screening questionnaire," n.d.): contained a total of 37 questions
2. Q1 sociodemographic questionnaire ("Q1 Sociodemographic and drug use questionnaire," n.d.): contained a maximum of 351 questions, with an expected average per participant of 50, due to adaptive questioning and questionnaire logic
3. Q2 sample submission questionnaire ("Q2 sample characteristics," n.d.): contained a total of 21 question
4. Q3a baseline drug effect questionnaire ("Q3a Experience report baseline," n.d.): contained a total of 12 questions
5. Q3b drug effect questionnaire given 24 hours after drug administration ("Q3b Drug effects assessment," n.d.): contained a total of 39 questions
6. Q4 1-year follow-up questionnaire ("Q4 follow up questionnaire," n.d.): contained a maximum of 351 questions, with an expected average per participant of 50, due to adaptive questioning and questionnaire logic.

The number of pages and items on each page were optimized automatically by Qualtrics software and varied according to the screen size used to answer. The possibility to answer the questionnaires comfortably from the smartphone was assessed as essential by the research team.

More details regarding the visual analog scales selected to assess the substances subjective effects can be found in **annex 7.**

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## Substance samples' analysis:

Preliminary sample identification was performed by GC/MS (Gas chromatography coupled to mass spectrometry) using an Agilent 7890B gas chromatograph coupled to a 5977A quadrupole mass spectrometer detector (Agilent). The gas chromatograph was fitted with a G4513A auto-sampler injector. Insert liners packed with salinized glasswool were used, and the injector and interface were operated at 280°C.

Samples were injected in split mode into a 0.25 milimetersfilm thickness (5% phenylmethylsilicone) column (HP-5MS, Agilent Technologies). Helium was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was initially maintained at 90°C for 2 minutes and programmed to reach 320°C at 20°C/min. It was finally maintained at 320°C for 9.5 minutes (total run time was 21.5 min). The mass spectrometer was operated in the electron impact ionization mode at 70 eV.

To confirm the mass spectra, 4 libraries were used: the Searchable Mass Spectral Library NIST (National Institute of Standards and Technology)/EPA (Environmental Protection Agency)/NIH (National Institute of Health) Mass Spectral Library, Data Version: NIST (National Institute of Standards and Technology) 14; Searchable Mass Spectral Library Version 2.3 ("SWGDRUG Mass Spectral Library," n.d.); Searchable Mass Spectral Library Cayman Spectral Library ("Home | Cayman Chemical," n.d.); and EC's internal mass spectral library.

Confirmation (when needed) was performed by liquid chromatography coupled to tandem MS (LC/MS/MS) using an Agilent 1100 series HPLC (High Performance Liquid Chromatography) chromatograph (Agilent Technologies) and an Esquire 3000 plus mass spectrometer MRM (Bruker Daltonic GmbH). Chromatography was performed using a Poroshell 120 EC-C18 column (100 milimeterslength×2.1 milimetersi.d., 2.7 milimetersparticle size) at 30°C. The mobile phases consisted of 1% formic acid  and 1% formic acid in methanol . The following gradient elution was used: at time 0 minute, 15% B was changed to 90% B in 7 minutes, held for 1 minute, and changed back to the initial conditions in 1 minute. Before injection of the next sample, the column was re-equilibrated for 7 minutes. The flow rate was 0.35 mL/min. The electrospray source was operated in the positive ionization mode. Product ions that were obtained by collision-induced dissociation allowed the MS/MS to be operated in the multiple reaction monitoring mode. The dwell time was set at 0.25 seconds. The desolvation gas was nitrogen set at 365°C and delivered at a flow rate of

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9 L/min. The capillary voltage was 3.90 kV, and the collision gas was helium. The Bruker Compass Hystar system software Version 3.2-SR2 was used for instrument control and identification.

### GRASP fòrum

Secondary data about the participants' discussions on the study platform were supported by a licensed discourse (Civilized Discourse Construction Kit, Inc.) account and the software used to build the platform. Qualtrics (SAP Global Corporate Affairs) data were downloaded for analysis, which was conducted using the institutionally licensed Microsoft Excel (Microsoft Corporation) from the IMIM and R, which is a free software environment for statistical computing and graphic that does not require a license.

## Data management and statistical analysis

Data was stored in Qualtrics software and was only used according to the goals of the study. Most of the data was entered directly by the participants, and the principal investigator (MG) screened every questionnaire for consistency,asking the participants for clarifications in case of suspected errors in data entry or reporting.

Data on participant performance was be entered manually by one researcher in an internal Excel database. No personal information was be stored other than the safe email address asked in the study advertisement and the nickname the participant chose to use in the forum. This ensured the maintenance of pseudo-anonymity, as information about the online persona was stored, but the link between the online identity and the real identity was be impossible to establish with the collected information. This procedure was designed according to the consistent recommendation from previous research (Aldridge, Stevens, & Barratt, 2017; Barratt, 2011; Chiauzzi et al., 2013; Sutherland et al., 2017).

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Data was managed and processed using Qualtrics software and initially analyzed using Excel by the research team. At the same time, an independent and blind statistician used R software to analyze the same data and corroborate the obtained results. Only fully completed and valid questionnaires will be analyzed. The validity of the questionnaires was assessed by a research team member based only on the consistency and completeness of the participants' answers.

Data analysis procedures were intended to be mainly descriptive statistics, to maximize an adequate visualization of the data collected within the minimum space. In addition, as there was

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no control group, statistical tests were limited to potential comparisons to assess bias on the results, such as comparing data from participants who completed the study with participants who droped out or were excluded. However, no statistical corrections will be applied to adjust the representativeness of the sample, as there were no intention to make inferences dependent on external validity.

The nonparametric Wilcoxon signed-rank analysis were used to perform a mean comparison of the quantitative data and the chi-square test for qualitative data. In addition, factor analysis was attempted to study the relationship between all the visual analog scales used to assess the subjective drug effects.

The data analysis focused on the outcomes of the participants who completed the study. Data entries containing evident errors or inconsistent information were discarded and accounted for in the results section. The raw data was screened for duplicate responses that may result from data glitches or respondents completing the survey more than once (either accidentally or intentionally). A key validity issue for web surveys is ensuring that participants only complete the survey once. Also, to ensure data consistency, questions which should be dependent on an answer to an earlier question are cleaned according to sets of rules; for example, if a respondent reports first trying a substance through an online drug market but does not report ever use of LSD in the earlier drug screen, the later data are removed, given primacy to the accuracy of the earliest response. (Barratt et al., 2017).

## Systematic literature review:

A systematic literature review was performed for each substance that was correctly sent, analyzed, ingested, and reported. The literature review was included while the data was being analyzed, to show the relevance of the presented results. All searches were conducted in the *pubmed* database during April 2021.

The search terms were (for each of the 40 substances correctly analyzed and reported):

1. The standard chemical nomenclature
2. Up to three commonly used scientific abbreviations or street names. Date of the search.

The selection criteria for the articles selected were: Inclusion criteria were:

- Studies written in English.
- Studies referring to the analyzed substance as a new psychoactive substance.

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- Studies presenting original data on human subjects or their biological samples.

Exclusion criteria were:

- Publications in which another publication from the same authors was found in the latter day, and on which the initial dataset was included along new original data.
- Publications where the same dataset was presented.

The results that yielded for each substance were assessed by one researcher, excluding only the ones that did not meet the inclusion criteria. The results were recorded in excel. A senior researcher then checked the included papers, excluding the ones that met exclusion criteria, resulting in the total number of selected studies for each substance.

The selected studies were then classified into 5 previously defined categories, according to their goals and methodology:

1. Studies that made analytical characterization of a substance using one particular methodology. Samples were biological matrices or inorganic powder.
2. Studies that made prevalence or epidemiological estimations analyzing biological matrices or inorganic substances (e.g. urine, wastewater compounds).
3. Studies that analyzed already existing data from online forums.
4. Studies that used online surveys and interviews to gather information.
5. Studies that reported the effects of the substances on humans, including case reports, cohort studies and clinical trials.

Independently of the previous classification, the studies that reported subjective effects from the selected substances were identified. From these studies, the ones that also provided analytical confirmation of the reported substance were identified.

Both independent classifications were assessed independently by two researchers, obtaining the same results.

These were the terms used to search for each substance:

1. **3-MMC** or 3-Methilmethcathinone or metaphedrone, or 2-(Methylamino)-1-(3-methylphenyl)propan-1-one
2. **4-HO-MET** metocin or methylcybin or 3-{2-[Ethyl(methyl)amino]ethyl}-1H-indol-4-ol
3. **4-HO-MiPT** or miprocin or 3-{2-[methyl(propan-2-yl)amino]ethyl}-1*H*-indol-4-ol

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4. **2-FDCK** or Fluoroketamine or 2-Fluorodeschloroketamine or 2-(2-Fluorophenyl)-2-methylamino-cyclohexanone
5. **C-D** or dimoxamine or BL-3912 or 1-(2,5-Dimethoxy-4-methylphenyl)butan-2-amine
6. **Methallylescaline** or 2-{3,5-dimethoxy-4-[(2-methylprop-2-en-1-yl)oxy]phenyl}ethanamine
7. **2C-T-7** or Blue Mystic or 2-[2,5-Dimethoxy-4-(propylsulfanyl)phenyl]ethan-1-amine
8. **2C-E** or Aquarust or 2-(4-Ethyl-2,5-dimethoxyphenyl)ethanamine
9. **2C-D** or 2C-M or 2-(2,5-Dimethoxy-4-methylphenyl)ethan-1-amine
10. **2C-B** or Bromo Mescaline or 2,5-dimethoxy-4-bromophenethylamine or 2-(4-Bromo-2,5-dimethoxyphenyl)ethanamine
11. **3-MeO-PCP** or 3-Methoxyphencyclidine or 1-[1-(3-methoxyphenyl)cyclohexyl]-piperidine
12. **ETH-LAD** or N-Ethyl-nor-LSD or (6a*R*,9*R*)-*N*,*N*-diethyl-7-ethyl-4,6,6a,7,8,9-hexahydroindolo-[4,3-*fg*]quinoline-9-carboxamide
13. **25E-NBOH** or 2C-E-NBOH or NBOH-2C-E" or "2-({[2-(4-ethyl-2,5-dimethoxyphenyl)ethyl]amino}methyl)phenol"
14. **2C-C** or 2-(4-Chloro-2,5-dimethoxyphenyl)ethan-1-amine or 4-Chloro-2,5-dimethoxyphenethylamine
15. **2-FA** or 2-Fluoroamphetamine or 1-(2-Fluorophenyl)propan-2-amine
16. **2-FMA** or 2-Fluoromethamphetamine or (RS)-1-(2-Fluorophenyl)-N-methylpropan-2-amine
17. **3-FPM** or 3-Fluorophenmetrazine or 3-FPH or PAL-593 2-(3-Fluorophenyl)-3-methylmorpholine
18. **3-HO-PCE** or 3-Hydroxyeticyclidine or *3-[1-(Ethylamino)cyclohexyl]phenol*
19. **3-MeO-PCE** or 3-Methoxyeticyclidine or Methoxieticyclidine orN-Ethyl-1-(3-methoxyphenyl)cyclohexan-1-amine
20. **4-AcO-MALT** or 4-Acetoxy-MALT or 3-(2-(allyl(methyl)amino)ethyl)-1H-indol-4-yl acetate
21. **4-AcO-MET** or 4-Acetoxy-MET or Metacetin or 3-{2-[Ethyl(methyl)amino]ethyl}-1H-indol-4-yl acetate
22. **'-Chlorodiazepam** or 7-Chloro-5-(4-chlorophenyl)-1-methyl-3H-1,4-benzodiazepin-2-one
23. **4F-MPH** or 4-Fluoromethylphenidate or 4-FMPH or Methyl 2-(4-fluorophenyl)-2-(piperidin-2-yl)acetate
24. **5-MeO-DALT** or N,N-Diallyl-5-methoxytryptamine or N-[2-(5-methoxy-1H-indol-3-yl)ethyl]-N-(prop-2-en-1-yl)prop-2-en-1-amine

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25. **5-MeO-DMT** or 5-methoxy-N,N-dimethyltryptamine or O-methyl-bufotenin or 2-(5-Methoxy-1H-indol-3-yl)-N,N-dimethylethanamine
26. **5-MeO-MiPT** or 5-Methoxy-N-methyl-N-isopropyltryptamine or N-[2-(5-methoxy-1H-indol-3-yl)ethyl]-N-methylpropan-2-amine
27. **6-APB** or 6-(2-Aminopropyl)benzofuran or Benzofury or 1-(1-Benzofuran-6-yl)propan-2-amine
28. **alpha-PHP** or α-PHP or α-Pyrrolidinohexanophenone or (RS)-1-Phenyl-2-(pyrrolidin-1-yl)hexan-1-one
29. **AMT** or alpha-Methyltryptamine or α-Methyltryptamine or 1-(1H-Indol-3-yl)propan-2-amine
30. **BOD** or 4-methyl-2,5,β-trimethoxyphenethylamine or 2-(2,5-Dimethoxy-4-methylphenyl)-2-methoxyethan-1-amine
31. **DOC** or 2,5-Dimethoxy-4-chloroamphetamine or 1-(4-Chloro-2,5-dimethoxyphenyl)propan-2-amine
32. **DOF** or 2,5-Dimethoxy-4-fluoroamphetamine or 1-(4-Fluoro-2,5-dimethoxyphenyl)propan-2-amine
33. **DOiP** or 2,5-Dimethoxy-4-isopropylamphetamine or 1-[2,5-Dimethoxy-(propan-2-yl)phenyl]propan-2-amine
34. **DOPr** or 2,5-Dimethoxy-4-propylamphetamine or 1-(2,5-Dimethoxy-4-propylphenyl)propan-2-amine
35. **MEAI** or 5-methoxy-2-aminoindane or 5-MeO-AI or Chaperon
36. **Methoxetamine** or 3-MeO-2'-Oxo-PCE or MXE or Mexxy or (R/S)-2-(3-Methoxyphenyl)-2-(ethylamino)cyclohexanone
37. **MiPT** or Methylisopropyltryptamine or N-Methyl-N-isopropyltryptamine or N-[2-(1H-indol-3-yl)ethyl]-N-methylpropan-2-amine
38. **MXPr** or methoxpropapine or 2-Oxo-3'-methoxy-PCPr or 2-(3-Methoxyphenyl)-2-(propylamino)cyclohexanone
39. **O-Desmethyltramadol** or Desmetramadol or O-DSMT or 3-(2-((dimethylamino)methyl)-1-hydroxycyclohexyl)phenol
40. **O-PCE** or 2'-Oxo-PCE or N-ethyldeschloroketamine or eticyclidone or 2-(ethylamino)-2-phenyl-cyclohexanone

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## Dissemination policy

All individual and collective data, as well as all the results derived from the study, will be strictly protected, and will only be published with the authorization of the principal investigator and the affected participants. All relevant findings will be sent for publication in suitable journals and submitted for presentation at relevant scientific meetings.

The funding organizations will have no role in the publication process. In addition, data without identifiable information will be shared with study participants after assessment and approval by the research team.

Finally, all results published in the scientific literature will also be made available in lay language to the communities of origin of the participants. Authorships in the publications will be determined by the amount of scientific and academic contributions of the members of the research team, including external collaborators. There are no plans to make the data sets publicly available.

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# Results

## Study feasibility:

The main result of this study is that its potentially complex execution proved to be achievable. To our knowledge, this is the very first study published using methodology

As shown in *figure 1*, 184 candidates were screened. From those, 17 became fully committed participants. Committed participants were defined as those who completed at least one self-administration trial (SAT). Despite losing 91% of the candidates, the compliance and committed of the participants allowed for the collection of 64 valid self-administration trials (SATs) during the data collection period, which was from August 2019 to October 2020 (14 months). It must be noted that 34.4% of the self-administration trials (SATs), come from the same participant.

Six candidates were excluded, as also shown in figure 1. The reasons to exclude these 6 candidates were diverse: One reported being underage, one failed to send a valid informed consent, one reported a psychiatric history that was assessed as severe dysfunction and was referred to their mental health services. Finally, three candidates reported no previous use of NPS

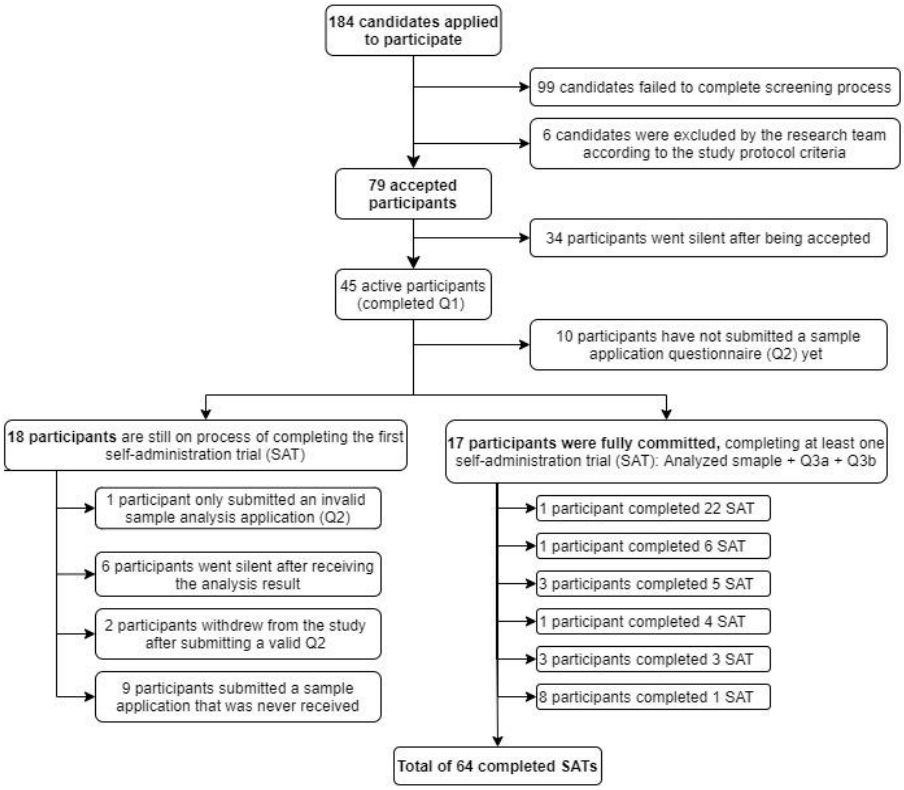


*Figure 1: Participants' flow-chart*

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and/or no previous plans to use them in the following year, so they were excluded to preserve the naturalistic orientation of the study.

The second finding of the study is that it is possible to collect a significant part of the intended data, but changes in the study design and execution may be necessary to obtain all the intended data.

As shown in figure 2, 136 samples were submitted for analysis, from which 92 (54+38) were received and analyzed, providing valuable information of the NPS used by the participants, their expectations and laboratory results, during the data collection period. Additionally, from these samples, 54 had a valid report on their effects, resulting on the same amount of 64 self-administration trials (SATs) already reported in figure 1.

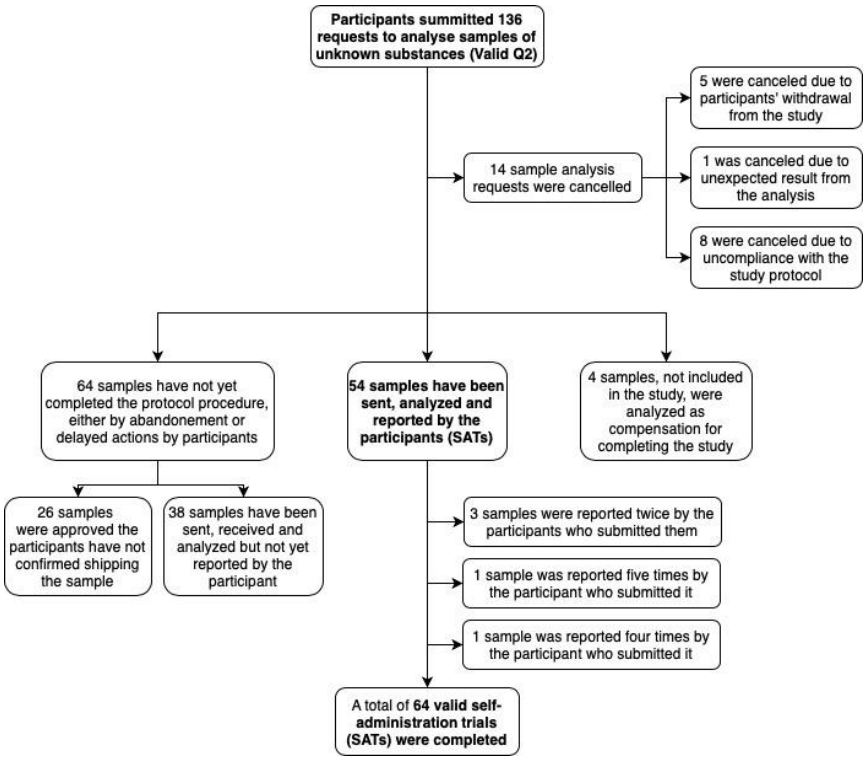


*Figure 2: Substances' samples' flow-chart*

The main questionnaire characteristics are shown on Table 1.

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*Table 1: Characteristics of the used questionnaires*

| Qs | Nº of submitted Qs | Nº of valid Qs answered | Valid Qs compl. rate | Number of questions | Average duration to complete in (min) | Standard deviation (min) |
|---|---|---|---|---|---|---|
| Q0 | 99 | 79 | 79.8% | 37 | 13.4 | 11.5 |
| Q1 | 46 | 45 | 97.8% | 351 | 19.7 | 19.4 |
| Q2 | 145 | 136 | 93.8% | 21 | 6.4 | 8.9 |
| Q3a | 87 | 72 | 82.8% | 12 | 10.6 | 6.2 |
| Q3b | 82 | 66 | 80.5% | 39 | 23.6 | 14.8 |
| Q4 | 15 | 12 | 80.0% | 351 | 36.6 | 30.7 |

Each participant answered a maximum of 800 survey questions along the different questionnaires, from which a significant proportion was not displayed to them according to their precious answers. Details are shown on *Table 1*. Five fully committed participants may have answered less questions due to the failure to fill the Q4 follow-up questionnaire, but 7 of those participants completed more than one self-administration trial (SAT). The participants spent, on average, between 10 and 30 minutes to answer the questionnaires.

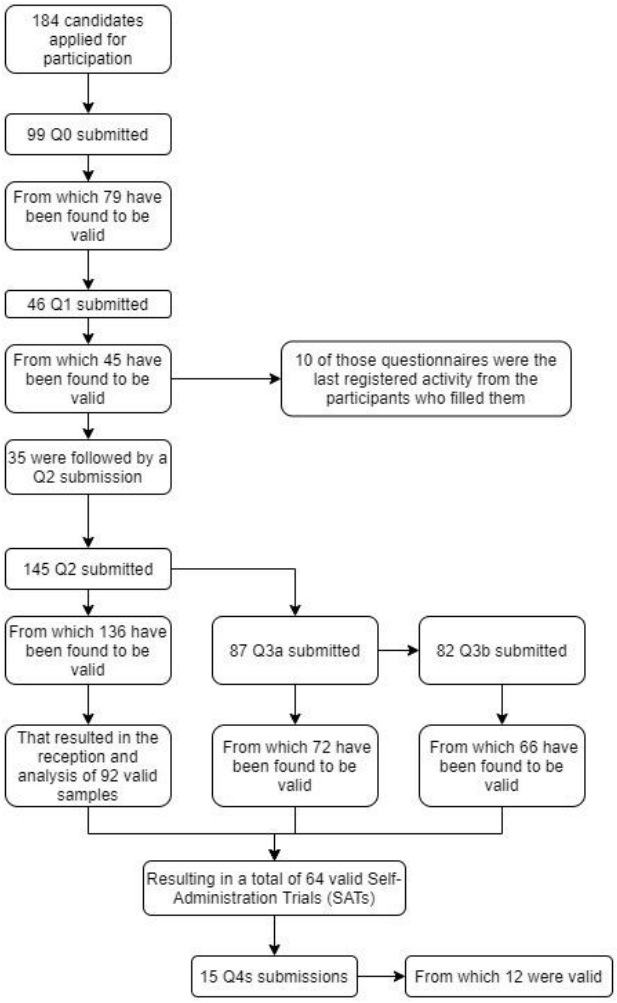


*Figure 3: Questionnaires' flow-chart*

The mean time that passed from the baseline questionnaire (Q3a) and the drug effects questionnaire (Q3b) was of 24.1 hours, with an interquartile range of 15.2 hours. Up to 71% of the self-administration trials (SATs) were filled within the 24 hours following the drug self-administration.

Additionally, the integrated information of participants, substances' samples and questionnaires can be found in Figure 3.

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Finally, an unanticipated finding, shown on *figure 4*, has been that data collection was not significantly altered by the COVID-19 pandemic or the different lockdowns that took place in the same period.

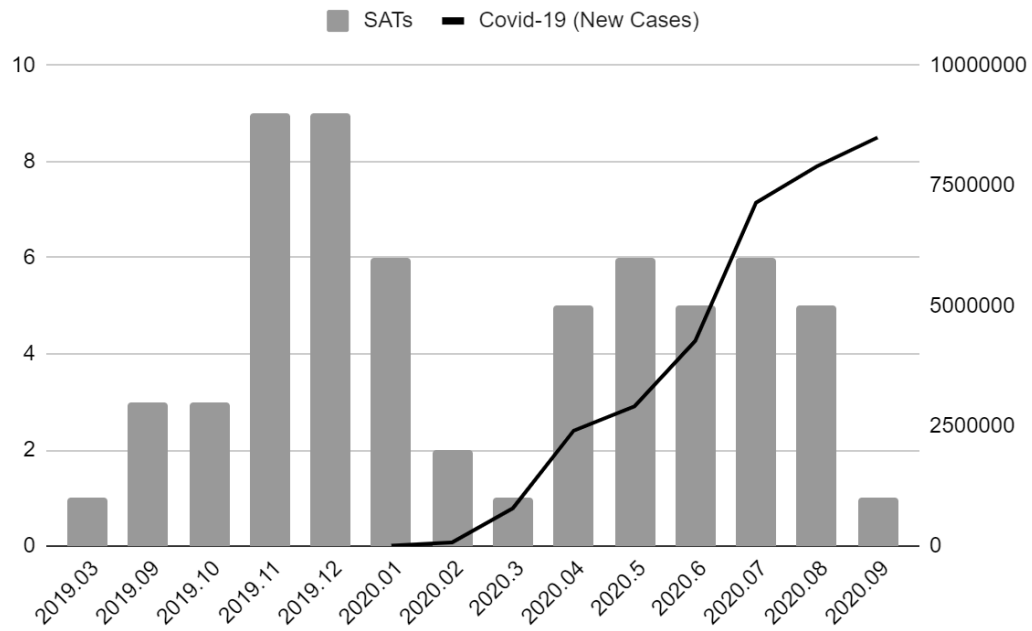


Fig.4: Self administration trials over time and over number of detected COVID cases worldwide

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## Participants' characteristics.

The second main finding of the study was that the recruited participants met the defining characteristics of e-psychonauts. As shown on *table 2*, they reportedly had tried an average number of 23.2 NPSs, with a clear tendency to psychedelics and mind-exploring substances. Only one committed participant had tried more stimulants, which correlated negatively with psychedelic use in all participants (*see figure 2*). Also, in addition to the time spent filling the questionnaires, they spent on average 5 and a half hours participating on the study online platform. The interactions in the study platform were indistinguishable from the interactions that occur on the main online drug forums.

| Descriptive statistics | | Top 5 used NPS | n |
|---|---|---|---|
| Mean | 23.2 | 4-HO-MET | 13 |
| St Dev | 19.9 | AL-LAD | 11 |
| Q1 | 8 | 1P-LSD | 10 |
| Q3 | 31 | 5-MeO-MiPT | 10 |
| Median | 20 | 4-Acetoxy-DMT | 9 |

*Table 2: Characteristics of previous NPS use by committed participants*

As shown in *table 3 and table 4*, the participants were mainly white, 30-year-old males with annual incomes around 30.000 EUR. typically associated with solvent personal economies. All participants had at least completed mandatory school, with 90 % having higher education. A third had a bachelor's degree or similar and 10% had a masters or an MBA.

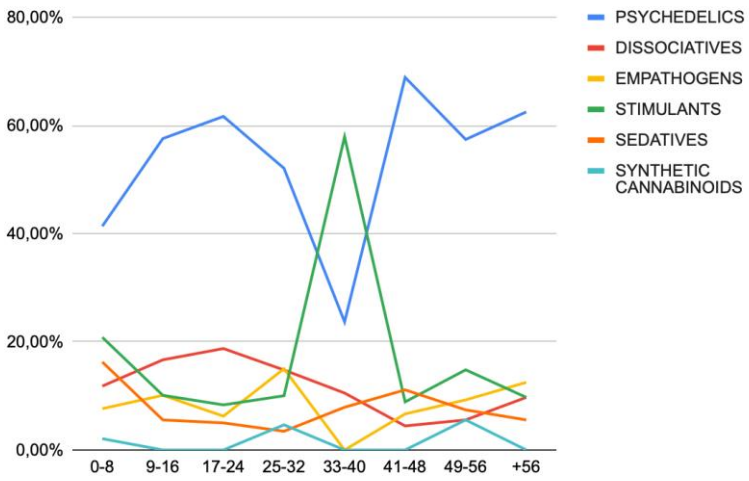


Fig. 3: Relative amount of reportedly used by the total number of NPS tried NPS before entering the study.

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Table 3: Quantitative sociodemographics of non-commited and committed participants.

| | Non-committed participants (n=28) | | Committed participants (n=17) |
|---|---|---|---|
| Age | | | |
| Mean | 28.4 | | 27.8 |
| Median | 26 | | 26 |
| Q1 | 22 | | 22 |
| Q3 | 31.3 | | 32 |
| *p-value* | | 0.79 | |
| Height | | | |
| Mean | 177.6 | | 179.3 |
| Median | 180 | | 180 |
| Q1.1 | 173.8 | | 175.8 |
| Q3.1 | 184 | | 183 |
| *p-value* | | 0.48 | |
| Weight | | | |
| Mean | 75.9 | | 75.2 |
| Median | 72.5 | | 75 |
| Q1.2 | 64.8 | | 70 |
| Q3.2 | 82.5 | | 80 |
| *p-value* | | 0.85 | |
| Annual income | | | |
| Mean | 27713.4 | | 85203.4 |
| Median | 15244.6 | | 23000 |
| Q1.3 | 9659.5 | | 4829.8 |
| Q3.3 | 40000 | | 72000 |
| *p-value* | | 0.18 | |

| | Non-Comitted. participants (n=28) | | Comitted participants (n=17) | | *p-value* |
|---|---|---|---|---|---|
| Forum | n | % | n | % | |
| Bluelight | 4 | 14.3 | 4 | 23.5 | 0.70 |
| Reddit | 24 | 85.7 | 13 | 76.5 | |
| Sex | n | % | n | % | |
| Female | 2 | 7.1 | 0 | 0 | 0.70 |
| Male | 26 | 92.9 | 17 | 100 | |
| Gender | n | % | n | % | |
| Femenine | 3 | 10.7 | 1 | 5.9 | 0.51 |
| Masculine | 24 | 85.7 | 14 | 82.3 | |
| Other | 1 | 3.6 | 2 | 11.8 | |
| Ethnicity | n | % | n | % | |
| Caucasian | 26 | 92.8 | 15 | 88.2 | 0.49 |
| Other | 2 | 7,2 | 2 | 11.2 | |
| Studies | n | % | n | % | |
| Primary school | 0 | 0 | 0 | 0 | |
| Mandatory school | 3 | 10.7 | 1 | 5.9 | |
| Advanced secondary | 10 | 35.6 | 3 | 17.7 | |
| Associate's degree level | 1 | 3.8 | 2 | 11.8 | |
| Bachelor's degree or | 9 | 32.1 | 8 | 47.1 | 0.45 |
| Masters degree or MBA | 3 | 10.7 | 3 | 17.7 | |
| Ph.D. | 2 | 0 | 0 | 0 | |

Table 4 Qualitative sociodemographic variables of committed non-committed participants.

The participants came only from two of the four forums where recruitment was intended. One of the forums where recruitment was expected (drugs-forum), rejected to promote the study among its members after the main moderator discussed the possibility with the principal investigator. Another forum (DNStarsVIP) was closed by the authorities at the beginning of the recruitment process.

*Table 5* shows how the participants reported very sporadic use of traditional drugs (except from cannabis and ayahuasca). Also, the table shows statistically significant differences in the use of traditional drugs between the participants who committed to the study and those who did not. The committed participants reported having different patterns of use from 9 out of the 35 drugs and groups of substances assessed. From these 9 different substance use patterns, the majority (n=4) could be comprised in the opioid family.

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| | Non-Committed participants (n=28) | | | | **Committed participants (n=17)** | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | In the last month | In the last year | More than 1 year ago | Never | **In the last month** | **In the last year** | **More than 1 year ago** | **Never** | *p-value* |
| Alcohol | 22 | 2 | 3 | 1 | 15 | 1 | 1 | 0 | 0,94 |
| Tobacco | 13 | 4 | 7 | 4 | 8 | 4 | 5 | 0 | 0,51 |
| Cannabis | 18 | 6 | 4 | 0 | 11 | 3 | 2 | 1 | 0,75 |
| Caffeine | 27 | 1 | 0 | 0 | 16 | 1 | 0 | 0 | 0,96 |
| NPS | 22 | 5 | 1 | 0 | 13 | 4 | 0 | 0 | 0,94 |
| Amphetamine | 11 | 10 | 3 | 4 | 1 | 7 | 7 | 2 | 0,04* |
| MDMA | 3 | 12 | 8 | 5 | 1 | 11 | 3 | 2 | 0,64 |
| Cocaine | 1 | 5 | 10 | 12 | 1 | 5 | 4 | 7 | 0,74 |
| Methamphetamine | 2 | 4 | 2 | 20 | 1 | 1 | 2 | 13 | 0,86 |
| Methylphenidate | 5 | 13 | 10 | 0 | 5 | 6 | 6 | 0 | 0,81 |
| Over-the counter meds | 5 | 5 | 2 | 16 | 3 | 2 | 12 | 0 | <0.001* |
| Diet.pills | 9 | 4 | 2 | 13 | 3 | 3 | 2 | 9 | 0,76 |
| Other.stim | 8 | 5 | 1 | 9 | 2 | 4 | 4 | 4 | 0,18 |
| Heroin | 2 | 2 | 24 | 0 | 1 | 2 | 14 | 0 | 0,95 |
| Morphine | 1 | 4 | 6 | 17 | 1 | 2 | 14 | 0 | <0.001* |
| Opium | 1 | 1 | 4 | 22 | 2 | 6 | 9 | 0 | <0.001* |
| Codeine | 2 | 8 | 10 | 8 | 3 | 9 | 5 | 0 | 0,08 |
| Methadone | 1 | 27 | 0 | 0 | 1 | 16 | 0 | 0 | 0,96 |
| Fentanyl | 2 | 3 | 23 | 0 | 1 | 16 | 0 | 0 | <0.001* |
| Other.opi | 6 | 2 | 4 | 11 | 2 | 2 | 9 | 0 | <0.008* |
| LSD | 4 | 9 | 4 | 11 | 1 | 10 | 2 | 4 | 0,41 |
| Mescaline | 3 | 3 | 16 | 6 | 2 | 3 | 11 | 1 | 0,61 |
| Ayahuasca | 1 | 1 | 24 | 2 | 14 | 2 | 0 | 0 | <0.001* |
| Psilocybin | 4 | 9 | 4 | 11 | 1 | 8 | 2 | 6 | 0,78 |
| 2C-B | 3 | 8 | 11 | 6 | 2 | 9 | 3 | 3 | 0,38 |
| Other.psy | 4 | 4 | 9 | 4 | 2 | 3 | 3 | 4 | 0,72 |
| Ketamine | 7 | 8 | 9 | 4 | 6 | 5 | 4 | 2 | 0,89 |
| PCP | 2 | 2 | 23 | 1 | 1 | 16 | 0 | 0 | <0.001* |
| Poppers | 1 | 2 | 18 | 7 | 1 | 2 | 8 | 6 | 0,71 |
| Nitrous oxide | 9 | 4 | 10 | 5 | 4 | 8 | 5 | 0 | 0,09 |
| Benzodiazepines | 10 | 8 | 4 | 6 | 5 | 6 | 3 | 3 | 0,94 |
| GHB/GBL | 3 | 5 | 1 | 19 | 5 | 1 | 2 | 9 | 0,24 |
| Antihistamines | 7 | 7 | 5 | 9 | 4 | 2 | 2 | 9 | 0,57 |
| Barbiturates | 1 | 3 | 6 | 18 | 2 | 4 | 11 | 0 | <0.001* |
| Other.sed | 3 | 25 | 0 | 0 | 17 | 0 | 0 | 0 | <0.001* |

Table 5 Committed and non-committed participants history of drug use (excluding NPS)[2].

When analyzing the harm reduction strategies (shown in table 6), no statistically significant differences can be found between committed and non-committed participants. The general tendency is that most of the study participants report using most of the asked harm reduction strategies. All participants report using at least 3 of those strategies and 70% of the participants that use drug checking report changing their behavior as a result, either adjusting the dose or discarding the substance. Also, it's worth noting that all the participants used internet to learn about the psychoactive substances they intend to use.

[2] *Means statistical differences between the pattern of use of committed and non-committed participants.

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| | Non committed Participants (n=28) | | Committed Participants (n=17) | | |
|---|---|---|---|---|---|
| | n | % | n | % | *p-value* |
| Discarded NPS due forum info | 11 | 39.3 | 10 | 58.8 | 0.33 |
| Use of drug checking | 14 | 50.0 | 11 | 64.7 | 0.51 |
| -from which NPS discarded | 8 | 57.1* | 8 | 72.7* | 0.69 |
| -from which NPS adjusted dose | 7 | 50* | 3 | 27.3* | 0.46 |
| -from which almost 1 answer | 10 | 71.4* | 8 | 72.7* | |
| Avoid use without drug testing | 12 | 42.9 | 7 | 41.2 | 1 |
| Ask friend who tried same NPS | 12 | 42.9 | 9 | 53.0 | 0.73 |
| Ask dealer about NPS details | 12 | 42.9 | 3 | 17.7 | 0.18 |
| Consume a test dose | 24 | 85.7 | 11 | 64.7 | 0.2 |
| Examine the substance | 24 | 85.7 | 13 | 76.5 | 0.7 |
| Assess quality based on price | 10 | 35.7 | 4 | 23.5 | 0.6 |
| Use internet to educate before | 28 | 100.0 | 17 | 100.0 | |
| Regeant testing | 19 | 67.9 | 12 | 70.6 | 1 |
| Other | 5 | 17.9 | 6 | 35.3 | 0.34 |
| 3 or more | 28 | 100.0 | 17 | 100.0 | |
| 4 or more | 26 | 92.7 | 17 | 100.0 | |
| 5 or more | 20 | 71.4 | 15 | 88.2 | |
| 6 or more | 18 | 64.3 | 8 | 47.1 | |
| 7 or more | 12 | 42.9 | 7 | 41.2 | |
| 8 or more | 7 | 25.0 | 5 | 29.4 | |

**Table 6**: Reported Harm reduction strategies by committed and non-committed participants.

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## Substance sample characteristics

The submitted samples that were ingested and reported are compared with the ones that were submitted without being ingested and reported by the participants. Their origin, type of dealer, and means of acquisition did not show statistically significant differences among the two groups using Chi tests to compare. The samples were bought mainly from Europe (n=79), using trusted dealers (n=67), and using the regular surface web (86,79%). Details are shown on Table 7.

Another finding to highlight is that the participants suspected higher adulteration of their samples than what was found (n=23 vs n=14).

In Tables 8 and 9 is shown the expected adulteration and the actual adulteration of the samples received. In the first table, the difference between the samples of the participants of the self-administration trials (SATs) and the non-participants are statistically significant. In the second table it is shown that the relation between expected adulteration and the actual adulteration is significant too.

| | Samples with at least one SAT (n=54) | | Samples without any SAT (n=82) | | All (n=136) | |
|---|---|---|---|---|---|---|
| Origin | *p-value = 1* | | | | | |
| Europe | 33 | 61,1% | 46 | 56,1% | 79 | 58,1% |
| Belgium | 0 | 0,0% | 1 | 1,2% | 1 | 0,7% |
| Eastern | 3 | 5,6% | 0 | 0,0% | 3 | 2,2% |
| Finland | 0 | 0,0% | 2 | 2,4% | 2 | 1,5% |
| France | 0 | 0,0% | 1 | 1,2% | 1 | 0,7% |
| Germany | 4 | 7,4% | 1 | 1,2% | 5 | 3,7% |
| Netherlands | 21 | 38,9% | 38 | 46,3% | 59 | 43,4% |
| Spain | 4 | 7,4% | 1 | 1,2% | 5 | 3,7% |
| Sweden | 0 | 0,0% | 1 | 1,2% | 1 | 0,7% |
| UK | 1 | 1,9% | 1 | 1,2% | 2 | 1,5% |
| America | 16 | 29,6% | 28 | 34,1% | 44 | 32,4% |
| Canada | 8 | 14,8% | 11 | 13,4% | 19 | 14,0% |
| USA | 8 | 14,8% | 17 | 20,7% | 25 | 18,4% |
| Asia | 3 | 5,6% | 3 | 3,7% | 6 | 4,4% |
| China | 3 | 5,6% | 3 | 3,7% | 6 | 4,4% |
| Others | 2 | 3,7% | 5 | 6,1% | 7 | 5,1% |
| Self made | 0 | 0,0% | 1 | 1,2% | 1 | 0,7% |
| Unknown | 1 | 1,9% | 3 | 3,7% | 4 | 2,9% |
| Not | 1 | 1,9% | 1 | 1,2% | 2 | 1,5% |
| Vendor | *p-value = 0.14* | | | | | |
| Friend | 2 | 3,7% | 7 | 8,5% | 9 | 6,6% |
| Other | 5 | 9,3% | 2 | 2,4% | 7 | 5,1% |
| Trusted Dealer | 30 | 55,6% | 37 | 45,1% | 67 | 49,3% |
| Unknown Dealer | 17 | 31,5% | 34 | 41,5% | 51 | 37,5% |
| Not answered | 0 | 0,0% | 2 | 2,4% | 2 | 1,5% |
| Setting | *p-value = 0.44* | | | | | |
| Meeting up with | 0 | 0.0% | 3 | 3.7% | 3 | 2.2% |
| On the Clearnet | 36 | 66.7% | 56 | 68.3% | 92 | 67.6% |
| On the Darknet | 12 | 22.2% | 13 | 15.9% | 25 | 18.4% |
| On the Street | 0 | 0.0% | 1 | 1.2% | 1 | 0.7% |
| Other | 5 | 9.3% | 8 | 9.8% | 13 | 9.6% |
| Not answered | 1 | 1.9% | 1 | 1.2% | 2 | 1.5% |

**Table 7.** Characteristics of all the substances' samples' requests received grouped by the ones that have a self-administration trial (SAT) and the ones that have not.

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| | Sample submission request (Q2) with at least one SAT (n=54) | | Sample submission request (Q2) without any SAT (n=82) | | All sample submission requests (n=136) | |
|---|---|---|---|---|---|---|
| **Expected adulteration** | | | | | *p-value = 0.03** | |
| No | 50 | 92.6% | 62 | 75.6% | 112 | 82.4% |
| Yes | 4 | 4.4% | 19 | 23.2% | 23 | 16.9% |
| Unanswered | 0 | 0.0% | 1 | 1.2% | 1 | 0.7% |
| **Actual adulteration** | | | | | *p-value = 0.11* | |
| Adulterated | 2 | 3.7% | 6 | 7.3% | 8 | 5.9% |
| Expected | 50 | 92.6% | 32 | 39.0% | 82 | 60.3% |
| Not analyzed | 0 | 0.0% | 12 | 14.6% | 12 | 8.8% |
| Substituted | 2 | 3.7% | 4 | 4.9% | 6 | 4.4% |
| Unknown | 0 | 0.0% | 2 | 2.4% | 2 | 1.5% |
| Waiting sample arrival | 0 | 0.0% | 26 | 31.7% | 26 | 19.1% |

**Table 8.** Expected adulteration and actual adulteration of submitted NPS samples' requests grouped by the ones that have a self-administration trial (SAT) and the ones that have not.

| | | Substances' samples expected to be adulterated by participants (Q2) | |
|---|---|---|---|
| | | No | Yes |
| Substances' samples that contained other or another substance than the expected | Yes | 10 | 6 |
| | No | 70 | 11 |
| | | *p-value = 0.05* | |

**Table 9** Expected adulteration of the samples by the users compared to the adulteration detected in the NPS sample analysis[3].

In general, the participants expect their samples not to be adulterated or substituted (n=80; 82,5%). Moreover, we found statistical evidence that the relationship between their predictions about the analysis and the analysis result could not be explained by chance.

[3] Shows the statistical significance of the expected adulteration versus the actual adulteration. The categories of Adulterated, Substituted and Unknown from table 8 are grouped under the Adulterated tag. The samples waiting arrival and those not analyzed were not considered.

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| | Sample submission requests with at least one SAT (n=54) | Sample submission requests without any SAT (n=82) | All (n=136) |
|---|---|---|---|
| **Sample ingested before submission** | | | ***p-value = 0.97*** |
| No | 28 | 41 | 69 |
| Yes | 26 | 41 | 67 |
| **Administration route** | | | ***p-value = 1.00*** |
| Intravenous | 1 | 1 | 2 |
| Nasal | 7 | 6 | 13 |
| Oral | 16 | 27 | 43 |
| Other | 1 | 1 | 2 |
| Rectal | 0 | 2 | 2 |
| Sublingual | 1 | 1 | 2 |
| Vaporized | 0 | 3 | 3 |
| **Sample not ingested since submission until lab result given** | | | ***p-value = 0.12*** |
| No | 11 | 8 | 19 |
| Yes | 17 | 33 | 50 |

**Table 10.** Shows if the participants had tried or not the samples sent, the route of administration if they tried and the will to wait for the results if they have not tried the sample.

| | | **n** | **%** |
|---|---|---|---|
| Sample submission requests containing only the substance expected | | 82 | 60.3% |
| Sample submission requests without sample analysis | | 40 | 29.4% |
| Sample submission requests containing the expected and others | | 8 | 5.9% |
| *Expected and detected substance* | *Other substances found* | | *n* |
| DOF | DOiP | | 1 |
| 4-HO-MPT | Unknown substance | | 1 |
| bk-2C-B | Unknown substance | | 1 |
| 2C-T-2 | 2C-C | | 1 |
| Escaline | Unknown substance | | 1 |
| DOC | DOB | | 1 |
| Harmine | Harmaline | | 1 |
| 2-FA | Unknown substance | | 1 |
| Sample submission requests containing other substances | | 6 | 4.4% |
| *Expected substance* | *Laboratory result* | | *n* |
| 6-APB | 4-EMC | | 1 |
| 2c-ef | 2C-T-7 | | 1 |
| DCK | 2-OXO-PCE | | 1 |
| 2C-YN | 2C-D | | 1 |
| MiPLA | LSD and Iso-LSD | | 1 |
| BOHB | BOH-2CB | | 1 |
| Total sample submission requests | | 136 | 100% |

**Table 11**. Shows the sample submission requests received according to their laboratory analysis and the participant expectation of their composition

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In Table 10 can be seen that half of the participants had already tried the submitted substance before entering the study. However, most of the participants that had not tried the sample before sending it for analysis, waited for the analysis result before consuming the substance. The preferred route of administration was oral, followed by the intranasal, being the other routes used anecdotally.

*Table 11* shows that 93.8% of the analyzed substances contained what the user expected (excluding the 40 samples that were submitted for analysis but were not received or analyzed). Only 8 samples (8.3% of received samples) containing the expected substance and others and only 6 samples (6.3% of received samples) containing only other substances than the ones expected. In two occasions, it was impossible to identify the substance present in the sample sent. This was due to the lack of known patterns in the GC/MS libraries, and they may be still identified in the future using MNR spectroscopy.

| | Number of SATS (n=64) |
|---|---|
| ROA | |
| Intravenous | 2 |
| Oral | 47 |
| Rectal | 1 |
| Smoked | 1 |
| Snorted | 8 |
| Sublingual | 3 |
| Used precision scale to dose? | |
| No | 5 |
| Yes | 57 |
| Consumed more than once? | |
| No | 51 |
| Yes | 11 |
| Consumed after knowing the lab result? | |
| Yes | 39 |
| No | 23 |

Table 12: Self-administration trial (SAT) characteristics

In Table 12 we can see that most of the participants reporting on the self-administration of previously analyzed substances used a precision scale to measure the dose they intended to consume (n=57; 92%). Additionally, most of the participants only performed one self-administration in the reported session (n=51; 82.3%)

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## Self-administration trials (SATs)

The fourth main finding is that most self-administration trials (SATs) reported on different substances, having identified 40 different substances in 64 self-administration trials. From those trials, the majority (n=40; 62.5%) reported on different substances. More than one self-administration trial (SAT) was collected only for the following 12 substances: 3-MMC, 4-HO-MET, 4-HO-MiPT, 2-FDCK, 4C-D, Methallylescaline, 2C-T-7, 2C-E, 2C-D, 2C-B, 3-MeO-PCP and ETH-LAD.

See table 12 for further detail. The substances are listed according to the sample size, following a numeric-alphabetic order in case of equal sample size. The same order is maintained in table 14.

Most participants used a scale to dose the substance they used in the self-administration trial (SATs) (data from table 13). Also, and a clear majority waited until the laboratory analysis was available to proceed with the SAT.

Table 13: Laboratory results of the samples with valid self-administration trial (SAT)

| Lab result | SATs (n=64) per sample | SATs (n=64) per substance |
|---|---|---|
| 3-MMC | 2 | 6 |
| | 4 | |
| 4-HO-MET | 5 | 5 |
| 4-HO-MiPT | 1 | 4 |
| | 1 | |
| | 1 | |
| | 1 | |
| 2-FDCK | 1 | 3 |
| | 1 | |
| | 1 | |
| 4C-D | 2 | 3 |
| | 1 | |
| Methallylescaline | 1 | 3 |
| | 1 | |
| | 1 | |
| 2C-T-7 | 1 | 2 |
| | 1 | |
| 2C-E | 1 | 2 |
| | 1 | |
| 2C-D | 2 | 2 |
| 2C-B | 2 | 2 |
| 3-MeO-PCP | 1 | 2 |
| | 1 | |
| ETH-LAD | 1 | 2 |
| | 1 | |
| 25E-NBOH | 1 | 1 |
| 2C-C | 1 | 1 |
| 2-FA | 1 | 1 |
| 2-FMA | 1 | 1 |
| 3-FPM | 1 | 1 |
| 3-HO-PCE | 1 | 1 |
| 3-MeO-PCE | 1 | 1 |
| 4-ACO-MALT | 1 | 1 |
| 4-ACO-MET | 1 | 1 |
| 4'-chlorodiazepam | 1 | 1 |
| 4F-MPH | 1 | 1 |
| 5-MeO-DALT | 1 | 1 |
| 5-MeO-DMT | 1 | 1 |
| 5-MeO-MiPT | 1 | 1 |
| 6-APB | 1 | 1 |
| alpha-PHP | 1 | 1 |
| AMT | 1 | 1 |
| BOD | 1 | 1 |
| DOC | 1 | 1 |
| DOF | 1 | 1 |
| DOiP | 1 | 1 |
| DOPr | 1 | 1 |
| MEAI | 1 | 1 |
| Methoxetamine | 1 | 1 |
| MiPT | 1 | 1 |
| MXPr | 1 | 1 |
| O-Desmethyltramadol | 1 | 1 |
| O-PCE | 1 | 1 |

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## Systematic Literature review of subjective effects in humans:

From the substances with a self-administration trial (SAT) describing its subjective effects, only 27 (67,5%) had been previously reported in the scientific literature, identifying 13 (32,5) substances consumed as NPS with no previous scientific literature.

From the 27 substances with previous scientific publications, their subjective effects were reported only for 15 (37.5% of the total identified substances and 55,6% of the substances previously reported). See *table 14*.

| Substances detected in self-administration trials | Results yielded by search criteria | Biological sample analysis | | Case Reports, Cohort studies and Clinical Trials[4] | Online forum analysis | Surveys and interviews | Total of selected studies | Studies reporting subjective effects |
|---|---|---|---|---|---|---|---|---|
| | | Analytical characterization | Prevalence Estimation | | | | | |
| 3-MMC | 249 | 10 | 4 | 11 | 2 | 2 | 29 | 0 |
| 4-HO-MET | 7 | 1 | 1 | 1 | 2 | 0 | 5 | 1 |
| 4-HO-MiPT | 3 | 0 | 0 | 1* | 0 | 0 | 1 | 1 |
| 2-FDCK | 3 | 2 | 0 | 0 | 0 | 0 | 2 | 0 |
| 4C-D | 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Methallylescaline | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2C-T-7 | 34 | 10 | 0 | 0 | 1 | 0 | 11 | 0 |
| 2C-E | 55 | 6 | 0 | 2+1* | 0 | 1 | 10 | 3 |
| 2C-D | 94 | 4 | 0 | 0 | 0 | 0 | 4 | 0 |
| 2C-B | 128 | 11 | 7 | 7+2*+1** | 1 | 7 | 36 | 5 |
| 3-MeO-PCP | 28 | 6 | 0 | 11 | 1 | 0 | 18 | 7 |
| ETH-LAD | 4 | 1 | 0 | 0 | 0 | 1 | 2 | 1 |
| 25E-NBOH | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2C-C | 65 | 1 | 0 | 1 | 0 | 0 | 2 | 0 |
| 2-FA | 294 | 2 | 0 | 0 | 0 | 0 | 2 | 0 |
| 2-FMA | 5 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| 3-FPM | 13 | 0 | 0 | 4 | 0 | 0 | 4 | 3 |
| 3-HO-PCE | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3-MeO-PCE | 5 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| 4-ACO-MALT | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-ACO-MET | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4-Chlorodiazepam | 17 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| 4F-MPH | 6 | 1 | 0 | 2 | 0 | 0 | 3 | 0 |
| 5-MeO-DALT | 21 | 0 | 1 | 2 | 0 | 0 | 3 | 2 |
| 5-MeO-DMT | 25 | 0 | 0 | 2+1* | 0 | 3 | 6 | 5 |
| 5-MeO-MiPT | 14 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| 6-APB | 87 | 7 | 5 | 4+1** | 1 | 1 | 19 | 5 |
| Alpha-PHP | 33 | 3 | 0 | 5 | 0 | 0 | 8 | 3 |
| AMT | 24 | 0 | 0 | 1+1** | 1 | 1 | 4 | 2 |
| BOD | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DOC | 10 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| DOF | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DOiP | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DOPr | 133 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| MEAI | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Methoxetamine | 25 | 2 | 2 | 3 | 2 | 0 | 9 | 3 |
| MiPT | 299 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MXPr | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| O-Desmethyltramadol | 190 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| O-PCE | 11 | 0 | 1 | 3 | 0 | 0 | 4 | 2 |

Table 14: Number of studies from each type by each different analyzed substance

[4] *Clinical Trial; **Cohort study

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## Measurement of subjective effects

### Reliability of the used instruments

To assess the subjective effects collected using Instrument 1 and 2, both instruments have been compared. These analyses were intended to explore the reliability of the results and the internal structure of the Visual Analogue Scales in the Instruments.

Cronbach's Alpha analysis was used to have an initial approximation to the reliability of both instruments. Cronbach's Alpha for instrument 1 resulted in 0,91 (0.88-0.94 in 95% confidence interval), and in 0.93 (0.91-0.95) for instrument 2.

To further assess the structure and validity of both instruments, two other tools were used.

A principal component analysis showed that instrument 1 had a structure of 13 factors, which could explain 76.91% of the total variance. Meanwhile, instrument 2 showed a 11-factor structure, that could explain 76.49% of total variance.

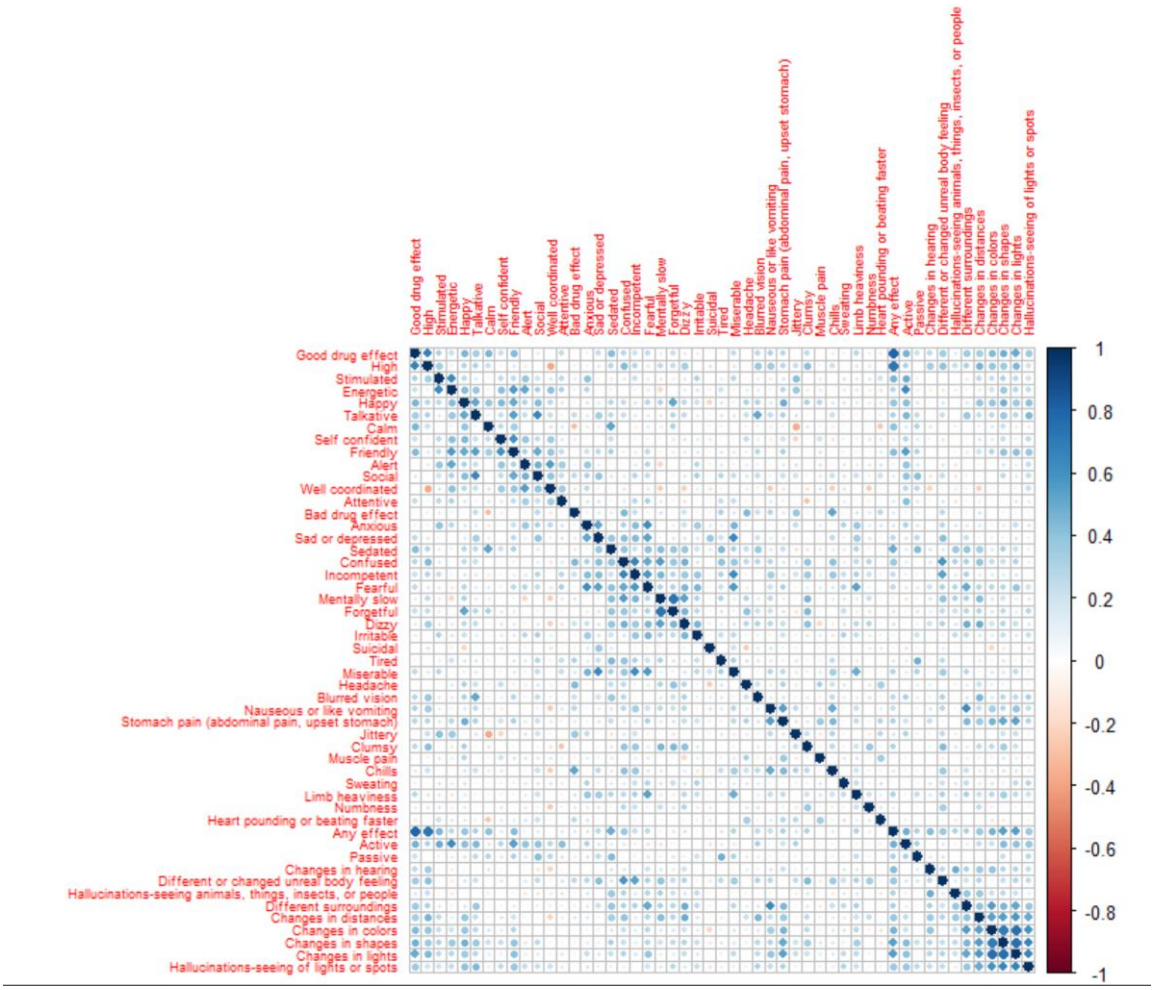

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**Figure 6** Shows the correlation between the visual analogue scales of Instrument 1.

A correlation analysis was also used, with the specific aim to identify redundant visual analogue scales. They are shown in figures 6 and 7. Note that the most positively correlated items are shown in progressively darker shades of blue. The items most negatively correlated are shown in progressively dark shades of red.

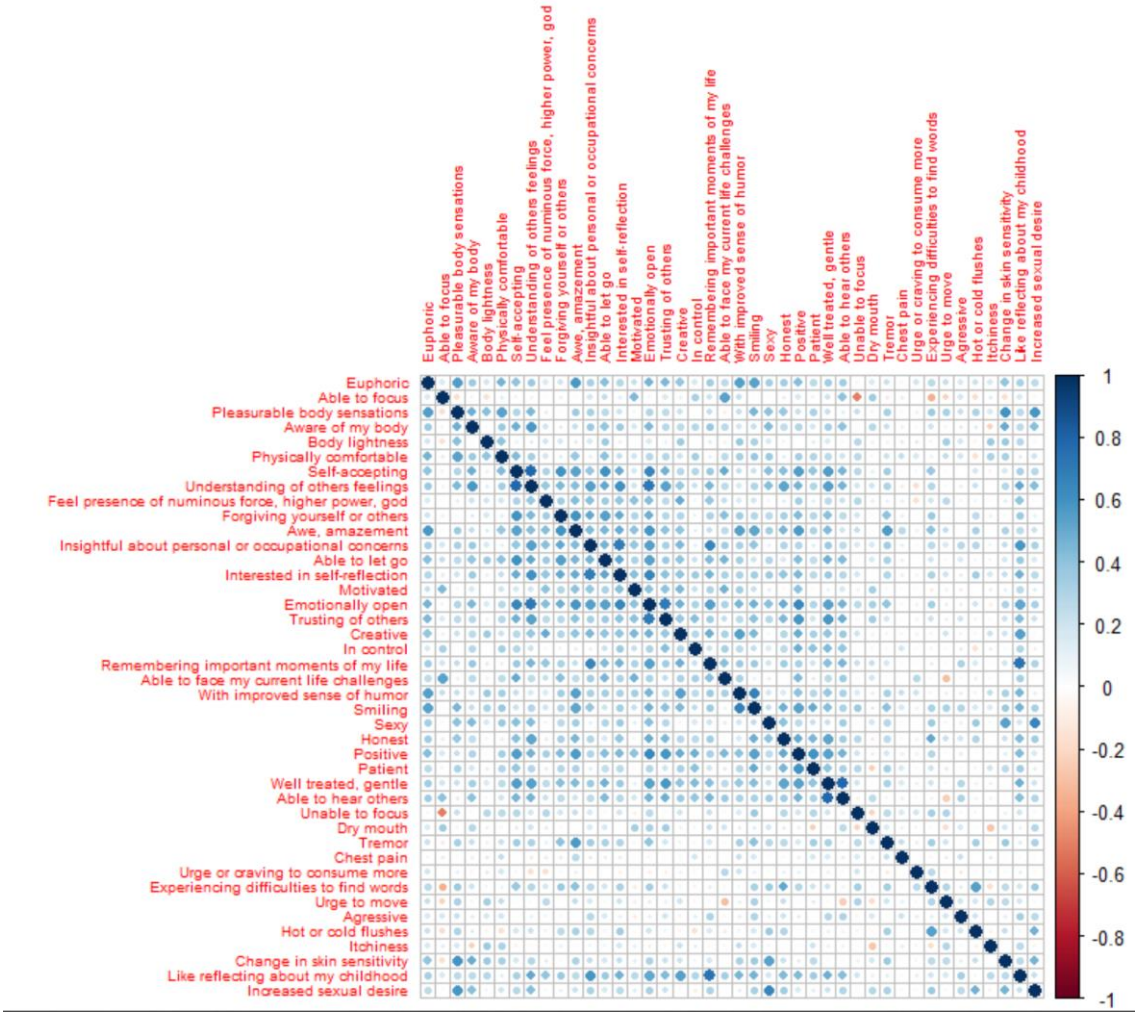


**Figure 7** Shows the correlation between the visual analogue scales of Instrument 2.

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## Psychopharmacological profile by type of substance.

The subjective effects of the confirmed reports, when analyzed by group, followed the expected patterns, discriminating between psychedelics, stimulants, dissociatives and sedatives

In figure 8, these patterns can be easily visualized. All types of substances score high "good drug effect", but when looking at items like "Energetic", "Alert", "Attentive", and "Well coordinated", stimulants and psychedelics have high scores while dissociatives and sedatives have negative scores. Moreover, when looking at typically psychedelic effects such as "changes in colors", the groups with high scores are psychedelics and dissociatives, while stimulants and sedatives tend to zero effect. Muscle pain is only reduced by sedatives, while other groups score 0.

The represented scales have been selected by the researchers to provide a preliminary visual representation of the data. Additionally, the authors have not accounted for the different doses reported by the participants.

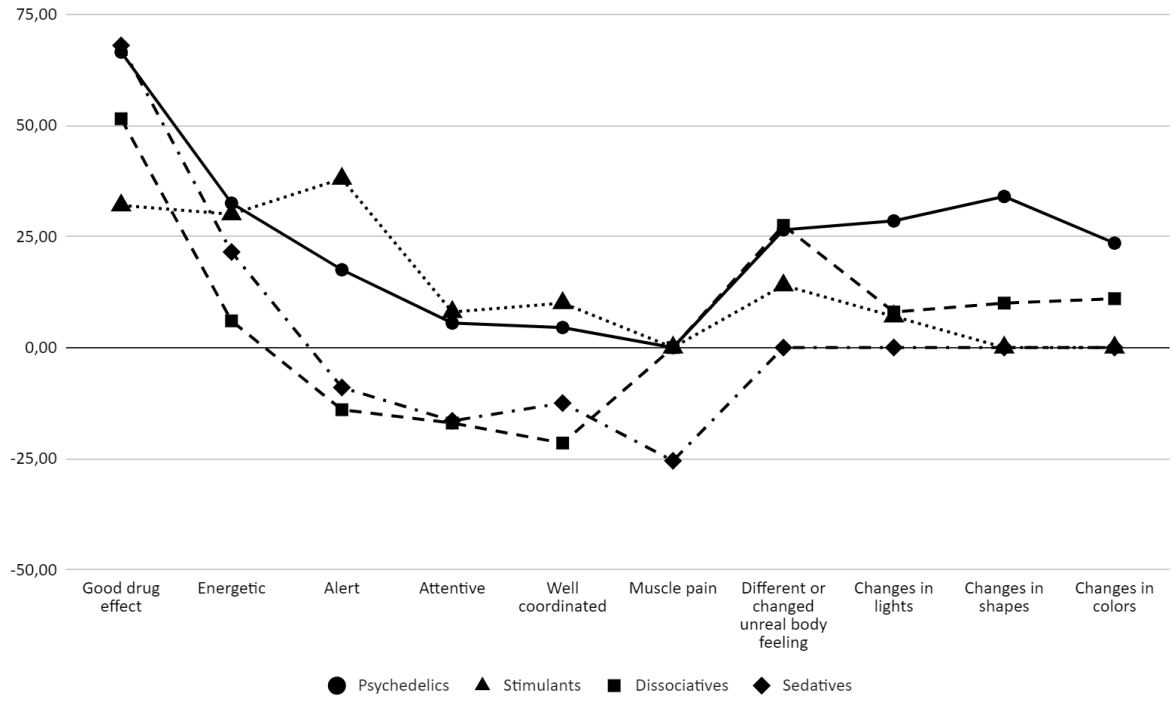


Fig. 8: Selected subjective effects of the analyzed and reported samples by type of substance[5].

[5] The represented outcome is the difference in mm from the visual analog scale from Q3b minus the visual analogue scale (VAS) from the Q3a baseline.

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# Discussion

This design has resulted in the first internet-based, multi-national, longitudinal study on a key sentinel population of e-psychonauts reporting on the effects of the analytically confirmed NPS they consumed over one year. The study has allowed the identification of 40 different substances, from which 13 (32.5%) had not been previously reported in the scientific literature and another 12 (30.0%) had been reported but without descriptions of their subjective effects. This demonstrates the potential of this study design to identify and potentially characterize the subjective effects of emerging NPS before other methodologies like case reports or wastewater analysis can.

## Feasibility:

The study proved to be feasible despite its complexity and numerous limitations, some inherent by design and some that seem to be possible to address easily when replicating the methodology. Also, this study points a way to develop similar research methodologies in other fields, as digitalization keeps changing our lives, so our research methods should be changed accordingly (Guaritaa et al., 2021).

The importance of proving this study design feasible is notably, as the advantages of conducting surveys of hidden populations online are numerous: large and geographically and linguistically diverse samples can be obtained relatively easily, responses can be gathered more rapidly, costs and other resource demands are relatively low, transcription and data entry are automated, and flexibility and convenience are enhanced for both respondents and researchers (Barratt et al., 2017).

Apart from the digitalization of data collection, this study was also designed to produce results that interested the study participants, with their (or their peers) active collaboration in all the study phases. Allowing them to discuss the questionnaire structure, the outcome measurements, and the participant retention strategies, among others. It is likely that successful research will shift in that direction, because as digitalization spreads, participants demand to be more involved in science, and this methodology provides the possibility to engage the studied population as active research collaborators, empowering them instead of expropriating their knowledge without their consent or benefit (Peacock et al., 2019). Furthermore, the increasing use of

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patient-reported outcome measures in both clinical trials and usual clinical practice shares key aspects with the study design, which further aligns itself with the future trends in research (Trujols et al., 2013).

Participant attrition is generally high in internet-based prospective studies (usually greater than 25%), and among those participants who are retained, engagement rates typically drop over time (Bennett & Glasgow, 2009). Eysenbach argued that high rates (considered 40%-50%) of participant attrition, in the form of both dropouts and losses to follow-up, rep- resent one of the "fundamental characteristics" of Internet interventions (Eysenbach & Wyatt, 2002). Despite predicting high dropout rate, losing 91% of the candidates was initially unexpected, but then the high commitment from the participants was also shocking. The high completion rate of valid questionnaires (average among questionnaires of 85.7%) and the consistency regarding the number of questions and the time spent answering, points to a remarkably high level of compliance and internal validity for a non-supervised setting. These phenomena could be understood from the perspective of a good recruitment strategy followed by a high demand participation, also linked with a high level of sustained interaction with the research team to sustain the 17 committed participants during a 1 year follow up. It is consistently reported that dropouts increase over time, which is contradictory with the study data, where most dropouts occur during the engagement process.

Given their potential for low costs, scalability, adaptability, and effectiveness, Internet interventions may be appropriate How- ever, each of these settings varies considerably with regard to their resources, expertise, interest, and ability to implement Internet interventions independently (Bennett & Glasgow, 2009). A multidisciplinary team with a wide variety of skills is necessary to start the study, while then, it may be maintained with less personal.

There are other innovative digital approaches capable of pioneer identification of emerging NPS, such as massive data scraping from online communities without their knowledge. *Blankers* et al and *Gouwe* et al 2019 first detected the use of 4-FA and up-LSD, respectively. However, forum data scraping cannot provide analytical confirmation and may rise ethical concerns about privacy and harvesting individuals' data without their consent (Guaritaa et al., 2021). Other NPS-monitoring approaches such as wastewater analysis are already being used widely, but they depend upon other methodologies to choose the priority substances they will be analyzing the wastewater for. In that case, both methodologies would combine synergically, as prevalence

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estimation would be always impossible using self-selected samples while being one of the strengths of the wastewater analysis (Bade et al., 2021; Castiglioni et al., 2021).

The COVID-19 pandemic will have a profound and long-lasting impact on the entire scientific endeavor. Studies that were under way when the current crisis began will be truncated, resulting either in work that cannot be published or in work whose true impact is difficult to accurately assess. Scientists already are adapting research programs to face this new challenge to conduct in-person assessments (Feil-Seifer, Haring, Rossi, Wagner, & Williams, 2021). The execution of the GRASP study, however, was proven immune to the effects of the pandemic and the following lockdowns. This adds another layer of security when investing in these kinds of new methodologies. Not only we tend to a more digitalized and decentralized world, but also, we tend to a world where unexpected massive catastrophes may increase.

## Participants

From the 184 candidates that applied for participating in the study, only a minority of 17 has been finally considered committed participants, defined as a participant who has at least sent a sample that has been received and that has then completed the subjective effects questionnaires correctly. The sample size of participants is markedly lower when compared with other studies on the same field, but it must be noted that these studies have virtually always cross-sectional or transverse designs, instead of the one year follow up of this study (Davey, Schifano, Corazza, & Deluca, 2012; Matthews et al., 2017; O'Brien et al., 2014; Orsolini et al., 2015; Peacock et al., 2019; van der Gouwe, Brunt, van Laar, & van der Pol, 2017a).

The participants' sociodemographic data matches with precision the previously reported in the literature regarding e-psychonauts. Additionally, to further support their condition of e-psychonauts, all of them report using NPS and using the internet to educate themselves regarding their use. As is often reported, a high proportion of these e-psychonauts report using harm reduction strategies. In this study, all participants used at least 4 of the 8 harm reduction strategies assessed, dropping progressively to a 30% of the participants that reported using all of the suggested strategies (n=8; 100%) (Davey et al., 2012; Higgins et al., 2021; O'Brien et al., 2014; Orsolini et al., 2015). To further stress this point, about two thirds of the study participants waited to receive the sample analysis result before ingesting the substance and completing the report and the most used route of administration was orally. Additionally, recent research points out that the people who use NPS may not exhibit significant sociodemographic or patterns of drug

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use than the population of people who use multiple illicit drugs, both reporting the use of psychoactive substances to achieve personal goals in early adulthood, such as developing or retaining social networks (Higgins et al., 2021). All these characteristics, despite being consistent with all published research, still are contradictory to the popular beliefs and stereotypical ideas of some researchers. These ideas have historically misrepresented the profiles of the people who use non-regulated psychoactive substances (Barratt et al., 2017).

These incompatible negative believes might be a reason as strong as legal consequences to hide the use of NPS, isolating the person and sustaining the stigma (Ross et al., 2020). The social stigma seems to be the cause of the difficulty of reaching this high-functioning populations of people who use drugs. Thus, it has been previously discussed the inadequacy of using representative sample frames for their study, if the aim is not prevalence estimation (Barratt et al., 2017).

## Samples

From a total of 138 sample submission requests, 92 NPS samples (67%) were received and analyzed. The sample size of NPS samples was notably higher than the sample size of committed participants.

A total of Most of the samples were reportedly bought on Clearnet 66% only 20% on darknet or crypto-markets, this is consistent with data from drug-checking services such as DIMS. Whereas illicit drugs are typically bought in person or in the darknet, NPS, thanks to their gray legal status, are usually purchased using the regular internet (van der Gouwe, Brunt, van Laar, & van der Pol, 2017b). However, this is still surprising, as different initiatives to shut down these regular websites have been reported to work (Guaritaa et al., 2021).

Both from the database of user expectations and sample analysis result, we did not find any substance that could be labeled as a synthetic cannabinoid nor synthetic opioid. This results differ from the high detection rate of both groups (usually more than 50% combined) when monitoring NPS using other methods such as hair analysis (Florou & Boumba, 2021). This is relevant because those are precisely the NPS groups that have been more clearly associated to both reported and detected negative consequences, especially health and social harms (Higgins et al., 2021).

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Up to 94,3% of the analyzed samples contained the substances expected by the participant. This contributes to the partial and apparently opposed picture provided by the published evidence. Usually, the drug-checking services such as Energy Control, find NPS as adulterants of other drugs, mainly MDMA. And the adulteration of MDMA, for example, which is among the lowest, ranges from 10 to 50% (excluding data from 2009). In summary, drug-checking services mainly detect NPS as adulterants of illicit drugs, which are regularly more adulterated than the samples of NPS sent as NPS collected in the GRASP study (Brunt et al., 2016; Giné, Espinosa, & Vilamala, 2013; van der Gouwe et al., 2017b). When comparing with data from other drug-checking services such as DIMS, the adulteration of NPS sent as such is much higher there (between 28% and 75%) (van der Gouwe et al., 2017b). However, when comparing with crypto-market adulteration of illicit drugs (not NPS), the results are similar, despite most of the GRASP samples coming from the regular internet (Caudevilla et al., 2016).

Most participants expected the analysis to confirm their idea about the composition. Moreover, there were significant statistical evidence that the relationship between their predictions and the samples' compositions could not be attributed to chance. When predicting no adulteration, they seemed to be highly accurate, but when predicting adulteration, the accuracy was low. This may indicate that the participants develop an expectation of their samples' adulteration based of some sort of knowledge, like when they trust the source, they can be confident but when they don't, they don't really know. The authors have not found other research that combines the adulteration of samples with the user expectation to compare these results. To these facts, we should add that in the same line, the most common way of buying through an online trusted vendor 55%, this is inconsistent with previous literature, that reports purchasing from friends as the main source. However, in the cited literature the recruitment doesn't have to be online, despite aiming for the same population (C van Amsterdam ab et al., 2015). Finally, it should be noted that among the samples expected to be adulterated, the proportion of completed self-administration reports is significantly lower. Sadly, the meaning of these data will remain speculative until similar study designs are published.

Most of the samples are reportedly from Europe, which is where we would guess the study participants to be, too. The same can be said about North America, that mimics Europe but in second place. We think this indicates more where this elite population of e-psychonauts live rather than the sites of production or shipping of a significant proportion of the NPS.

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**One of the most important results of the study is that it has identified 13 NPS that had not been previously reported in the scientific literature.** Moreover, it has recorded the subjective effects of 25 NPS with no previous publications reporting them. Abundant research supports the importance of identifying and characterizing the emerging NPS, both for political and clinical purposes, while acknowledging that the current means of responding to emerging substances might no longer be fit for purpose (Ramos et al., 2020).

## Subjective effects

The sample of 17 committed participants, provided with a sample of 92 NPS samples, which resulted in an apparent respectable sample size of 64 valid self-administration trials (SATs). However, when considering that these 64 reports are from a variety of 40 samples, we can conclude that the sample of reports for each substance is too scarce to draw differentiated conclusions. We hope that more data will be collected in the future using compatible assessment tools to provide consistent psychopharmacological effects for the different from which those are unknown.

### Validity of the selected measurements for assessing the NPS subjective effects

For the reason stated above, it is of the outmost importance to analyze the validity of our subjective effects measurements, so future studies will be able to contribute to the existing database.

The use of the visual analog scale has been the gold standard in psychopharmacology to measure the subjective effects. For this reason, we developed Instrument 1 with the most popular visual analog scales used in previous literature to facilitate comparing datasets. The main differences between those studies and this one are the following:

1) The scales are not given printed in paper to a present participant
2) There is no one from the research team to verify how and when the participant fills the scales.

Multiple studies and a review by *Krantz and Dalal* have shown that web-based data collection and traditional methods (e.g. paper and pencil) result in equivalent conclusions, demonstrating the validity and reliability of online data collection for research (Ip et al., 2010). Additionally, when compared to paper-based methods, participants using the internet reported lower social anxiety (Miller & Sønderlund, 2010)

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The problem of the lack of supervision, however, clearly limits our measurements' validity. However, when aiming to develop comprehensive psychopharmacological knowledge about the NPS, some authors have suggested that the only way to keep up may be to establish an acceptable 'tarnished gold' to substitute the glorified 'gold standard' (Barratt et al., 2017). Pointing to this yet-to-be-defined acceptable 'tarnished gold', it should be highlighted that most of the participants used precision scale to report the dosing, and most self-administered only once, these results being the closest this study design might get to the controlled conditions of an in-patient trial.

Regarding the validity and reliability of the visual analog scales that comprised instrument 1, we have not been able to find assessments of their validity or reliability in the previous literature, from which the scales were replicated (Camí et al., 2000; Comer et al., 2001; Daniel Kleinloog, Frits Roozen, Willem de Winter, Jan Freijer, 2015; De La Torre et al., 2000; Farré et al., 1998; Farre et al., 1993; González, Torrens, & Farré, 2015; Haney, Ward, Comer, Foltin, & Fischman, 1999; Hart, Van Gorp, Haney, Foltin, & Fischman, 2001; Hart, Ward, Haney, Nasser, & Foltin, 2003; Papaseit et al., 2018c; Riba, 2003).

Regarding the instrument 2, constructed with added positive scales to match the predominantly negative scales from the previous literature, there were no differences in the multiple analysis made to compare their validity and reliability. However, an apparent higher number of redundancies were identified in the specifically developed instrument 2 when comparing with instrument 1.

## Subjective effects of the reported NPS

The subjective effects of the confirmed reports, when analyzed by group, followed the expected patterns, discriminating between psychedelics, stimulants, dissociatives and sedatives.

This indicates the validity of this methodology to discriminate the different drug effects, at least when grouped in these categories. Further research is needed to be able to better characterize these substances and provide data like the existing database of randomized controlled trials. In any case, the existing data, despite its great limitations, is still the only available data right now regarding the subjective effects of at least 25 new psychoactive substances, and probably the best data available for the other 15 identified NPS.

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## Limitations

As expected, this new study design has multiple limitations. Most of those, however, are limitations intrinsic to the study design, representing choices consciously made by the author to achieve overcoming the other limitations that have plagued the field of NPS research. No set of limitations or strengths is seen better than the other, as all might be the best designs for different specific situations.

Among these study design intrinsic limitations, the following should be highlighted:

1. The sample was self-selected and thus subjected to the voluntary bias: The committed participants were only those included participants who would really want to participate in a high commitment study with the only motivations of getting their drugs tested and contributing to science. This limitation, however, is widely discussed if sample representativeness is not necessary (e.g. to produce prevalence estimates). When the research has other aims, including measuring relationships between variables or in-depth profiling of sub-populations, the use of probability-based sampling frameworks is often inefficient, may be unnecessary or even better avoided (Barratt et al., 2017).
2. Unknown response rate, as the size of the population from which the sample is recruited cannot be estimated. In any case, as stated above, the aim of the study was never the representation of the population from which the participants' sample was drawn. The main interest of the study was on the sample of analyzed NPS sample and the sample of self-reported administration trials (Barratt et al., 2017; Palamar et al., 2016).
3. The huge over representation of well-educated mid-income white males produces a bias that should be always kept in mind, despite being highly expected based on previous research. The persistent lack of race, gender, sexual orientation, and socioeconomic status in scientific research is a more general worrying phenomenon. However, some evidence points that the majority of US drug users resemble online samples more than they do clinical populations of drug users (Miller & Sønderlund, 2010).
4. No controllable intervention was made when administering the substance, so multiple factors could not be controlled. Additionally, the physical absence of a researcher when the drug was administered limited the control over other factors. This last lack of control, however, could also be seen as less interference in the natural setting on which the drug would be administered, having a resultant of unknown direction either to strengthen or weaken the collected dataset.
5. There was no control group.

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The limitations not intrinsic in the study design might be addressed in potential future replications of the study, where execution can be improved or recruitment period extended, for example. From those limitations, the following should be highlighted:

- Low sample size of participants but decent aggregated sample size of analyzed samples and of self-administration trials reported. Still far too low SAT sample size for each substance. This last limitation might have been avoided if the huge diversity of samples had been expected beforehand.
- Some questionnaires have incomplete data. This was allowed to minimize dropouts during a questionnaire and to the decision to err on the side of reducing annoyance for the respondent who may wish to skip a section without being forced to answer it (Barratt et al., 2017)
- Only 2 of the four (50%) targeted communities could be included in the study. One community disappeared at the beginning of the recruitment process apparently due to law enforcement intervention, and another rejected our approach advertise the study in their platform. The scarce stability of websites an online community is well known in the field, as studies tend to last longer than some internet-based structures (Davey et al., 2012).
- The same participant produced the 34.4% of the self-administration trials (SATs). The fact that deleting this participants data could significantly change the results of the study pose a serious limitation for the feasibility results.
- The internal biases produced in selecting the committed participants are discussed below:
    a. Importantly, no sociodemographic differences between the two groups.
    b. Statistically significant differences in previous drug use were detected, but were considered not relevant, in addition of being detected mostly in rarely used substances. They could also be interpreted as artifacts due to the high number of comparisons made (4 options for each of 35 drug categories between two groups)
    c. The bias between the samples without a subjective effects report and the samples with it was only potentially detected regarding the expectation of adulteration: The NPS samples with reported SAT were expected to be less adulterated that the samples that were not reported after being analyzed, but there were no differences regarding actual adulteration.

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## Goals and hypothesis:

In summary, the initial questions that motivated this exploratory study would be answered as follows:

1) **Is it possible to collect reliable data using this innovative and methodology with the collaboration of the e-psychonaut communities?**

Yes, it is. There are some intrinsic limitations we could not overcome, and other more concerning limitations found could be overcome by improving the study execution and funding.

2) **What are the characteristics of the recruited participants?**

The same as the previously reported in the literature as e-psychonauts or cyber-psychonauts. Despite the impossibility of knowing the population from which the sample is drawn, the sample is consistent with the other samples from studies with the same sample target.

3) **What substances will they intend to use during the data collection period, and will those be what they expect?**

A wide variety of them. 40 different NPS among 17 participants. From those samples a shockingly high proportion have no previous reports or no previous reports about their effects.

4) **What are the subjective effects of the analytically confirmed substances they ingest and report on during the study duration?**

Their subjective effects are the expected by the type of substance (stimulant, psychedelic, dissociative or sedative). However, individual substance profiles were not possible to produce due to limitations of sample size for each of the 40 different substances.

Finally, the relationship between the previously recorded hypothesis and the results will be discussed here using the previously used structure:

A. General hypothesis 1/3: It is possible to recruit e-psychonauts
    1. Key stakeholders of the internet communities of e-psychonauts have been successfully engaged in half (n=2) of the targeted communities. Rapport has been established and they contributed to improve study design and execution.
    2. Our recruitment strategy provided 184 (230% out of 80) applications from candidates, more than doubling expectations.
    3. From these candidates, almost the double of the expected (79 out of 80) was accepted in the study.

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B. General hypothesis 2/3: 17 participants completed the study protocol.
    1. All the accepted participants matched as much as could be reasonably expected the sociodemographic profile of e-psychonauts described in the literature.
    2. From the accepted participants, 21,5% (17 out of 79) showed a high level of commitment, completing the protocol until the one-year follow-up. This represents less than half of the 50% expected to complete the study.
    3. Only one fifth of the expected participants completed the study, providing 64 self-administration trial reports, the 16% of the 400 expected. The unexpected low number of reports combined with the high variety of substances reported resulted to be one of the main avoidable limitations of the study.
    4. We could not perform statistical analysis to assess if the participants' expectations were a predictive factor of the composition, but we could determine that there was a statistically significant association between their expectation and the sample analysis result. The study data supports the pre-established hypothesis, without being able to provide definitive confirmation.
C. General hypothesis 3/3: Data on previously unknown NPS was successfully collected, exceeding expectations.
    1. The adulteration of the analyzed samples was clearly lower than any other estimates found in the literature regarding the adulteration of illicit drugs commonly sold in the street level. The study hypothesis could not be rejected.
    2. As predicted, most of the detected substances were psychedelics and cathinones, and most of the time the NPS were consumed orally. Further research is warranted, as this data is inconsistent with previous literature.
    3. Data on previously unknown NPS was collected for 13 substances. Subjective effects data on substances with no previous reports of such data was collected for 12 more different substances. These results exceeded the non-quantified expectations of the stablished hypothesis.
    4. The collected data on NPS' subjective effects from previously used scales and the specifically designed scales showed similar validity and reliability in our analysis. However, the analysis showed that the new scales were more redundant than those used from previous literature. Additionally, aggregated data by type of substance showed the expected distinct effect patterns between psychedelics, stimulants, sedatives and dissociatives. Within the limitations of this exploratory

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study, this data is considered to fully support the hypothesis regarding the validity and reliability of the subjective effects measurements.

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# Conflict of interest:

The authors report no conflict of interest.

# Abbreviations:

**NPS**: New Psychoactive substance

**RCT**: Randomized controlled trial

**GRASP**: Global research and analysis of new substances project

**EC-ABD**: Energy Control-Associació Benestar i Desenvolupament

**CHERRIES:** Checklist for Reporting Results of Internet E-Surveys

**STROBE:** Strengthening The Reporting of OBservational Studies in Epidemiology

**SPIRIT:** Standard Protocol Items: Recommendations for Interventional Trials

**CEIC**: Clinical Research Ethics Committee (local IRB)

**RGPD**: reglamento general de protección de datos (general law about data protection)

**PGP**: Pretty Good Privacy. An encryption method that provides cryptographic privacy and authentication.

**Q0**: Screening questionnaire

**IMIM**: Hospital del Mar research institute in Barcelona

**SAT:** Self-administration trial

**Q3a**: drug effect baseline questionnaire

**Q3b**: drug effect questionnaire

**Q1**: sociodemographic and drug use history questionnaire

**Q2**: sample submission questionnaire

**Q4**: Follow up questionnaire

**MBA:** Mater on Business Administration

.

**GC/MS:** Gas chromatography coupled with mass spectrometry

**LC/MS/MS:** liquid chromatography coupled to tandem mass spectrometry

**MNR:** Nuclear Magnetic Resonance

**3-MMC:** 3-Methylmethcathinone

**4-HO-MET:** 4-hydroxy-N-methyl-N-ethyltryptamine

**4-HO-MiPT:** 4-hydroxy-N-methyl-N-isopropyltryptamine

**2-FDCK:** 2-Fluorodeschloroketamine

**4-CD:** 4-Chlorodimethylcathinone

**2C-T-7:** 2,5-Dimethoxy-4-propylthiophenethylamine

**2C-E:** 2,5-Dimethoxy-4-ethylphenethylamine

**2C-D:** 2,5-Dimethoxy-4-methylphenethylamine

**2C-B**: 4-Bromo-2,5-dimethoxyphenethylamine

**3-MeO-PCP**: 3-Methoxyphencyclidine

**ETH-LAD:** 6-Ethyl-6-nor-lysergic acid diethylamide

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# Annex 1: Information to the participant

TITLE OF THE STUDY: Validation and application of a new online methodology to study the effects of new psychoactive substances

SHORTENED TITLE: : GRASP (Global research and analysis of substances project)

PROTOCOL VERSION AND CODE: 4th version; May 27, 2019; 2018/8283/I

PRINCIPAL INVESTIGATOR: Marc Grifell Guàrdia

RESEARCH CENTER: Hospital del mar research institute (IMIM) in collaboration with Energy Control (EC), Universitat Autònoma de Barcelona (UAB) and Columbia University (CU)

**Purpose of Study:**

The purpose of this research project is to study the subjective effects of new psychoactive substances and the characteristics of a population of committed participants in the main online communities of drug users.

You are confirming that you are not currently seeking treatment for your drug use, and if you are a woman that you are not pregnant. If you are interested in treatment for your drug use, please let us know and we can help you find it.

This study is funded by research funds from Hospital del Mar research institute (IMIM),

Energy Control (EC) and Columbia University (CU).

**Importance of the study**:

Information regarding new psychoactive substances is insufficient. Online surveys and self-reported data offer now timely information but sadly not reliable enough, due to the impossibility to confirm the actual substances consumed and asses the conditions in which they are consumed. Information from clinical trials, in the rare case it exists, takes too long to reach both the scientific community and the communities of drug users who want to make informed decisions.

This project pretends to fill the evidence gap between this two types of research in new psychoactive substances. To do so, a new methodology of study will be implemented with a threefold purpose:

To study the population of committed members of the main online communities of drug users

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To characterize the subjective effects of the new psychoactive substances emerging during the period of study

To try this new methodology by comparing the results of the reported effects of traditional drugs with the existing database of laboratory studies with traditional substances

**Voluntary participation:**

Participation in this study is voluntary, and you may refuse to participate or stop participating at any time without loss of benefits to which you are otherwise entitled. You will be informed of any new findings or risks that arise that may affect your willingness to continue in this study. If you decide not to participate in this study or to withdraw at any time, this will not affect your present or future enrollment in other studies conducted by these same institutions or researchers. The investigator may also decide that your participation should be discontinued, if he/she thinks that this is better for you.

**Alternative to Participating in This Study**

This is not a treatment or intervention study. Information being collected is for research purposes only. The alternative to participating would be simply not to participate.

**Study Procedures**

If you agree to participate, you will fill a baseline form assessing your sociodemographic characteristics, drug use experience and medical history. This may involve questions about your mental health, physical health, drug use, sexual activity, legal history, and any problems you might be having.

Although we prefer that you answer all of the questions, you do not have to answer any questions that make you feel uncomfortable.

Once you have agreed to participate and signed the consent form, you will have access to an online platform where you will be able to discuss with other participants or the investigators any concern that you might have during the study. Participation regarding the study design and the study results will also be encouraged there. Experiences regarding the effects of the studied samples will not be allowed as discussion topic to avoid contamination among participants.

At any point that you intend to consume a substance during the period of study, you will be asked to fill a very short form informing which substance do you intend to use and if you want to include it in the study. In case the episode of drug use is included in the study you will be asked to fill an

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extensive report regarding the context of use and the subjective effects of the substance. Also, you will be asked to send a sample for chemical analysis performed either by GC/MS (gas cromathography / mass spectrometry) and HPLC (high pressure liquid cromathography). The sample will be analyzed for free and you will receive a report within a few days. Although it is recommended to wait for the report before ingesting the substance, this decision will be yours, as this is not an intervention study and we only intend to study a naturally occurring phenomena. The research team will be able to deny the free analysis of the substance after the short form is submitted and before the sample is sent (and the questionnaire filled). This measure is only intended to prevent the flooding of the analysis service by the same type of sample or under the suspicion of fraudulent use, that will be discussed with the user.

After one year of initiating your participation in the study, this will formally end, and participants will be expected to fill another extensive questionnaire about their drug use and personal situation at that moment. If permission is granted by the ethics committee and funding is available, participants might be given the opportunity to continue with the study for more time, although this possibility is not granted.

**Risks If you participate in the study:**

Because all of the potential risks of the studied behaviors to an unborn baby, women should not be in this study if they are pregnant, breastfeeding, or possibly pregnant. If you think you might be pregnant at any point, please tell the investigator.

The following risks may be involved with the study procedures.

Some people have found the questionnaires to be uncomfortable and/or tiring. Some of the questions we may ask could be embarrassing. You can refuse to answer any questions.

The study might be time consuming, with an expected dedication of 4-6 hours a month.

As the study is a naturalistic study, it will not substantially modify the risks of the behaviors the participants usually engage in.

**Confidentiality**

The treatment, communication and cession of the data gathered in the study will be according to the new European data protection regulation, Regulation (EU) 2016/679 of the European Parliament and the council of April 27, 2016 (RGPD).

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Additionally, we will never ask you for your name, address or any information that can be related to your physical identity.

We will need you to provide us with an email address that you will need to confirm and use for the study proceedings. This e-mail address can be yours or specifically set up for this study to protect your identity.

Once you enter the study you will be assigned a study code and you will choose a study username.

You will be able to exert your right to the destruction of all information relative to your participation at any point during the study, which would also imply your withdrawal from it. You will only need to contact the research team through the information provided below to exert that right.

Specific collected data without identifiable information might be shared with other researchers. This data would not contain either your email or the chosen username.

**Final remarks**:

If you have any questions, please ask. The investigators will answer to the best of their abilities any questions you may have now or in the future about the study procedures.

You should contact the Principal Investigator, Dr. Marc Grifell Guàrida at admin@grasp.pw if you have any questions.

The hospital del mar research institute research ethics committee has approved the recruitment of participants for these studies. A research ethics committee is a committee that protects the rights of participants in research studies. If you have any questions about your rights as a research participant or any complaints, you may contact the committee at +34 93 316 06 79 from 9am to 2pm (Spanish time).

Please save a copy of this document for your personal use.

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# Annex 2. Informed consent.

**Title of the study**: Validation and application of a new online methodology to study the effects of new psychoactive substances.

**Shortened title:** GRASP (Global research and analysis of substances project).

I have discussed this study with _____ to my satisfaction.

To the best of my knowledge, I am not pregnant.

I understand my participation is voluntary.

The investigator has explained to me the risks derived from consuming new psychoactive substances with unknown effects, I am aware of these risks and here I testify that I have decided to consume NPS at my own responsability independently of my particpation in the study.

Signing this form does not waive any of my legal rights.

I may choose not to participate or to discontinue my participation at any time without penalty or loss of benefits to which I am otherwise entitled. I voluntarily agree to participate in the research study described above.

Participant username _____

I have discussed the proposed research with this participant and, in my opinion: this participant understands the benefits, risks, and alternatives (including non-participation) and is capable of freely consenting to participate in this research.

Investigator_____

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# Annex 3 Announcement for candidates

Research project needs experienced NPS users to collaborate

The principal investigator of this study is Marc Grifell Guàrdia, from Hospital del Mar research Institute (IMIM) and Autonomous University of Barcelona (UAB). The research team is comprised of leading figures in the field like Dr. Carl Hart from Columbia University and Dra. Mireia Ventura from Energy Control.

The goal is to provide fast and reliable information about subjective effects of new drugs when they emerge in internet communities.

We believe that there is a gap between no-analytically confirmed trip reports or surveys and clinical trials with healthy volunteers. We want to implement a new methodology combining the speed of the self-reported information with the reliability of clinical trials. For this reason, we have asked forum moderators to contact committed and experienced members of their communities to help us develop this project.

If you decide to participate, you will be invited to a private forum where free drug checking services will be provided to all participants (GC/MS and HPLC). There, you will asked to systematically report the effects of the NPS you have decided to test. Also, we hope you will engage in a discussion with the research team to design the second phase of this study.

We believe that people like you can make a difference in the development of scientific knowledge if provided with the necessary tools. With your help, we hope to make self-experimentation a valid source of scientific knowledge. We would like that the reliable, balanced and timely information produced in this study will be able to shape drug policy.

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Please send an email to admin@grasp.pw if you are interested. We want to protect both your virtual and physical identity, so the only identifiable information will be the email address from which you send the message. Do not add any other information. Please make sure it's anonymous and you check it regularly. We'll send a time-sensitive invitation there.

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# Annex 4: Study procedures summaries

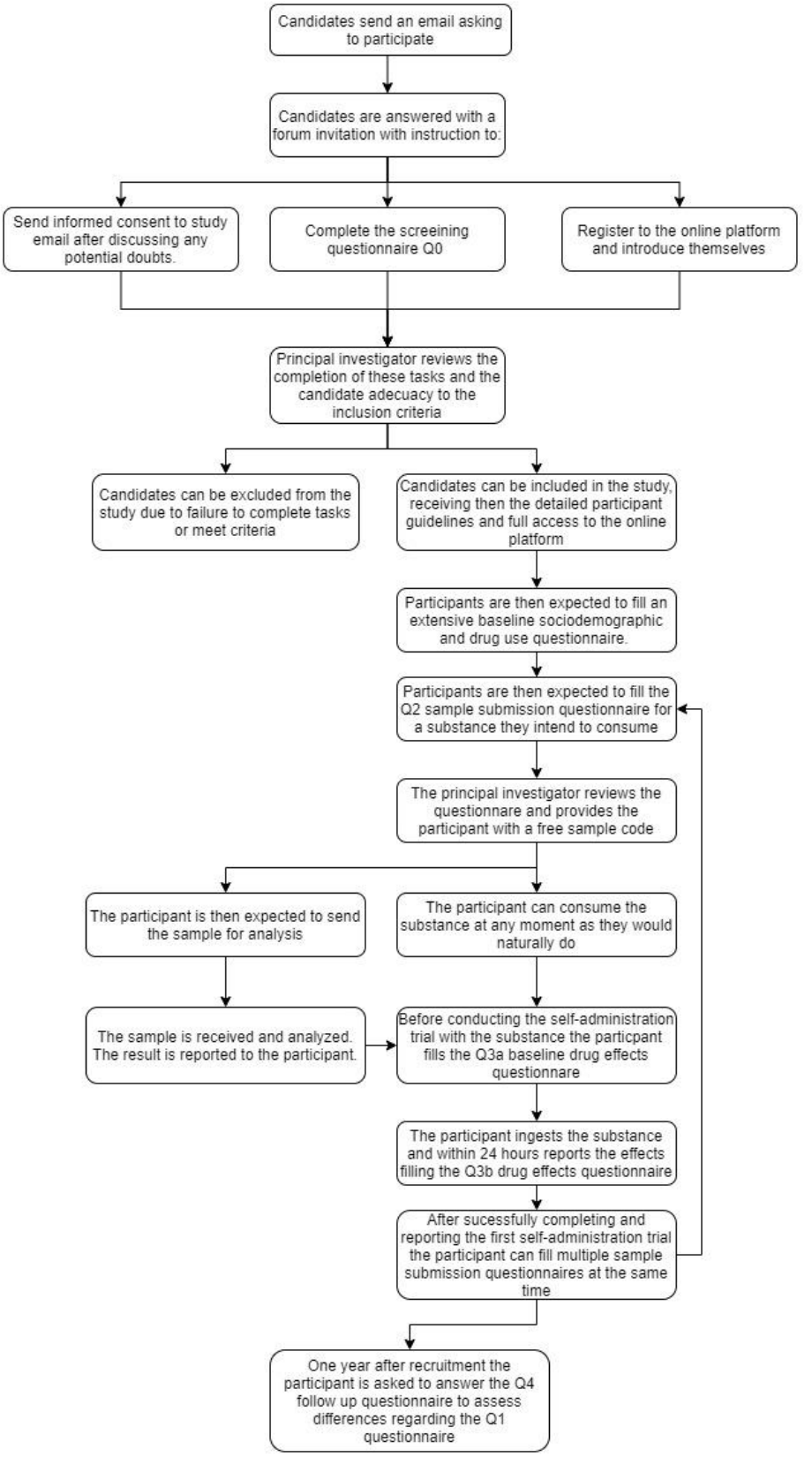


*Figure 4 Participant expected procedures during the study*

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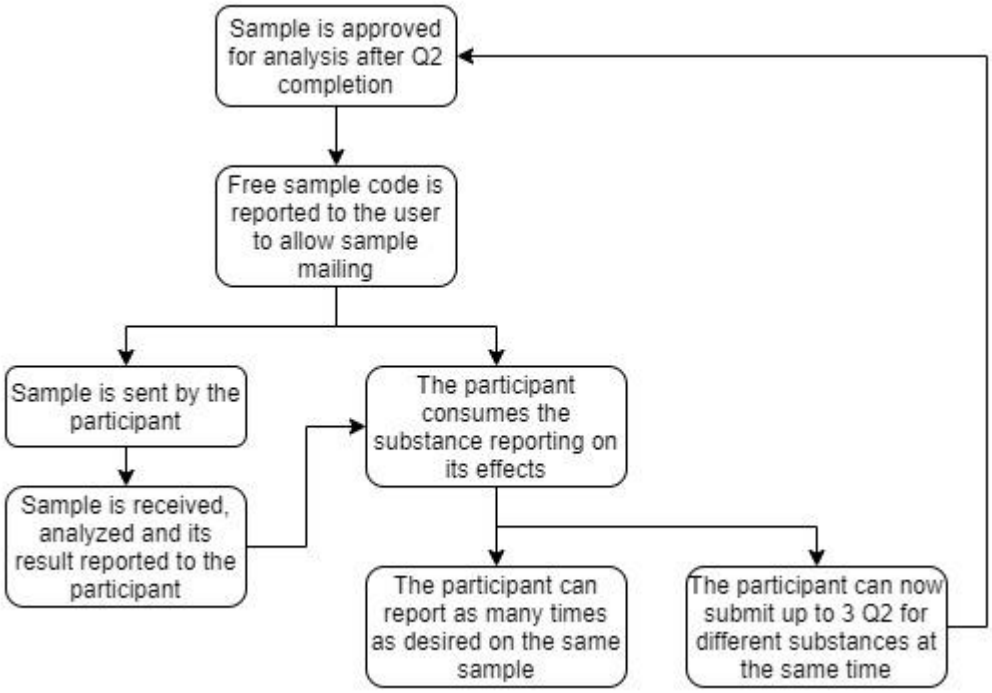


*Figure 5 Sample processes flow-chart*

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# Annex 5: Main outcome measurement

**Table 1.** Sociodemographics

| Domain | Measurement | Variable characteristics | Timepoints | Analysis metric |
|---|---|---|---|---|
| Age | Absolute number of years | Numeric | Q0 | Mean, Standard deviation |
| Sex | Self-perceived sex | Three categories (M/F/other) | Q1, Q4 | Percentatge |
| Geographic location | Self-reported country | Free text | Q1, Q4 | Percentatge |
| Education | Completed studies | 7 fixed categories | Q1, Q4 | Percentatge |
| Income | Self-reported last year annual income | Numeric | Q1, Q4 | Mean, median, Q1, Q3 and range (Box plot) |

**Table 2.** Medical history:

| Domain | Measurement | Variable characteristics | Timepoints | Analysis metric |
|---|---|---|---|---|
| Medical or psychiatric history | Self-reported | Open text in structured battery for common symptoms, diseases and milestones such as hospitalizations | Q1,Q4 | Perfentage; Difference Q1 to Q4 |

**Table 3.** Patterns of drug use (for each substance or substance category)

| Domain | Measurement | Variable characteristics | Timepoints | Analysis metric |
|---|---|---|---|---|
| Age of first consumption | Self reported age | Numeric | Q1 | Mean, median, Q1, Q3 and range (Box plot) |
| Route of administration | Self reported | 7 fixed categories | Q1, Q4 | Perfentage; Difference Q1 to Q4 |
| Frecuency of drug use | Self reported use | 4 fixed categories (last month, last, year, more | Q1,Q4 | Perfentage; Difference Q1 to |

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| | | | | |
|---|---|---|---|---|
| | in time-period | than one year ago, never) | | Q4 |
| Drug related problems | Self reported for the 11 SUD criteria | Dicothomic (yes/no) | Q1, Q4 | Perfentage; Difference Q1 to Q4 |

**Table 4.** Self administration trial (SAT) report

| **Domain** | **Measurement** | **Categories** | **Timepoints** | **Analysis metric** |
|---|---|---|---|---|
| Route of administration | Self-reported | 7 fixed categories | Q3a | Perfentage; |
| Ingested dose | Self-reported | Numerical (mg) or approximation | Q3a | Mean, median, Q1, Q3 and range (Box plot) |
| Duration and intensity of effects | Self reported | Visual analogue scale (VAS) of intensity of effects at each hour for 12 hours | Q3b | Mean and Standard deviation for each timepoint |
| Craving at comedown | VARS mm | Numerical | Q3b | Mean, median, Q1, Q3 and range (Box plot) |
| Subjective drug effects | Visual Analog scales (see next section for detail) | Numerical | Q3a Q3b | Difference from baseline. (Q3b-Q3a) |
| Ingested substance | GC/MS; LC/MS | Categorical, list of NPS | Depending on when the sample is sent and duration of analysis | Percentage, difference from expectation, |

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## Subjective effects assessed using visual analog scales (Q3a and Q3b)

| | | | | |
|---|---|---|---|---|
| Good drug effect | High | Stimulated | Euphoric | Energetic |
| Happy | Talkative | Calm | Self confident | Friendly |
| Alert | Social | Able to focus | Well coordinated | Self-accepting |
| Understanding of others feelings | Physically comfortable | Interested in self-reflection | Pleasurable body sensations | Forgiving yourself or others |
| Able to face my current life challenges | Please mark the minimum value (validity item) | Feel presence of numinous force, higher power, god | Remembering important moments of my life | Insightful about personal or occupational concerns |
| Able to hear others | Well treated, gentle | With improved sense of humor | Aware of my body | Emotionally open |
| Trusting of others | Creative | In control | Body lightness | Motivated |
| Attentive | Able to “let go” | Smiling | Honest | Positive |
| Patient | Sexy | Awe, amazement | | |
| Bad drug effect | Anxious | Sad or depressed | Sedated | Confused |
| Incompetent | Fearful | Mentally slow | Forgetful | Dizzy |
| Nauseous or like vomiting | Limb heaviness | Stomach pain | Tired | Miserable |
| Headache | Blurred vision | Irritable | Suicidal | Dry mouth |
| Jittery | Clumsy | Tremor | Muscle pain | Chills |
| Sweating | Aggressive | Numbness | Chest pain | Urge to move |
| Please mark the maximum value (validity item) | Experiencing difficulties to find words | Heart pounding or beating faster | Urge or craving to consume more | Hot or cold flushes |
| Itchiness | | | | |
| Any effect | Active | Changes in lights | Changes in colors | Passive |
| Increased sexual desire | Change in skin sensitivity | Different surroundings | Changes in distances | Changes in hearing |

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| | | | | |
|---|---|---|---|---|
| Please mark between the middle and the maximum (validity item | Hallucinations-seeing animals, things, insects, or people | Hallucinations-seeing of lights or spots | Different or changed unreal body feeling | Like reflecting about my childhood |

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# Annex 6: Ethical analysis by principles

## Non-maleficence

No harms to the study participants are expected. The cost for the participants will be their time answering the questionnaires and the shipment expenses related to sending the samples to the study laboratory. Their privacy will be kept using different overlapping strategies, such as log-in records, use of personal usernames and passwords for different research members with different access to data and encryption using PGP, among others. Also, the participants will only relate with the researchers with their virtual identities, which will also be protected. This has been described as pseudo-anonymous participation [25]. Finally, when the study ends, the only information linking the virtual identity of the participants (the email address) will be removed from the study databases.

The interactions in the GRASP platform will be monitored to avoid interventions that can lead to increased or harmful drug use. This will be done by the weekly review of all content and the eventual elimination of messages or participants that might cause harm to others. The lack of physical presence of the researchers and the absence of a controlled environment similar to the traditional clinical trials setting clearly reduces subjects perception of a hierarchical relationship between researchers and them, something that in previous research has been reported as positive, leading to a more symmetrical relationship between both roles [22,23].

## Respect for persons and autonomy

As commented above, the increased symmetry between researchers and participants will secure their autonomy and dignity. Also, active efforts will be made to enroll participants in a pro-active attitude towards all aspects of the study, including having access to the data and manuscripts before publication so their opinion and voice will be heard. This approach has been described by some authors as necessary for all human research in the 21st century [62].

## Individual subject benefits

The study participants will have the opportunity to use free drug checking services during their participation int he study. This is an established harm reduction practices that has shown to improve user's safety and, in some cases, save their lives [26, 27, 63, 64]. Thanks to free drug checking, the study participants will have the opportunity to discard adulterated samples or

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mislabeled samples containing other substances than expected, increasing the safety of a risky behavior they were already engaged in [27].

## Justice:

All researchers except the principal investigator will be blinded to the gender, race, or origin data of the participants, minimizing the potential for discrimination. However, the recruitment will happen globally, and it is likely that the recruited sample will not be representative of the global diversity in its multiple layers and intersections. The researchers have not been able to avoid this limitation and hope that the results of the study will help to better understand the profile of the population to better inform future studies in that regard.

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# Annex 7: Origin of the selected visual analog scales and assessed connotation

*Table 1.*

| | | "Please rate to which extent you feel..." | References |
|---|---|---|---|
| ITEMS WITH AT LEAST ONE REFERENCE IN LITERATURE | 1 | Good drug effect | *Hart, 2001, 2003; Haney, 1999; Brower, 1988; Riba, 2003; Camí, 2000; Farré, 1993, 1998; Gonzalez, 2015; Papasseit, 2018* |
| | 2 | High | *Hart, 2001, 2003; Haney, 1999; Brower, 1988; Riba, 2003; Camí, 2000; Farré, 1993, 1998; Gonzalez, 2015; Papasseit, 2018; Kleinloog, 2015; Bowdle, 1998* |
| | 3 | Stimulated | *Hart, 2001, 2003; Haney, 1999; Brower, 1988; Riba, 2003; Camí, 2000; Farré, 1993, 1998* |
| | 4 | Energetic | *Hart, 2001, 2003; Farré, 1993; Bond and Lader, 1974* |
| | 5 | Happy | *Bond and Lader, 1974* |
| | 6 | Talkative | *Hart, 2001, 2003* |
| | 7 | Calm | *Riba, 2003; Camí, 2000; Farré, 1998; Bond and Lader, 1974* |
| | 8 | Self confident | *Hart, 2001, 2003* |
| | 9 | Friendly | *Hart, 2001, 2003* |
| | 10 | Alert | *Hart, 2001, 2003; Bond and Lader, 1974* |
| | 11 | Social | *Hart, 2001, 2003* |
| | 12 | Well coordinated | *Bond and Lader, 1974* |
| | 13 | Attentive | *Bond and Lader, 1974* |
| | 14 | Bad drug effect | *Hart, 2001, 2003; Riba, 2003; Camí, 2000; Farré, 1993, 1998; Gonzalez, 2015; Papasseit, 2018* |
| | 15 | Anxious | *Hart, 2001, 2003; Haney, 1999; Brower, 1988; Farré, 1993; Kleinloog, 2015; Bowdle, 1998* |
| | 16 | Sad or depressed | *Hart, 2001, 2003; Haney, 1999; Brower, 1988; Riba, 2003; Camí, 2000; Gonzalez, 2015; Papasseit, 2018; Bond and Lader, 1974* |
| | 17 | Sedated | *Hart, 2001, 2003* |
| | 18 | Confused | *Hart, 2001, 2003; Riba, 2003; Camí, 2000; Gonzalez, 2015; Papasseit, 2018* |
| | 19 | Incompetent | *Bond and Lader, 1974* |
| | 20 | Fearful | *Riba, 2003; Camí, 2000; Gonzalez, 2015; Papasseit, 2018* |
| | 21 | Mentally slow | *Bond and Lader, 1974* |
| | 22 | Forgetful | *Hart, 2001, 2003* |
| | 23 | Dizzy | *Hart, 2001, 2003* |
| | 24 | Irritable | *Hart, 2001, 2003; Haney, 1999; Brower, 1988* |
| | 25 | Suicidal | *Hart, 2001, 2003* |
| | 26 | Tired | *Hart, 2001, 2003* |
| | 27 | Miserable | *Hart, 2001, 2003* |
| | 28 | Headache | *Hart, 2001, 2003* |
| | 29 | Blurred vision | *Hart, 2001, 2003* |
| | 30 | Nauseous or like vomiting | *Hart, 2001, 2003* |
| | 31 | Stomach pain (abdominal pain, upset stomach) | *Hart, 2001, 2003* |
| | 32 | Jittery | *Hart, 2001, 2003* |
| | 33 | Clumsy | *Hart, 2001, 2003; Bond and Lader, 1974* |
| | 34 | Muscle pain | *Hart, 2001, 2003* |
| | 35 | Chills | *Hart, 2001, 2003* |
| | 36 | Sweating | *Hart, 2001, 2003* |
| | 37 | Limb heaviness | *Hart, 2001, 2003; Haney, 1999; Brower, 1988* |
| | 38 | Numbness | *Hart, 2001, 2003* |
| | 39 | Heart pounding or beating faster | *Hart, 2001, 2003* |

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|---|---|---|---|
| | 40 | Any effect | *Riba, 2003; Camí, 2000; Farré, 1993, 1998* |
| | 41 | Active | *Riba, 2003; Camí, 2000; Farré, 1998* |
| | 42 | Passive | *Riba, 2003; Camí, 2000; Farré, 1998* |
| | 43 | Changes in hearing | *Riba, 2003; Camí, 2000; Gonzalez, 2015; Papasseit, 2018* |
| | 44 | Different or changed unreal body feeling | *Riba, 2003; Camí, 2000; Gonzalez, 2015; Papasseit, 2018* |
| | 45 | Hallucinations-seeing animals, things, insects, or people | *Riba, 2003; Camí, 2000; Gonzalez, 2015; Papasseit, 2018* |
| | 46 | Different surroundings | *Riba, 2003; Camí, 2000; Gonzalez, 2015; Papasseit, 2018; Kleinloog, 2015; Bowdle, 1998* |
| | 47 | Changes in distances | *Riba, 2003; Camí, 2000; Gonzalez, 2015; Papasseit, 2018* |
| | 48 | Changes in colors | *Riba, 2003; Camí, 2000; Gonzalez, 2015; Papasseit, 2018* |
| | 49 | Changes in shapes | *Riba, 2003; Camí, 2000; Gonzalez, 2015; Papasseit, 2018* |
| | 50 | Changes in lights | *Riba, 2003; Camí, 2000; Gonzalez, 2015; Papasseit, 2018* |
| | 51 | Hallucinations-seeing of lights or spots | *Riba, 2003; Camí, 2000;Gonzalez, 2015; Papasseit, 2018* |

Table 1. This table shows the items used in the Instrument 1 and its references in literature

*Table 2.*

| | | **CONNOTATION** | |
|---|---|---|---|
| | Positive | Negative | Neutral |
| ***ITEM*** ***" Please rate to which extent you feel..."*** | *Good drug effect* | *Bad drug effect* | *High* |
| | *Stimulated* | *Anxious* | *Alert* |
| | *Energetic* | *Sad or depressed* | *Blurred vision* |
| | *Happy* | *Sedated* | *Chills* |
| | *Talkative* | *Confused* | *Sweating* |
| | *Calm* | *Incompetent* | *Limb heaviness* |
| | *Self confident* | *Fearful* | *Any effect* |
| | *Friendly* | *Mentally slow* | *Changes in hearing* |
| | *Social* | *Forgetful* | *Hallucinations-seeing of lights or spots* |
| | *Well coordinated* | *Dizzy* | *Different surroundings* |
| | *Attentive* | *Irritable* | *Changes in distances* |
| | *Active* | *Suicidal* | *Changes in colors* |
| | | *Tired* | *Changes in shapes* |
| | | *Miserable* | *Changes in lights* |
| | | *Headache* | *Hallucinations-seeing animals, things, insects, or people* |
| | | *Nauseous or like vomiting* | |
| | | *Stomach pain (abdominal pain, upset stomach)* | |
| | | *Jittery* | |
| | | *Clumsy* | |
| | | *Muscle pain* | |
| | | *Numbness* | |
| | | *Heart pounding or beating faster* | |
| | | *Passive* | |
| | | *Different or changed unreal body feeling* | |
| *Total* | **12** | **24** | **15** |
| *%* | **20.53%** | **47.06%** | **29.41%** |

Table 2. This table shows the items of Instrument 1 and their connotation grouped in positive, negative and neutral. This shows that there are as many negative connotation items than those positive and neutral combined.

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*Table 4.*

| ITEM "Please rate to which extent you feel..." | CONNOTATION | | |
|---|---|---|---|
| | Positive | Negative | Neutral |
| | *Euphoric* | *Tremor* | *Aware of my body* |
| | *Able to focus* | *Chest pain* | *Body lightness* |
| | *Pleasurable body sensations* | *Urge or craving to consume more* | *Remembering important moments of* |
| | *Physically comfortable* | *Experiencing difficulties to find* | *Dry mouth* |
| | *Self-accepting* | *Urge to move* | *Change in skin sensitivity* |
| | *Understanding of others feelings* | *Aggressive* | *Like reflecting about my childhood* |
| | *Forgiving yourself or others* | *Hot or cold flushes* | *Feel presence of numinous force, higher* |
| | *Awe, amazement* | *Itchiness* | |
| | *Insightful about personal or* | *Unable to focus* | |
| | *Able to "let go"* | | |
| | *Interested in self-reflection* | | |
| | *Motivated* | | |
| | *Emotionally open* | | |
| | *Trusting of others* | | |
| | *Creative* | | |
| | *In control* | | |
| | *Able to face my current life* | | |
| | *With improved sense of humor* | | |
| | *Smiling* | | |
| | *Sexy* | | |
| | *Honest* | | |
| | *Positive* | | |
| | *Patient* | | |
| | *Well treated, gentle* | | |
| | *Able to hear others* | | |
| | *Increased sexual desire* | | |
| *Tot* | **26** | **9** | **7** |
| *%* | **61.9%** | **21.43%** | **16.67%** |

Table 4. This shows the new items grouped by their connotation. The percentage of positive items is larger than shown in

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# Annex 8: Study published protocol

Please find published paper at the end of the document as supplementary material and also online using this link: https://www.researchprotocols.org/2021/7/e24433

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# Annex 9: References published by the Ph.D. candidate

These publications can be found at the end of the document after the aforementioned published protocol and also in the links detailed below:

1) Papaseit E, Farré M, Pérez-Mañá C, et al. Acute pharmacological effects of 2C-B in humans: An observational study. Front Pharmacol. 2018;9(MAR):1-10. doi:10.3389/fphar.2018.00206
    a. Link: https://repositori.upf.edu/handle/10230/34357
2) Papaseit E, Farré M, Pérez-Mañá C, et al. Acute pharmacological effects of 2C-E in humans: An observational study. Front Pharmacol. 2018;9(MAR):1-13. doi:10.3389/fphar.2018.00206
    a. Link: https://repositori.upf.edu/handle/10230/44402
3) Poyatos L, Papaseit E, Olesti E, et al. A Comparison of Acute Pharmacological Effects of Methylone and MDMA Administration in Humans and Oral Fluid Concentrations as Biomarkers of Exposure. Biology (Basel). 2021;10(8):788. doi:10.3390/biology10080788
    a. Link: https://www.mdpi.com/2079-7737/10/8/788
4) Papaseit E, Olesti E, Pérez-Mañá C, et al. Acute Pharmacological Effects of Oral and Intranasal Mephedrone: An Observational Study in Humans. Pharmaceuticals. 2021;11:1-13. doi:10.3389/fphar.2020.00233
    a. Link: https://www.mdpi.com/1424-8247/14/2/100

Protocol

# Self-reported Subjective Effects of Analytically Confirmed New Psychoactive Substances Consumed by e-Psychonauts: Protocol for a Longitudinal Study Using a New Internet-Based Methodology

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## Abstract

**Background:** During the last few years, the continuous emergence of new psychoactive substances (NPS) has become an important public health challenge. The use of NPS has been rising in two different ways: buying and consuming NPS knowingly and the presence of NPS in traditional drugs as adulterants. The rise of NPS use is increasing the number of different substances in the market to an extent impossible to study with current scientific methodologies. This has caused a remarkable absence of necessary information about newer drug effects on people who use drugs, mental health professionals, and policy makers. Current scientific methodologies have failed to provide enough data in the timeframe when critical decisions must be made, being not only too slow but also too square. Last but not least, they dramatically lack the high resolution of phenomenological details.

**Objective:** This study aims to characterize a population of e-psychonauts and the subjective effects of the NPS they used during the study period using a new, internet-based, fast, and inexpensive methodology. This will allow bridging an evidence gap between online surveys, which do not provide substance confirmation, and clinical trials, which are too slow and expensive to keep up with the new substances appearing every week.

**Methods:** To cover this purpose, we designed a highly personalized, observational longitudinal study methodology. Participants will be recruited from online communities of people who use NPS, and they will be followed online by means of a continuous objective and qualitative evaluation lasting for at least 1 year. In addition, participants will send samples of the substances they intend to use during that period, so they can be analyzed and matched with the effects they report on the questionnaires.

**Results:** The research protocol was approved by the Institutional Review Board of the Hospital del Mar Research Institute on December 11, 2018. Data collection started in August 2019 and was still ongoing when the protocol was submitted (September 2020). The first data collection period of the study ended in October 2020. Data analysis began in November 2020, and it is still ongoing. The authors expect to submit the first results for publication by the end of 2021. A preliminary analysis was conducted when the manuscript was submitted and was reviewed after it was accepted in February 2021.

**Conclusions:** It is possible to conduct an institutional review board–approved study using this new methodology and collect the expected data. However, the meaning and usefulness of these data are still unknown.

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**KEYWORDS**

psychotropic; psychoactive; psychonautic; longitudinal; observational; pharmacology; psychopharmacology; subjective effects; sentinel; mental health; public health; internet; eHealth; cathinones; drugs of abuse; psychedelics; mobile phone; smart phone; online recruitment; online forums

# Introduction

## Importance of New Psychoactive Substances

To date, new psychoactive substances (NPS) still represent a very important challenge to legislate, monitor, study, and develop health interventions. The understanding of use patterns remains poor, with most information being based on populations and settings where problems have already occurred [1].

The ever-increasing number of psychoactive substances used nowadays represents a new challenge for psychiatry, as the pharmacodynamics and pharmacokinetics of many NPS are not yet thoroughly understood [2]. In addition, NPS consumption rarely occurs in isolation from other habits but, on the contrary, is placed within a kaleidoscopic range of poly drug use trajectories. There seems to be no differential risk for NPS use compared with the use of traditional psychoactive substances such as alcohol, cannabis, or cocaine [1].

This new phenomenon represents an unprecedented challenge in the field of drug use as well as a fast-growing problem from social, cultural, legal, and political perspectives [3].

## NPS: Definition

NPS are substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances but which may pose a public health threat. It is important to note that different authors have previously referred to them as *designer drugs*, *legal highs*, *herbal highs*, *bath salts* and *research chemicals*. Moreover, the term *new* does not necessarily refer to new inventions but to substances that have recently emerged on the market [4]. Hence, *new* can include a failed pharmaceutical or an old patent that has been *rediscovered* for *recreational* substance [2].

Another distinction being made is between NPS and emerging psychoactive substances, where the latter term captures all NPS as well as drugs that may not be newly invented but have recently experienced a resurgence of, or increase in, use [2]. However, to simplify this work, only the term NPS will be used, also including all emerging psychoactive substances. Most NPS are the result of minor changes to the molecular structure of well-known legal or illegal drugs, such as opioids, ecstasy, or stimulants [5].

Between 2009 and 2017, 803 NPS were reported in 111 countries or territories [2,6]. In the European Union, by the end of 2017, the number of NPS was over 670, of which 632 were notified after 2004 [2,7]. However, evidence suggests that the NPS scenario could be much larger than that formally identified by international agencies. In a recent publication, Schifano et al [2] used a web search engine to identify NPS discussed online by NPS enthusiasts. Using this methodology, they identified a few thousand NPS, a number which is about 4-fold higher than the figures suggested by European and international drug agencies.

There is an ongoing debate on the scale of challenges posed by NPS, as the evidence on the prevalence of NPS use is scarce. For example, general population surveys suggest that the prevalence of NPS use is relatively low, with the best estimates found in the scientific literature being between 1% and 2% in the United Kingdom. However, the speed of technological innovation and the ease of synthesizing NPS present substantial challenges to regulatory authorities, researchers, and clinicians [5,8].

## NPS: Challenges

NPS may now pose a big challenge due to several factors:

1. NPS consist of several different classes of substances, which vary in their psychological and physiological effects. Treatment is often difficult because of the young age of most users and the possibility of concurrent polysubstance use. The pattern of use is often intermittent in social settings, so it may be perceived as less of a problem [9].
2. NPS appear into—and sometimes disappear from—the market very quickly, and as such, they are not significantly impacted by regulatory efforts. Currently, new substances are identified in Europe at a rate of one or more per week [10]. Several key studies have shown the continued use and popularity of mephedrone, a popular NPS, among specific drug-using populations after it was brought under control. The scheduling of new substances could even increase the speed at which manufacturers innovate, to bypass the law [11].

3. NPS are mainly distributed through the internet in a transnational market without solid information about their effects and risks [11]. During recent years, the widespread availability of internet access has led to a gradual, although only partial, shift from a *street* to a *web* market [12]. The increased web-based distribution has been seen in both the surface web and *dark net* [13].
4. NPS can substitute traditional drugs in times when their availability is restricted [8]. This could be problematic, as this substitution happens both by introducing new substances in the market as well as by selling NPS as traditional drugs, exposing large populations, unknowingly, to the effects of a new unstudied substance without previous experiences. This is especially dangerous, combined with the rapid turnover of NPS, as they change before we can obtain research data using conventional methodologies [5].
5. There is a concerted effort to grapple with the challenges of researching NPS, as traditional methodologies are too slow and expensive to generate relevant and timely data on the effects of NPS [5].
6. Clinicians are not usually able to identify a potential NPS user, and NPS usually produce negative results to traditional drug tests, which are designed to assess a very limited number of traditional substances [6]. On the other hand, NPS users rarely search for professional help linked exclusively to this problem, and clinicians are not trained to screen or identify NPS use.

## What Has Been Done and What Is Needed in NPS Research?

Despite the high number of publications about NPS during the last 20 years, especially after a sharp increase in 2010, there are still concerning *gaps in our understanding* of the phenomena. From the evidence map about the NPS research performed by Mdege et al [8], 2 things appear quite striking:

- First, most of the studies were performed in a general hospital population (118/294, 40.1%) or specialist settings (24/134, 18.2%), with relatively *low rates of studies coming from the internet population (*5/59, 8%). In addition, these studies mainly reported severe intoxication or other acute NPS-related problems.
- Second, the most frequent study design reported in the indexed peer-reviewed literature was case series and/or reports (n=367), followed by the literature review (n=243), the survey (n=130), and the secondary quantitative data analysis (n=99), with only 13 existing randomized controlled trials, 6 prospective cohort studies, and 1 case-control study [5,8].

There are also some *specific limitations* to the research performed till date. Although the most robust and representative data on NPS use are for mephedrone (surveys have been conducted in the United Kingdom since 2010), Mdege et al [8] acknowledge important limitations to this most robust research. For example, although participants may report using a substance, the names of NPS are sometimes used interchangeably, and *there is no analytical confirmation* of the true compound that was taken. Therefore, there is inherent uncertainty in the reported use of a particular NPS.

The same authors also reported that sentinel populations are likely to be at a greater risk of NPS use. However, it remains mostly limited to attendees of nightclubs where different sexual orientations are accepted. Other authors have also remarked that only a handful of studies have moved beyond prevalence to explore subjective user experiences and motivations [11].

Currently, the potential data sources that can provide some information on the acute effects of NPS consumption are as follows: (1) user self-reports on internet discussion forums, (2) surveys answered by users, and (3) fatal and nonfatal case reports.

Self-reports and surveys are mostly based on self-reported use rather than the analytical confirmation of the substances used. In contrast, case reports are usually generated from hospital settings in the context of an intoxication or overdose with multiple substances involved, so there is analytical confirmation of the substance but no self-reported effects. Unsurprisingly, the literature is dominated by studies investigating the problems associated with NPS (773/995, 77.7% of records). Therefore, caution is required when interpreting these data because of the following limitations:

- Users will report what they believe they have used, rather than whatever substance is actually taken [10].
- Intoxications with multiple substances in hospital settings do not target the information on psychopharmacological effects of a particular NPS [5,8].

In their empirical and conceptual review to produce research recommendations, Mdege et al [8] provide the following advice for research, among others:

1. The need to be aware of innovation opportunities, such as testing emerging NPS brands online as they become available.
2. Using cohort study designs to better understand the determinants of NPS use and related physical and mental health, psychosocial problems, and how patterns of involvement and consequences change over time.
3. What are the prevalence and patterns of NPS use in the general UK population and do they differ between subgroups of the population?
4. Are there sentinel populations capable of being monitored to provide early warnings of new trends?
5. Which acute intoxication problems are associated with NPS use?
6. Which promising approaches are currently available or can be made available in the United Kingdom for intervening with NPS use?

Finally, they concluded that there is a need for a major research effort to be directed at NPS, which should address NPS together with other forms of licit and illicit drug use [5,8].

## The e-Psychonaut Population

Both the limitations and recommendations stated above lead to the *necessity of conducting a longitudinal study in a specific and potential sentinel population*, such as internet NPS–consuming communities. This would allow for early assessments of the effects of recently emerged drugs and to

study the patterns of consumption, harm reduction strategies, and long-term drug-related problems.

Available data on sentinel populations are growing. For example, several studies of attendees of gay-friendly night clubs suggest that the trend in reduction of mephedrone witnessed nationally may also occur in this subgroup. However, the study of this sentinel population has failed to predict future harms and trends in the global NPS market [5].

Conversely, data on another sentinel population, namely, e-psychonauts, have been able to predict future NPS-related harms occurring in more general settings [2].

In fact, the sentinel population of *e-psychonauts* has been considered by several authors as potentially useful in identifying NPS availability, market, and diffusion in advance. This population is believed to be responsible for shaping and influencing the drug scenarios of the future [13]. In addition, Corazza et al [3] provided evidence supporting the claim that the online NPS scenario predicts the real-life NPS scenario.

The term psychonaut was first described by Newcombe [14] as an adult user of psychoactive drugs who takes these substances in *normal, everyday settings* with the intention of subjectively exploring their effects.

Some years later, O'Brien et al [11] coined the term *cyber-psychonauts* to refer to their sample composed predominantly of NPS consumers. Cyber-psychonauts are further defined by their commitment to harm reduction, to using NPS safely and responsibly, and to purchasing chemicals online [8].

Tackett-Gibson [15] also documents the existence of online communities populated by self-defined *experts* in using NPS, providing a contrasting narrative around drug use and risk to that established by the scientific community. A brief perusal of relevant websites confirms the existence of a great number of NPS-related discussion threads, suggesting the existence of an online community of more discerning NPS users [15].

Orsolini et al [13] also refer to this population in their more recent study, identifying *educated and informed* users within web-based drug forum communities, who can provide reliable information on psychoactive compounds. They refer to these users as *e-psychonauts*, providing the best characterization of the population to date [13]. The e-psychonauts appear to be mainly young and unmarried White males, presenting good or excellent employment conditions and with a set of key skills, including awareness to their inner *soul*; high standards of knowledge about drugs' chemical and pharmacological issues; and high levels of both technology-related skills. They are meant to have a wide vocabulary to define their own *on drug* experiences in the most subtle and precise way possible.

Among this population, the frequency of NPS use is high, with one-third of the participants reporting its use in the last week. They view themselves as knowledgeable consumers who use the internet to accumulate information about NPS and share their own experiences, informing fellow users of potential harms. However, other studies [16] reported possible stimulant dependence (3 or more dependence symptoms) in 30% of mephedrone users. Mdege et al [8] also found that NPS users often report substance use disorder symptoms, especially craving.

This community may have some other distinct characteristics. A minority of the sample reported that an NPS was the first drug that they had ever taken. Of those who ceased using NPS, majority found it either easy or very easy to stop. Most commonly, cessation was due to the side effects of NPS [17]. They also perceived internet forums as an important channel through which to communicate information on new drugs, and retailers reported monitoring forums to determine which drugs to stock in their store [8]. These users also tend to post online warnings based on first-hand experiences about the potential harms of the substances consumed and are willing to avoid harm to their peers. Orsolini et al [13] even stated that posting online the *on drug* experience report is arguably the *trait d'union* of all e-psychonauts, considering the intention behind using a substance is the most significant difference between a psychonaut and a typical drug user. O'Brien et al [11] also identified the role of e-psychonauts in disseminating emerging information about NPS-related harm and considered them well equipped to make a valuable contribution to NPS policy debates in general, and e-psychonauts are ideally placed to report on the effects of recent policy changes on NPS-related harms in particular.

Several authors have tried to engage cyber-psychonauts as research participants. Mdege et al [8] found difficulties in involving NPS users throughout the project due to a lack of willingness on the part of NPS users to be contacted in ways other than email. In addition, working with this population has inherent sampling problems: internet research participants are, by definition, a nonrandom and self-selecting sample, and it is very difficult to know the characteristics of the overall pool from which the sample is drawn [18,19].

Different authors believe that these internet communities are a huge opportunity for researchers. The qualitative analysis of how different groups interact with online communities may help to systematize and codify needs, values, and preferences that are relevant to the group [20]. In internet communities, researchers can simply recruit participants or even go further and engage drug users more fully in dialog [21]. Some authors even state that the lack of physical presence and separate physical settings all reduce researcher control and power, thereby potentially leading to a more balanced relationship between researchers and participants [22,23]. In any case, e-psychonauts are a hidden, hard-to-reach population that may have a significant influence on future drug trends.

Some authors even consider cyber-psychonauts to be ideally placed to become involved in the actual implementation of innovative responses to the increasing prominence of NPS markets, as it is difficult to imagine a more efficient method for the rapid dissemination of new information about things such as the adverse effects of new products to consumers [24].

The recent alarm related to the growth of the NPS market and the gradual shift from the street to the cyber-drug market may call for the implementation of preventive tools and practices tailored to these new drug users' characteristics [13].

Finally, in their empirical and conceptual revision of the NPS research field, Mdege et al [8] concluded that there was a clear need to move beyond an expert-driven discourse on NPS and involve people who use NPS as active and valuable research collaborators and stakeholders instead of passive research participants.

It is clear then that this sentinel population might be difficult to reach and retain in a highly structured study protocol [5,8,18,21,25]. However, the collaboration of the Energy Control (EC) International Drug Checking Service might provide a critical opportunity for recruitment, offering a free chemical analysis of the substance they want to consume. This service is already being used by this population in the main internet communities of psychonauts, and it has a well-regarded institutional presence in most of them [26,27].

### Relevance and Goals of This Study

In summary, NPS pose a public health threat at different levels, and there is a lack of research on the effects of the emerging substances as well as on which ones are appearing now. In addition, the research conducted to date has been unable to cover important gaps, such as studying relevant sentinel populations of e-psychonauts using new technologies, involving them in the research, and obtaining confirmatory analytical data of the substances studied. Old methodologies repeatedly fail to reach the skyrocketing turnover pace of newer NPS in the e-market. By the time old trials recruit the necessary drug *X* study participants and engage them in the old trial machinery, drug *X* has already become obsolete and has been replaced by *Y* and—possibly—even *Z* drugs.

This study aims to bridge the abovementioned evidence gaps. To do so, a naturalistic, observational, and longitudinal design has been adopted, recruiting e-psychonauts to gather information on their characteristics and the substances that they might be using before their popularization. This has been possible thanks to the development of a new online ad hoc tool designed for this project: an online platform thought to enhance communication with the e-psychonauts and allow community building. In addition, subjective effects on these substances have been studied, allowing the participants to send samples to a partner laboratory with gas chromatography (GC)/mass spectrometry (MS) in Barcelona and administering drug effect questionnaires in the most resembling way possible to the drug laboratory studies.

This design has resulted in the first internet-based, multinational study on a key sentinel population with laboratory confirmation on the composition of the reported samples.

### Study Objectives and Hypothesis

The study has been designed to answer 3 main research questions:

1. Who are the people who first try the new substances when they emerge in the market?
2. What are the substances emerging right now and their subjective effects?
3. Is it possible to collect reliable data to answer these questions using a low-budget internet platform and the design used in this study?

The researchers' initial hypotheses are as follows:

1. The population of e-psychonauts will be made up of functional and educated people who use drugs mainly in a recreational way.
2. During the study period, we will be able to identify a wide variety of different substances, some of which have never been reported before in the scientific literature or by the organizations aimed at controlling illicit drug supply.
3. The study design and implementation will attract enough research participants with sufficient commitment to provide valuable, reliable, and meaningful data to generate quality evidence.

## Methods

### Overview

This study aims to discover the characteristics of the e-psychonaut population and the effects of the NPS they use, with a longitudinal design and no control group. The study is conducted online, recruiting participants using an innovative and specifically developed platform as part of the study project: Global Research and Analysis of New Substances Project (GRASP).

The study has been designed and will be reported using the Checklist for Reporting Results of Internet E-Surveys [28] and the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement checklist for observational studies [29], with the support of the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) statement checklist of 2013 [30].

The study protocol was submitted in October 2018 and was approved by the Clinical Research Ethics Committee (Parc de Salut Mar, Barcelona, Spain, ref. 2018/8283/I) in January 2019. The study was conducted according to the Declaration of Helsinki recommendations and the emerging recommendations for online research on sensible topics [21,25]. All data collected online on the participants were encrypted according to the European and Spanish data protection regulations (2016/679 European Parliament and 27/4/16 reglamento general de protección de datos [general law about data protection] Spanish Royal Decree).

### Study Setting

The study is conducted mainly though internet, using 3 main tools:

1. The specifically designed GRASP platform
2. The *Qualtrics* survey service licensed though Columbia University
3. The Google Suite platform as an email service to contact candidates and attend to the private questions and concerns of the study participants.

In addition, the samples were received through traditional mail in the EC Headquarters in Barcelona, where they were initially

processed and identified. The samples were then transported to the EC laboratory at the Hospital del Mar Research Institute (IMIM), second floor, to be analyzed using the techniques described below. The research team worked at both the EC headquarters and the IMIM laboratories. During the COVID-related lockdown that was established in Spain in March 2020, the laboratory analysis was interrupted for 3 months.

The usability of the platform and the multiple automated processes, such as *sending an email with a specific link to a questionnaire*, and the logic pathways (adaptive questioning) and validation requirements used in the *Qualtrics* questionnaires were systematically tested by the research team. A checklist of all possible scenarios was devised, and they were all executed by a blind research team member and the principal investigator. Once errors were identified, they were corrected, and the process was repeated from the beginning. In addition, participants were encouraged to report any problems or ideas to improve the procedures, so changes could be implemented when needed during the study.

## Participants

All the participants were correctly and fully informed by writing (refer to the participant information sheet in Multimedia Appendix 1) and prompted to ask any questions by email. In that case, answers were provided until the candidate confirmed that they had no more questions and were satisfied with the information received. All participants indicated their agreement to participate and signed an informed consent (IC) form (Multimedia Appendix 2) that was sent to the project email address and checked by the principal investigator before inclusion. It was not possible for candidates or participants to answer any online questionnaires without previously receiving the specific link, which was sent by the research team only when the participant met the criteria to fill the questionnaire. Participants were asked to sign with their online usernames to further protect their physical identity, as recommended by Barratt et al [21,25]. Participants received no monetary compensation for their participation, but instead, they were offered the possibility to get the NPS they reported on analytically tested for free in the EC laboratory, located in the *IMIM*, Barcelona. The cost of this service is US $110 if contracted independently through the EC International Drug Checking Service.

This study included e-psychonauts, who have been defined as people with the following characteristics:

1. Previous experience with at least three NPS 12 months before the study inclusion
2. Activity on online communities where NPS consumption is discussed.

The inclusion and exclusion criteria for participants are provided in Textbox 1.

**Textbox 1.** Inclusion and exclusion criteria for participants.

**Inclusion Criteria**

- Meeting the operational definition of e-psychonaut
- Being 18 years or older at the time of recruitment
- Self-reported plans of maintaining the use of new psychoactive substances for the following 18 months

**Exclusion Criteria**

- Difficulties in communicating in English
- Difficulties in using new technologies to participate in the study
- Potentially pregnant women
- Potential presence of severe psychopathological symptomatology

Note that there is no restriction on the geographical location of the participants, as the study will not collect such data to further protect the participants' physical identity. Therefore, participants from around the globe could participate in the study. Both inclusion and exclusion criteria have been mainly assessed by direct self-reporting in an initial screening questionnaire (Q0), except the following:

1. Previous participation in forums has been assessed by self-report and exclusive advertisement of the study on these forums.
2. Difficulties in using new technologies have been assessed by the steps required to complete the screening process, such as sending an IC form in a particular format, registering to the platform, and following the instructions there to introduce themselves to the research team and other participants.
3. Difficulties in communicating in English and the presence of potential psychopathological impairments have also been evaluated by the principal investigator, assessing the answers to long and elaborate open questions in the screening questionnaire (Q0) and in the written introductions to the online platform.
4. The potential of being pregnant was assessed by indirect questioning using the same screening questionnaire (Q0) questionnaire.

## Recruitment

Recruitment ads were sent to the moderators of the selected online communities after establishing bilateral communication with them, mainly to ask permission and explain the goals of the project. To maximize interest in participating in the study, the only focus was on establishing rapport with community leaders, as if they share their interest in the study, they will be

able to transfer it to the rest of the community [31]. Each community moderator posted the research ad in the most appropriate way in their community after discussing it with the research team. Research ads were posted on all communities during the summer of 2019 (refer to Multimedia Appendix 3 for details). The recruitment was designed to be sequential until the designed sample size was reached or the study reached its duration limit.

The operational definition for online communities of people who use NPS has been adapted from the study by Barratt [21]:

1. Surface websites with at least 5 years of existence
2. Presence of participation forums dedicated to discussing the use of NPS
3. At least weekly activity on the community forum
4. The use of pseudo-anonymity by community members to identify themselves
5. The presence of official and analytical drug checking services in the community.

When the study design was completed (October 2018), there were 4 communities meeting the previously stated criteria [21]:

1. *Bluelight* [32]: Established in 1997, bluelight is probably the most prominent community of people who take illicit drugs, with approximately 250,000 members. Within the community, there is a subdivision in which the use of NPS is exclusively discussed. The community is known for its commitment to promoting risk management and harm reduction strategies among its participants as well as its formidable contributions to similar research projects [28]. Registration is required to access the content.
2. *Reddit* [33]: Established in 2010, this subreddit community allows almost any type of discussion regarding NPS. The community has approximately 90,000 members, but it is part of a broader community of people who use illicit drugs (not only NPS), with over 700,000 members. Both of these are part of the global reddit community, where all types of topics are discussed. The platform does not require registration to access the content.
3. *Drugs-forum* [34]: Established in 2003, this community also seems to have approximately 250,000 members. Registration is required to access the content.
4. *DNstarsVIP*: Established after discussing about NPS sources was banned on the *reddit* community, *DNstars* is a strongly emerging community with approximately 2000 users. Registration is required to access the content.

The main communities that were assessed and excluded were as follows:

1. *Legal-highs forum*: excluded because of the lack of weekly interactions, technical website problems, and impossibility to contact community managers.
2. *Erowid*: excluded because of the lack of an active forum.
3. *Psychonaut wiki*: excluded because of the lack of an active forum.
4. *Tripsit*: excluded because of the lack of an active forum, although there was an internet relay chat–supported chatroom.
5. *Dimethyltriptamine-nexus:* excluded because of the lack of a specific NPS subsection.
6. *Ecstasy data*: excluded because of the lack of an active forum and the lack of a specific NPS subsection.
7. *Shroomery:* Excluded because of the lack of a specific NPS subsection.

To the best of the authors' knowledge, the selected communities were the main ones at the moment when the selection occurred (October 2018), although it has to be acknowledged that this is a rapidly changing scenario; in a few years, this same process might produce different outcomes. At that moment, the authors were unable to find any contradicting information with that assumption. Soussan et al [35] referred to *bluelight*, drugs-forum, and legal-highs forum as the top 3 communities. However, they did not consider collaboration with drug checking organizations to be a relevant factor. Moreover, as stated above, these rankings are expected to change over relatively short periods [35].

The GRASP platform, which allowed for interaction among participants themselves and with the research team, was the main tool to promote participant engagement and minimize dropout rates.

## Sample Size

In the most recent review consulted by the authors, the sample sizes reached with web-based questionnaires in people who use illicit drugs ranged from 80 to 9867 [36]. The expected losses while filling these types of questionnaires are about 50% of the sample, but the authors have not found other online longitudinal studies including questionnaires like this one. According to the aforementioned review, the authors expected 80 candidates as the best possible estimate to achieve 40 final participants. Assuming that each participant takes one NPS every month during the duration of the study, the expected number of registered self-administration trials of NPS would be 480. As the study has been designed as exploratory, the sample size could not be determined based on the needs to perform specific statistical tests.

## Study Procedures and Timeline

The study's internal timeline and workflow are graphically represented in Multimedia Appendix 4. The first recruitment effort consisted of online discussions with forum moderators and posting the institutional review board (IRB)–approved announcement for candidates (displayed in its entirety in Multimedia Appendix 3) in those forums. In the announcement, potential candidates were instructed to send an email from a secure and nonidentifiable address to the research team (admin@grasp.pw). Candidates were then informed more broadly about the study. Candidates were informed homogeneously by sending the IRB-approved *information for candidates' sheet* to their email (the sheet used in this study can be found in Multimedia Appendix 1). The principal investigator then offered the candidates to answer any questions that might have arisen after reading the participant information sheet. When the participants had read and discussed the given information about the study with the principal investigator, they were asked to register on the GRASP platform and send the IRB-approved

IC form, completed with the registered username to the study email. In Multimedia Appendix 2, the IC form is available for consultation. Finally, the candidates were asked to complete the screening questionnaire (Q0) and introduce themselves on the platform without providing information that might reveal their real-world identities. When all these processes were complete, the principal investigator checked the IC form, the screening questionnaire, and the platform introduction to assess if the participant met the inclusion or exclusion criteria. Candidates were then informed by email about the results of the assessment, thus either being rejected or accepted as participants.

Once participants were accepted, they could interact with other participants on the online platform and received detailed instructions on how to conduct the study. However, NPS sourcing and the effects of the substances included in the study were not allowed. In case of a severe protocol violation such as this one, participants were immediately removed from the study and their information was deleted. In case of minor protocol violations, participants were notified and given the opportunity, if applicable, to amend their noncompliant behavior.

The first mandatory step was to fill a sociodemographic and drug use history questionnaire (Q1). This questionnaire was available to each participant through a participant-specific link, which was sent by email once they were accepted. After that, participants were asked to fill the sample submission questionnaire (Q2), where information about the sample they intended to consume was asked. This questionnaire was then reviewed by the principal investigator and approved if the substance met the study criteria of being a new psychoactive substance. The samples that did not meet the inclusion criteria were not accepted, and the participant was notified by email. The sample submission questionnaire (Q2) was available to all accepted participants as a link on the platform. All the questionnaire answers were reviewed weekly by a member of the research team to communicate to the participant the acceptance of the sample and to mark them as valid or invalid data for later analysis.

If the sample was approved, the participant received a specifically generated sample code with the instructions to send a small amount of the sample (approximately 30 mg, usually below the psychoactive threshold) via traditional mail to the laboratory at the IMIM. The sample was analyzed there, and the result was sent back to the user, along with harm reduction advice when appropriate.

Meanwhile, the users could consume the substance whenever they decided, as the study was intended to be observational. However, most of the participants waited until they had the result of the laboratory analysis to proceed with the self-administration trial. The self-administration trial started with the users filling the drug effect baseline questionnaire (Q3a), and then, they consumed the reported substance and filled the drug effect questionnaire (Q3b) 24 hours after filling the baseline questionnaire (Q3a). The links to these questionnaires were available for all participants in the forum, and the veracity of the information was ensured by asking information only available to each participant, such as the sample code of the reported sample.

Study recruitment began in August 2019 and is still ongoing. In August 2020, the first participant concluded the 1-year follow-up.

### IRB-Approved Protocol Changes During the Study

The protocol has been subjected to amendments twice, both approved by the IRB of the institution (Clinical Research Ethics Committee-IMIM).

The first amendment, submitted in January 2019, reported the following changes in the protocol:

1. Minor changes in the study advertisement sheet, participant information sheet, and IC form
2. The assessment of inclusion criteria was no longer done by the community moderators and was entirely assessed by self-reporting on online questionnaires
3. An increase in the required sample quantity to be sent to the laboratory from 30 to 50 mg by default, accepting exceptions depending on the substance potency
4. Addition of a key measurement timepoint at baseline before ingesting the substance.

The second amendment, submitted in November 2019, reported the following changes in the protocol:

1. Unblinding of the research team to the participant behavior and participation
2. Addition of an optional timepoint for data collection in the reporting of the subjective effects of the reported substances
3. Extension of the duration of the study from 6 months to 1 year for each participant
4. Reduction of the required age for inclusion from 21 to 18 years.

### Outcomes

The domains and measurements used in the study are based on previous laboratory studies, to maximize consistency in methodology and to eventually develop a validation study for this methodology. Multiple studies have shown that web-based data collection and traditional methods (eg, paper and pencil) result in equivalent conclusions, demonstrating the validity and reliability of online data collection for research [37,38].

Certain studies have been used as model references to select the measured outcomes [39-52]. However, new outcomes regarding the subjective effects of psychoactive substances have been added to balance the amount of positive and negative effects reported. The order of the questions was kept the same to facilitate reports to those participants who completed the same questionnaire multiple times. However, 3 questions to assess validity were present in both the Q3a and Q3b. More information about study outcomes, including assessed domains, chosen measurements, metrics, and time points, can be found in Multimedia Appendix 5.

## Data Collection

### *Questionnaires*

The study data were collected using a Columbia University *Qualtrics* license, a well-known questionnaire platform compliant with the guidelines to store sensible information about the research participants. The screening questionnaire (Q0) and the sociodemographic questionnaire (Q1) collected self-reported information about the participants' medical and drug history, psychosocial situation, and beliefs and behaviors related to drugs.

The subjective effects of drugs were assessed via visual analog scales, using the same parameters used in most laboratory studies to determine the subjective effects of drugs. The main outcome was the difference in mm from the drug effect questionnaire (Q3b) at 24 hours (referring to the peak experience) and the baseline questionnaire (Q3a).

Each questionnaire also had at least one validity entry to be filled by the researcher directly using the *Qualtrics* database. There were no automated consistency or completeness checks before the questionnaire was submitted, other than the validation criteria for certain questions. For example, the question *sample code reported* could not be submitted if the answer was not a 5-digit number.

The participants could go back through the questionnaire, but once submitted, they could not change their answers. A summary of the answers was not displayed either before or after submission. Two options were available to change the participants' answers if they were incorrect according to the participant or not valid according to the researcher. If the change was small, the researcher could just edit the participant response in the *Qualtrics* database according to the correct response provided by the participant using email or the platform's private messaging system. If the changes were relevant, the researcher could mark the questionnaire as invalid and provide another link to the participant.

Public links to copies of the used questionnaires can be found in the references cited below:

1. Q0, screening questionnaire [53]: contained a total of 37 questions
2. Q1, sociodemographic questionnaire [54]: contained a maximum of 351 questions, with an expected average per participant of 50, due to adaptive questioning and questionnaire logic
3. Q2, sample submission questionnaire [55]: contained a total of 21 questions
4. Q3a, baseline drug effect questionnaire [56]: contained a total of 12 questions
5. Q3b, drug effect questionnaire given 24 hours after drug administration [57]: contained a total of 39 questions
6. Q4, 1-year follow-up questionnaire [58]: contained a maximum of 351 questions, with an expected average per participant of 50, due to adaptive questioning and questionnaire logic.

The number of pages and items on each page were optimized automatically by *Qualtrics* software and varied according to the screen size used to answer. The possibility to answer the questionnaires comfortably from the smartphone was assessed as an essential by the research team.

### *Laboratory Analysis*

Preliminary sample identification was performed by GC coupled to MS using an Agilent 7890B gas chromatograph coupled to a 5977A quadrupole mass spectrometer detector (Agilent). The gas chromatograph was fitted with a G4513A auto-sampler injector. Insert liners packed with salinized glasswool were used, and the injector and interface were operated at 280 °C. Samples were injected in split mode into a 0.25 mm film thickness (5% phenylmethylsilicone) column (HP-5MS, Agilent Technologies). Helium was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was initially maintained at 90 °C for 2 minutes and programmed to reach 320 °C at 20 °C/min. It was finally maintained at 320 °C for 9.5 minutes (total run time was 21.5 min). The mass spectrometer was operated in the electron impact ionization mode at 70 eV. To confirm the mass spectra, 4 libraries were used: the NIST/EPA/NIH Mass Spectral Library, Data Version: NIST 14; Searchable Mass Spectral Library Version 2.3 [59]; Searchable Mass Spectral Library Cayman Spectral Library [60]; and EC's internal mass spectral library. Confirmation (when needed) was performed by liquid chromatography (LC) coupled to tandem MS (LC/MS/MS) using an Agilent 1100 series HPLC (high performance liquid chromatography) chromatograph (Agilent Technologies) and an Esquire 3000 plus mass spectrometer MRM (Bruker Daltonic GmbH). Chromatography was performed using a Poroshell 120 EC-C18 column (100 mm length×2.1 mm internal diameter; 2.7 mm particle size) at 30 °C. The mobile phases consisted of 1% formic acid and 1% formic acid in methanol. The following gradient elution was used: at time 0 minute, 15% B was changed to 90% B in 7 minutes, held for 1 minute, and changed back to the initial conditions in 1 minute. Before injection of the next sample, the column was re-equilibrated for 7 minutes. The flow rate was 0.35 mL/min. The electrospray source was operated in the positive ionization mode. Product ions that were obtained by collision-induced dissociation allowed the MS/MS to be operated in the multiple reaction monitoring mode. The dwell time was set at 0.25 seconds. The desolvation gas was nitrogen set at 365 °C and delivered at a flow rate of 9 L/min. The capillary voltage was 3.90 kV, and the collision gas was helium. The Bruker Compass Hystar system software Version 3.2-SR2 was used for instrument control and identification.

### *GRASP Forum*

Secondary data about the participants' discussions on the study platform were supported by a licensed *discourse* (Civilized Discourse Construction Kit, Inc) account and the software used to build the platform. *Qualtrics* (SAP Global Corporate Affairs) data were downloaded for analysis, which was conducted using the institutionally licensed Microsoft Excel from the IMIM and R (R Core Team), which is a free software environment for statistical computing and graphics that does not require a license.

### Data Management and Statistical Analysis

Data will be stored in *Qualtrics* software and will only be used according to the goals of the study described in the protocol. Most of the data will be entered directly by the participants, and a researcher will screen every questionnaire filled for consistency and ask the participants for clarifications in case of suspected errors in data entry or reporting. The principal investigator (MG) and 3 more researchers (GMF, XCM, and JGC) will have access to the complete data sets. Data on participant performance will be entered manually by one researcher in an internal Excel database. No personal information will be stored other than the *safe* email address asked in the study advertisement and the nickname the participant choses to use in the forum. This ensures the maintenance of pseudo-anonymity, as information about the online persona will be stored, but the link between the online identity and the real identity will not be impossible to establish with the collected information.

Data will be managed and processed using *Qualtrics* software and initially analyzed using Excel by the research team. At the same time, an independent statistician will use the same data from *Qualtrics* to perform a partially blind analysis using R. Only fully completed and valid questionnaires will be analyzed. The validity of the questionnaires will be assessed by a research team member based only on the consistency and completeness of the participants' answers.

As it is an exploratory study, the data analysis procedures will be mainly descriptive statistics, to maximize an adequate visualization of the data collected within the minimum space. In addition, as there is no control group, statistical tests will be limited to potential comparisons to assess bias on the results, such as comparing data from participants who complete the study with participants who drop out or are excluded. However, no statistical corrections will be applied to adjust the representativeness of the sample, as this validation will be done by comparing the sociodemographics of the study sample with the characteristics of the population of psychonauts widely reported in the literature. Statistical tests might also be used to compare information from the initial questionnaire Q1 and the data from the follow-up questionnaire Q4. The nonparametric Wilcoxon signed-rank analysis will be used to perform a mean comparison of the quantitative data and the chi-square test for qualitative data. In addition, factor analysis will be attempted to study the relationship between all the visual analog scales used to assess the subjective drug effects.

The data analysis will focus on the outcomes of the participants who completed the study. Data entries containing evident errors or inconsistent information will be discarded, and the extent to which this might have impacted the results will be reported.

### Ethical Considerations

This study was approved by the IRB of IMIM on December 11, 2018, after the first submission in October 2018, when clarifications were asked and delivered in November 2018.

The study has been designed and is being executed according to the basic principles of rights and dignity of the human being, as stated in the Helsinki declaration, and this study complies with all the current regulations that apply, including institutional, local, national, and international regulations.

All information is being handled confidentially according to the organic Spanish law 15/1999 and the European regulation 2016/679. The IRB has always granted access to any study information required.

All participants will receive the participant information sheet and will be required to read and fill the IC form, which will be sent to admin@grasp.pw. The principal investigator will be responsible for reviewing all candidates' IC forms and screening questionnaires for inclusion and exclusion criteria. All candidates and participants will have the opportunity to ask as many questions as needed before proceeding to any part of the study, both through the forum and through contacting the leading researcher email (admin@grasp.pw).

Participants will not be required to sign IC forms to protect their anonymity and avoid sharing data that could be used to track their physical identity. The identity that will be protected by the researchers will be the online one, as no other information relatable to the real identities will be given. This procedure is consistent with the methodology of previous studies [61].

The researchers will try their best to limit the influence of their interactions on the participants' behavior, especially the ones targeted in the study. However, as recommended by previous research, the participants will be involved in discussing the study design and incentivized to share their opinions on how the study could be improved [8,21].

Protocol changes will be communicated to the participants through the online platform once they are approved.

A more extensive ethical analysis by principles can be found in Multimedia Appendix 6 [26,27,39-52,62-64].

### Dissemination Policy

All individual and collective data, as well as all the results derived from the study, will be strictly protected and will only be published with the authorization of the principal investigator and the affected participants. All relevant findings will be sent for publication in suitable journals and submitted for presentation at relevant scientific meetings. The funding organizations will have no role in the publication process. In addition, data without identifiable information will be shared with study participants after assessment and approval by the research team. Finally, all results published in the scientific literature will also be made available in lay language to the communities of origin of the participants. Authorships in the publications will be determined by the amount of scientific and academic contributions of the members of the research team, including external collaborators. There are no plans to make the data sets publicly available.

## Results

The research protocol was approved by the IRB of the *IMIM* on December 11, 2018. Data collection started in August 2019 and was still ongoing when the protocol was submitted (September 2020), finalizing in October 2020.

Data analysis began in November 2020, and it is still ongoing. The authors expect to submit the first manuscript with preliminary results by the end of 2021.

From a total of 182 screened candidates, only 17 (9.3%) completed at least one self-administration trial, resulting in a total number of 64 self-administration trials. From these, 40 different substances were analyzed.

## Discussion

It is possible to conduct an IRB-approved study using this new methodology and collect the expected data. However, the meaning and usefulness of these data are still unknown.

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### Conflicts of Interest

None declared.

### Multimedia Appendix 1

Participant information sheet.
[DOC File , 50 KB-Multimedia Appendix 1]

### Multimedia Appendix 2

Informed consent form.
[DOC File , 33 KB-Multimedia Appendix 2]

### Multimedia Appendix 3

Announcement for candidates.
[DOC File , 33 KB-Multimedia Appendix 3]

### Multimedia Appendix 4

Summary of study procedures.
[DOC File , 192 KB-Multimedia Appendix 4]

### Multimedia Appendix 5

Main study outcomes.
[DOC File , 78 KB-Multimedia Appendix 5]

## Multimedia Appendix 6

Ethical analysis by principles.
[DOC File , 34 KB-Multimedia Appendix 6]

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## Abbreviations

**EC:** Energy Control
**EC–ABD:** Energy Control–Associació Benestar i Desenvolupament
**GC:** gas chromatography
**GRASP:** Global Research and Analysis of New Substances Project
**HPLC:** high performance liquid chromatography
**IMIM:** Hospital del Mar Research Institute
**IC:** informed consent
**IRB:** institutional review board
**LC:** liquid chromatography
**MS:** mass spectrometry
**NPS:** new psychoactive substances
**SPIRIT:** Standard Protocol Items: Recommendations for Interventional Trials
**STROBE:** Strengthening the Reporting of Observational Studies in Epidemiology

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# Acute Pharmacological Effects of 2C-B in Humans: An Observational Study

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2,5-dimethoxy-4-bromophenethylamine (2C-B) is a psychedelic phenylethylamine derivative, structurally similar to mescaline. It is a serotonin 5-hydroxytryptamine-2A (5-$HT_{2A}$), 5-hydroxytryptamine-2B (5-$HT_{2B}$), and 5-hydroxytryptamine-2C (5-$HT_{2C}$) receptor partial agonist used recreationally as a new psychoactive substance. It has been reported that 2C-B induces mild psychedelic effects, although its acute pharmacological effects and pharmacokinetics have not yet been fully studied in humans. An observational study was conducted to assess the acute subjective and physiological effects, as well as pharmacokinetics of 2C-B. Sixteen healthy, experienced drug users self-administered an oral dose of 2C-B (10, 15, or 20 mg). Vital signs (blood pressure and heart rate) were measured at baseline 1, 2, 3, 4, and 6 hours (h). Each participant completed subjective effects using three rating scales: the visual analog scale (VAS), the Addiction Research Centre Inventory (ARCI), and the Evaluation of the Subjective Effects of Substances with Abuse Potential (VESSPA-SSE) at baseline, 2–3 and 6 h after self-administration (maximum effects along 6 h), and the Hallucinogenic Rating Scale (maximum effects along 6 h). Oral fluid (saliva) was collected to assess 2C-B and cortisol concentrations during 24 h. Acute administration of 2C-B increased blood pressure and heart rate. Scores of scales related to euphoria increased (high, liking, and stimulated), and changes in perceptions (distances, colors, shapes, and lights) and different body feelings/surrounding were produced. Mild hallucinating effects were described in five subjects. Maximum concentrations of 2C-B and cortisol were reached at 1 and 3 h after self-administration, respectively. Oral 2C-B at recreational doses induces a constellation of psychedelic/psychostimulant-like effects similar to those associated with serotonin-acting drugs.

**Keywords: 2C-B (2,5-dimethoxy-4-bromophenethylamine), psychedelic, phenylethylamines, psychostimulants, cortisol**

## INTRODUCTION

Psychedelics have been traditionally classified by either their chemical structure or primary mechanism of action into two classes: serotonergic hallucinogens (indolamines, e.g., psilocybin and LSD) and phenylethylamines [e.g., mescaline and 2,5-dimethoxy-4-iodoamphetamine (DOI)] (Vollenweider, 2001; Aarde and Taffe, 2017). Recently, however, new psychoactive substances (NPSs) developed from both substitutions and well-known structures have emerged.

Such novel psychedelics include the 2C-series and its structural analogs, including *N*-Benzylphenethylamines (NBOMes) (Tracy et al., 2017). 2C-series, also called 2C-drugs/compounds, are a related group of substances with presumably psychedelic and psychostimulant properties. All of them are phenylethylamine derivatives structurally close to mescaline with methoxy substitutions at the 2 and 5 positions derived from the two carbon molecules between the benzene ring and the amino group. 2,5-dimethoxy-4-bromophenethylamine (2C-B, Nexus) is one of the oldest and best known 2C-type drugs. Despite its initial reputation as potential psychotherapeutical drug around the 1970s, and later as an aphrodisiac, over the last decade 2C-B has gained popularity among electronic music party goers as the replacement of choice for ecstasy (MDMA, Molly) and LSD, either alone or combined (González et al., 2013; Fernández-Calderón et al., 2017). Based on the abrupt introduction of 2-CB onto the drug market, 2C-B and any of its salts or isomers were added to Schedule II of the 1971 Convention on Psychotropic Substances by the UN Commission on Narcotic Drugs in 2001 (United Nations Office on Drugs and Crime [UNODC], 1971; de Boer and Bosman, 2004). The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and the United Nations Office on Drugs and Crime (UNDOC) classified it as an NPS (European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2011; United Nations Office on Drugs and Crime [UNODC], 2013). In some Latin American countries as Colombia, also 2C-B is considered an NPS due to its recent presence in the market (Colombia National Study of Psychoactive Substance Consumption, 2013).

From a pharmacological point of view, preclinical studies have demonstrated that 2C-drugs inhibit the norepinephrine (NE) and serotonin transporters (NET and SERT, respectively) with very low potency in comparison to amphetamines (Glennon et al., 1984; Vollenweider and Kometer, 2010). Regarding 2C-B, as other hallucinogenic phenethylamines, is a partial agonist of $5HT_{2A}$, $5HT_{2B}$, and $5HT_{2C}$ receptors (Rickli et al., 2015; Luethi et al., 2017). Other studies however have reported that may act as a $5HT_{2A}$ full antagonist (Villalobos et al., 2004). It elicits weak response (5–10%) in both phospholipase A2–arachidonic acid (PLA2–AA) release and phospholipase C-inositol phosphate (PLC-IP) accumulation on 5HT2A receptors (Kurrasch-Orbaugh et al., 2003; Moya et al., 2007). The metabolism of 2C-B has been studied in experimental animals and *in vivo* models (Kanamori et al., 2002, 2005; Carmo et al., 2004; Theobald et al., 2007). It is generally assumed that 2C-B is metabolized mainly by the monoamine oxidase enzymes, MAO-A and MAO-B, and, to a lesser degree, by the CYP450 system (Carmo et al., 2005; Pichini et al., 2008; Kanamori et al., 2013, 2017). Urine analysis from a 2C-B abuser have identified and quantified unchanged 2C-B and nine different metabolites suggesting that 2C-B is metabolized to an alcoholic metabolite [4-bromo-2,5-dimethoxyphenylethylalcohol (2C-B-ALC)], and a carboxylated metabolite [4-bromo-2,5-dimethoxyphenylacetic acid (2C-B-CBA) (Kanamori et al., 2013, 2017). Currently, there are not data available describing active metabolites that could contribute to overall effects, therefore, 2C-B seems the active one while the rest are inactive or nearly so.

Data concerning prevalence and patterns of use of novel psychedelics are limited. In 2013, almost half of the Global Drug Survey [GDS] (2013) respondents (2,282, 46.4%) reported lifetime use of at least one NPS. Of these, 21.7% described psychedelic phenethylamine lifetime use, with 18.4% corresponding to the 2C series, the most common being 2C-B ($n$ = 291, 12.97%) (Palamar et al., 2016). In Australia, a survey among regular ecstasy users showed that 44% had used an NPS in the last 6 months, mainly 4-iodo-2,5-dimethoxyphenethylamine (2C-I, 14%) and 2C-B (8%) (Burns et al., 2014). In a cross-sectional survey carried out at music festivals among 230 research chemical users, the most frequent substance employed was 2C-B (80.0%). Among the most frequent combinations were 2C-B with MDMA (28.3%), less prevalent were 2C-B with amphetamine, LSD, ketamine, and methylone (7.4, 5.7, 3.9, and 2.6%, respectively) (González et al., 2013). The latest data from the GDS, which included a non-representative sample of 115,000 subjects, suggest an increase in the consumption of drugs with a psychedelic effect profile (including LSD analogs), representing over 50% of the total NPSs. Estimated life-time and past year use of 2C-B was 5.1 and 2.7%, respectively (Global Drug Survey [GDS], 2017). Regarding trends in the United Kingdom, psychedelic use over the last 4 years was stable at around 7% with the exception of 2016 in which it rose to 9.8% (7.7-6.3-9.8-7.1%). Globally, 6.3% of the previous 12 month 2C-drug users suffered difficult/negative experiences while under the influence of psychedelics. In Central and South America, 2C-B has become a very popular nightlife NPS (Colombia National Study of Psychoactive Substance Consumption, 2013). 2C-B is usually taken orally in powder or tablet form, in doses of 10–30 mg. Tablets typically contain 5–10 mg of the substance. An oral low dose is considered to be 5–10 mg, a medium dose 10–25 mg, and a high dose 25–40 mg (Caudevilla-Gálligo et al., 2012; Nugteren-van Lonkhuyzen et al., 2015; Papoutsis et al., 2015).

With the exception of emerging 2C-B research performed in the 1950s–1970s by Shulgin, who reported a maximum oral dose of 100 mg without apparent harm (Shulgin and Carter, 1975; Shulgin and Shulgin, 1990), limited clinical research has been conducted in humans. Current evidence about 2C-B acute effects in humans comes from intoxications collected at Poison Information Centers (Burns et al., 2014; Srisuma et al., 2015), self-reports from research chemical recreational users (questionnaires and surveys) (Caudevilla-Gálligo et al., 2012; González et al., 2013), and intoxication cases (Ambrose et al., 2010; Huang and Bai, 2011; Hondebrink et al., 2015;

Liakoni et al., 2015; Caicedo et al., 2016), the clinical presentation including typical hallucinations (tactile, visual, and auditory) and neuropsychiatric symptoms (anxiety, agitation, and confusion). While a number of fatalities have been linked to other substances in the 2C-drug group none have been attributed to 2C-B alone.

We have recently published a manuscript about the acute pharmacological effects of 2C-B focused on emotions. Results showed a specific profile suggesting 2C-B classification as an entactogen drug with psychedelic properties (González et al., 2015). The purpose of the present study is to assess the acute pharmacological effects and oral fluid pharmacokinetics of 2C-B in humans.

# MATERIALS AND METHODS

## Participants

Sixteen healthy volunteers were included (eight males and eight females). Subjects were recreational drug users who reported having used 2C-B at least once in their lives. Exclusion criteria were history of any serious medical or mental disorder including drug dependence (except for nicotine), use of chronic medication, and serious adverse reactions with 2C-B.

Participants were recruited by worth-of-mouth through the Association for the Study of States of Consciousness (PHI). The protocol was approved by the Local Human Research Ethical Committee (CEIC Parc de Salut Mar, Barcelona, Spain) and all the participants were informed about the purpose and procedures of the study, and signed an informed consent prior to any study-related procedure. The study was conducted in accordance with the Declaration of Helsinki. Participants received financial compensation for their participation.

## Design and Treatments

A non-controlled prospective observational study was conducted. Each subject participated in one session. They ingested a capsule that they brought to the testing site themselves, which they had obtained from an unknown source. Although no information was available about the synthesis of the drug, similar capsules tested by Energy Control, a harm reduction organization that provides a Drug Checking Service for users, showed that the capsules contained 2C-B at 95% purity with no toxic adulterants. The 2C-B pill content was previously analyzed by means of gas chromatography associated with mass spectrometry (GC/MS). The method used permits to check for most common drugs of abuse including cocaine, MDMA, LSD, amphetamine and methamphetamine, heroin, 2C-B and other phenethylamines, DMT and other tryptamines, ketamine, psilocybin, salvinorin A, natural and synthetic cannabinoids, and most of the NPSs (Caudevilla-Gálligo et al., 2012; González et al., 2015; Grifell et al., 2017; Palma-Conesa et al., 2017; Quintana et al., 2017). Participants were given a choice of three doses to choose from, 10, 15 or 20 mg 2C-B, based on their stated preference from previous experience. They chose to take a mean 2C-B dose of 15.94 $\pm$ 4.17 mg (four subjects ingested 10 mg, five subjects 15 mg, and seven subjects 20 mg).

### Procedures

Prior to participation all subjects were trained with respect to the procedures, tests, and questionnaires employed in the study. Participants were requested to abstain from any drug use 48 hours (h) prior to the study session. Alcohol and caffeine-containing beverages were not allowed the previous 24 h (or the morning of the study session). Sessions took place on different days at the home of a member of the PHI Association. The setting included ambience music (except in the evaluation times). Subjects could read, talk, play table games during sessions and interact. They were instructed not to talk about the effects of the substance during the session. Assessments were performed at baseline (predose, immediately before 2C-B self-administration) and over 6 h after 2C-B self-administration. The experiments were conducted at the same time for all subjects, from 15:00 to 22:00 h. A light snack was ingested immediately after. Urine spot samples were collected before 2C-B administration to exclude drug use prior to the session (MDMA, amphetamines, barbiturates, benzodiazepines, cocaine, marijuana, morphine, methamphetamine, phencyclidine with Instant-View, Multipanel 10 Test Drug Screen Alfa Scientific Designs, Inc., Poway, CA, United States). 2C-B self-administration took place approximately at 16.00 h.

The sequence of procedures at each time point of the session was: vital signs, physiological effects, oral fluid collection, subjective effect scales and questionnaires.

### Vital Signs/Physiological Effects

Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured with an automatic Omron® monitor at baseline and 1, 2, 3, 4, and 6 h after self-administration.

### Subjective Effects

Subjective effects were recorded at baseline and 2 h after administration, at 6 h subjects were asked to report the maximum effects along 6 h (0–6 h). The Hallucinogenic Rating Scale (HRS) was completed only at 6 h post-administration.

Subjective effects of 2C-B were measured using a set of the visual analog scale (VAS), the Addiction Research Center Inventory (ARCI), the Evaluation of the Subjective Effects of Substances with Abuse Potential (VESSPA-SSE) questionnaires, and the HRS.

Visual analog scale (100 mm, from "not at all" to "extremely") were used to rate intensity; high; good effects; bad effects; liking; changes in distances; changes in colors; changes in shapes; changes in lights; hallucinations-seeing of lights or spots; hallucinations-seeing animals, things, insects, or people; changes in hearing; hallucinations-hearings of sounds or voices; drowsiness; dizziness; confusion; fear; depression or sadness; different body feeling; unreal body feeling; different surroundings; and unreal surroundings (González et al., 2015; Papaseit et al., 2016).

The ARCI is a true/false 49-item questionnaire is a sensitive instrument for determining subjective drug effects, and consists of five subscales: PCAG (pentobarbital-chlorpromazine-alcohol,

a measure of sedation), LSD (lysergic acid diethylamide group, a measure of dysphoria and somatic symptoms), MBG (morphine-benzedrine group, a measure of euphoria), BG (benzedrine group, a stimulant scale consisting mainly of items relating to intellectual efficiency and energy), and A (amphetamine, an empirically derived scale sensitive to the effects of D-amphetamine) (Haertzen, 1965; Lamas et al., 1994).

The VESSPA-SE questionnaire measures changes in subjective effects caused by a number of drugs. It includes six subscales: sedation (S), psychosomatic anxiety (ANX), changes in perception (CP), pleasure and sociability (SOC), activity and energy (ACT), and psychotic symptoms (PS) (González et al., 2015; Papaseit et al., 2016).

The HRS includes 100 items distributed in six scales: (i) somaesthesia (reflecting somatic effects including interoceptive, visceral, and tactile effects); (ii) affect (sensitive to emotional and affective responses); (iii) volition (indicating the subject's capacity to willfully interact with his/her 'self' and or the environment); (iv) cognition (describing alterations in thought processes or content); (v) perception (measuring visual, auditory, gustatory, and olfactory experiences); and (vi) intensity (which reflects the strength of the overall experience) (Strassman et al., 1994; Riba et al., 2001a).

### Oral Fluid Concentrations of 2C-B and Cortisol

Oral fluid (saliva) was collected with Salivette® tubes to assess 2C-B and cortisol concentrations at baseline, 1, 2, 3, 4, 6, 16, and 24 h after administration ($n$ = 8 for cortisol). Oral fluid samples were centrifuged and frozen at −20°C until analysis. 2C-B concentrations were quantified with gas chromatography–mass spectrometry (GC–MS) (González et al., 2015). Cortisol samples were analyzed with the AxSYM Cortisol Assay (Abbott Diagnostics, Abbott Park, IL, United States) which utilizes fluorescence polarization immunoassay (FPIA) according to the manufacturers' instructions.

## Statistical Analysis

Differences with respect to baseline were calculated for vital signs (SBP, DBP, and HR) and subjective effects (VAS, ARCI, and VESSPA). Maximum effects ($E_{max}$) and the time needed to reach maximum effects ($t_{max}$) were also calculated for the mentioned variables. The area under the curve of the concentrations (AUC) using the trapezoidal rule were calculated for vital signs.

The AUC, the maximum concentration ($C_{max}$) and the time needed to reach the maximum concentration ($t_{max}$), elimination half-life ($t_{1/2}$) and elimination constant ($K_e$), from 2C-B and cortisol oral fluid concentrations over time were determined using Pharmacokinetic Functions for Microsoft Excel (Joel Usansky, Atul Desai, and Diane Tang-Liu, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA, United States).

Firstly, a two-way analysis of variance (ANOVA) test was conducted to study the influence of dose and gender in the different parameters calculated. Because the results showed only marginal statistically significant results for interactions between dose and gender, dose or gender, the analysis was rejected (nine variables showed significant results for a total number of 198 comparisons). Subsequently, the statistical analysis presented was performed without considering these factors.

$E_{max}$ values of vital signs and cortisol were compared with baseline data using a paired samples $t$-test. Furthermore, a detailed comparison between different time points was performed by means of a one-way repeated measures ANOVA, with time condition as factor. When the time condition was statistically significant, a Dunnett *post hoc* test was performed to compare the different time points with baseline.

For subjective effects, a one-way repeated measures ANOVA was performed with time condition as factor (baseline, 2 and 6 h). When ANOVA has been statistically significant a Dunnett *post hoc* test was performed to compare 2 and 6 h with baseline. No comparison between 2 and 6 h (maximum effects from 0 to 6 h) was performed because both measures although obtained in different time points are an approximation of the same parameter ($E_{max}$).

Statistical analysis was performed using PAWS Statistics version 18 (SPSS, Inc., Chicago, IL, United States). A value of $p < 0.05$ was considered statistically significant and it was adjusted for the multiple comparisons.

# RESULTS

## Participants

A total of 16 healthy subjects participated in the study (eight males and eight females). They had a mean age of 33.25 ± 3.71 years (range: 27–39), weighed 63.81 ± 11.59 kg (range: 44–84), and their mean body mass index (BMI) was 21.69 ± 2.49 kg/m$^2$ (range: 18.6–27). The mean 2C-B weight-adjusted dose was 0.27 ± 0.09 mg/kg (range: 0.12–0.45). They reported an average previous 2C-B use of 3 (range: 1–20) times during their lifetime. All volunteers had recreational experience with MDMA, amphetamines, hallucinogens, cocaine, and cannabis. 12 were current tobacco smokers (range: 5–30 cigarettes/day) and all of them consumed alcohol (mean: 1 unit/day). Baseline drug urine tests were negative.

## Vital Signs/Physiological Effects

Changes in vital signs/physiological outcomes are shown in **Table 1** and **Figure 1A**. 2C-B produced an increase in SBP, DBP, and HR. Maximum effects ($E_{max}$) were +19 mmHg, +13 mmHg, and +13 bpm, respectively. Compared to baseline values, statistically significant differences were detected for SBP from 1 to 4 h and from 1 to 3 h for DBP and HR. For both SBP and HR, median $t_{max}$ values ranged from 1 to 4 h whereas for DBP $t_{max}$ ranged from 1 to 2 h. Time course of changes were similar to oral fluid concentration of 2C-B (see below, **Figure 1B**).

## Subjective Effects

2C-B produced robust changes in most subjective effects measured by VAS, ARCI, and VESSPA-SEE. **Table 2** shows the results for the different subscales of the questionnaires.

2C-B self-administration increased the score in all the outcomes measured with VAS. The highest scores (a difference

**TABLE 1 |** Summary of result on the systolic and diastolic blood pressure (SBP, DBP) and heart rate (HR) ($n$ = 16) observed after self-administration of 2C-B.

| Vital signs/physiological effects | Parameter | ANOVA/T student | | Comparison to baseline | Mean ± *SD* |
|---|---|---|---|---|---|
| | | F/T | *p*-Value | Dunett's test | |
| SBP | $E_{max}$ | 5.740 | <0.001 | | 19.25 ± 13.41 |
| | T-C (df = 1,75) | 7.119 | <0.001 | 1, 2, 3, 4 h | |
| DBP | $E_{max}$ | 5.910 | <0.001 | | 13.13 ± 8.88 |
| | T-C (df = 1,75) | 6.460 | <0.001 | 1, 2, 3 h | |
| HR | $E_{max}$ | 6.060 | <0.001 | | 12.63 ± 8.33 |
| | T-C (df = 1,75) | 6.813 | <0.001 | 1, 2, 3 h | |

*$E_{max}$ = peak effects 0–6 h (differences from baseline) measured by mmHg (SBP, DBP), bpm (HR). T-C = time course from 0 to 6 h. For T-C an ANOVA and a post hoc Dunnett's test for multiple comparisons was used.*

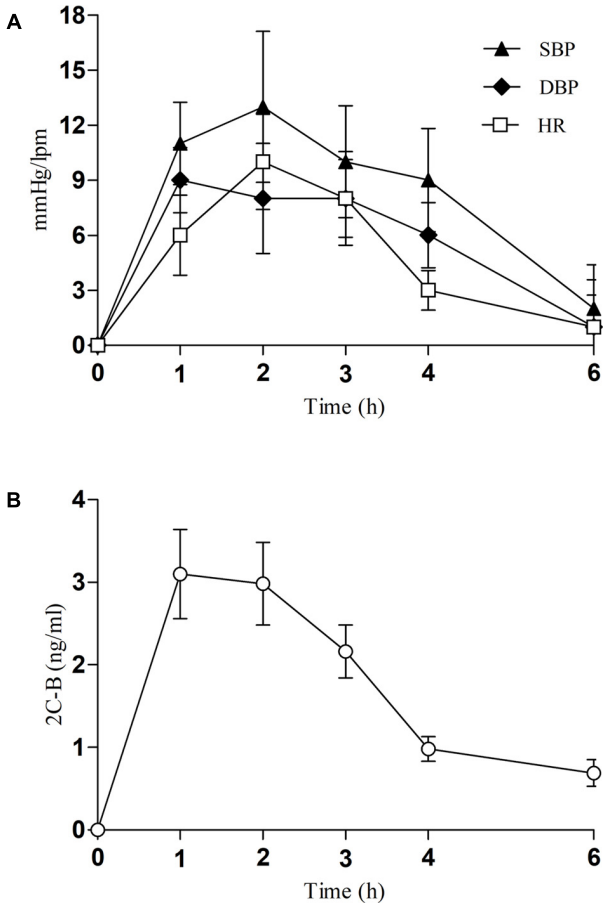


**FIGURE 1 | (A)** Time course of changes from baseline for systolic and diastolic blood pressure (SBP, DBP, mmHg) and heart rate (HR, bpm, beats per minute) ($n$ = 16, mean, standard error). **(B)** Time course of 2C-B concentrations in oral fluid ($n$ = 16, mean, standard error).

of >50 mm from baseline) were obtained for intensity, high, good effects, and different body feeling scales. Differences of >25 mm from baseline were obtained for changes in distances, colors, shapes, and light scales while moderate (<15 mm) and small changes (<10 mm) were found in the scales measuring hallucinations, changes in hearing, and unreal surroundings. In comparison to baseline, statistical significant changes were detected for all VAS scales with the exception of bad effects, hallucinations-seeing animals, things, insects, or people, hallucinations-hearings of sounds or voices, dizziness, fear, and depression or sadness. In fact only five of the subjects described clear hallucinogenic effects in VAS.

Regarding the effects measured with the ARCI questionnaire, after 2C-B administration significant changes in all ARCI subscales were observed, expect for PCAG (sedation). The most marked increases compared to baseline were found for MBG (euphoria) and A (amphetamine) subscales. Modest increases were detected for LSD (dysphoria and somatic symptoms) and BG (intellectual efficiency and energy) subscales.

In relation to the VESSPA-SP questionnaire, 2C-B induced significant increases compared to baseline in all subscales. The main changes were observed in SOC (pleasure and sociability), ACT (activity and energy), and S (sedation) subscales.

For several subjective outcomes, mean peak effects reported at 6 h (summary effects of the 0–6 h) were slightly lower than scores obtained at 2 h after administration (see **Table 2**). Nevertheless, when statistical differences from baseline were observed at 2 h, the same occurred for 6 h (maximum effects from 0 to 6 h). These differences between 2 and 6 h could be explained due to memory bias.

With respect to the HRS, the highest scores were obtained for intensity, volition and affect subscales (see **Figure 2**).

## Oral Fluid Concentrations

2C-B oral fluid concentrations increased quickly after 2C-B ingestion, reaching a peak ($t_{max}$) 1 h after self-administration (**Figure 1B**). Concentrations decreased rapidly from 2 to 6 h after ingestion and could be detected in oral fluid up to 24 h in half of the volunteers. $C_{max}$ reached was 4.19 ± 1.86 ng/ml and AUC from 0 to 24 h was 19.54 ± 4.72 ng × h/ml. 2C-B elimination half-life ($t_{1/2}$) in oral fluid was 2.48 ± 3.20 h. Data from one volunteer were excluded due to outlier concentrations (an analytical error was suspected). As mentioned previously, both concentrations and vital signs time course were similar (**Figures 1A,B**).

Cortisol concentrations were measured in a subset of eight volunteers. Cortisol baseline concentrations were 0.64 ± 0.46 μg/dl. After 2C-B administration concentrations reached a $C_{max}$ of 1.13 ± 0.23 μg/dl at 3 h ($t_{max}$) (not statistically

**TABLE 2 |** Summary of result on subjective effects ($n$ = 16) observed after self-administration of 2C-B.

| Subjective effects | Time (h) | Mean ± *SD* | ANOVA | | Comparison to baseline |
|---|---|---|---|---|---|
| | | | *F* | *p*-Value | Dunett's test |
| **Visual analog scale (VAS)** | | | | | |
| Intensity | 2 | 45.13 ± 21.07 | 64.622 | <0.001 | <0.001 |
| | 6 | 59.68 ± 20.18 | | | <0.001 |
| High | 2 | 69.44 ± 17.36 | 189.515 | <0.001 | <0.001 |
| | 6 | 67.75 ± 17.40 | | | <0.001 |
| Good effects | 2 | 68.25 ± 19.26 | 176.377 | <0.001 | <0.001 |
| | 6 | 70.00 ± 15.43 | | | <0.001 |
| Bad effects | 2 | 3.63 ± 5.23 | 3.023 | 0.064 | |
| | 6 | 10.50 ± 21.33 | | | |
| Liking | 2 | 78.00 ± 19.77 | 185.113 | <0.001 | <0.001 |
| | 6 | 76.69 ± 23.41 | | | <0.001 |
| Changes in distances | 2 | 28.19 ± 26.72 | 14.252 | <0.001 | <0.001 |
| | 6 | 25.38 ± 23.10 | | | <0.001 |
| Changes in colors | 2 | 37.50 ± 29.68 | 20.220 | <0.001 | <0.001 |
| | 6 | 34.44 ± 27.75 | | | <0.001 |
| Changes in shapes | 2 | 36.94 ± 32.39 | 10.567 | <0.001 | <0.001 |
| | 6 | 32.25 ± 29.66 | | | <0.001 |
| Changes in lights | 2 | 42.19 ± 31.12 | 18.549 | <0.001 | <0.001 |
| | 6 | 35.63 ± 26.57 | | | <0.001 |
| Hallucinations-seeing of lights or spots | 2 | 13.50 ± 21.90 | 6.138 | 0.006 | 0.036 |
| | 6 | 18.50 ± 28.27 | | | 0.004 |
| Hallucinations-seeing animals, things, insects, or people | 2 | 6.75 ± 21.61 | 1.609 | 0.217 | |
| | 6 | 5.37 ± 15.55 | | | |
| Changes in hearing | 2 | 9.25 ± 15.91 | 3.883 | 0.032 | 0.020 |
| | 6 | 6.38 ± 6.90 | | | 0.125 |
| Hallucinations-hearings of sounds or voices | 2 | 3.25 ± 7.90 | 2.021 | 0.150 | |
| | 6 | 1.69 ± 3.18 | | | |
| Drowsiness | 2 | 13.19 ± 20.37 | 5.918 | 0.007 | 0.027 |
| | 6 | 16.50 ± 22.66 | | | 0.005 |
| Dizziness | 2 | 8.75 ± 21.29 | 2.060 | 0.145 | |
| | 6 | 7.00 ± 16.12 | | | |
| Confusion | 2 | 6.81 ± 9.11 | 4.115 | 0.026 | 0.103 |
| | 6 | 9.63 ± 17.19 | | | 0.017 |
| Fear | 2 | 0.69 ± 1.54 | 1.086 | 0.350 | |
| | 6 | 2.25 ± 7.44 | | | |
| Depression or sadness | 2 | 1.38 ± 2.92 | 1.163 | 0.326 | |
| | 6 | 3.88 ± 13.66 | | | |
| Different body feeling | 2 | 60.38 ± 21.69 | 53.946 | <0.001 | <0.001 |
| | 6 | 56.50 ± 26.34 | | | <0.001 |
| Unreal body feeling | 2 | 13.06 ± 21.66 | 3.533 | 0.042 | 0.024 |
| | 6 | 7.19 ± 13.52 | | | 0.262 |
| Different surroundings | 2 | 29.19 ± 28.51 | 12.102 | <0.001 | <0.001 |
| | 6 | 19.94 ± 22.97 | | | 0.005 |
| Unreal surroundings | 2 | 3.19 ± 5.65 | 3.476 | 0.044 | 0.027 |
| | 6 | 2.13 ± 3.80 | | | 0.165 |
| **ARCI questionnaire** | | | | | |
| PCAG | 2 | 4.19 ± 2.86 | 0.121 | 0.887 | |
| | 6 | 4.31 ± 3.14 | | | |
| MBG | 2 | 7.13 ± 3.65 | 45.812 | <0.001 | <0.001 |
| | 6 | 6.19 ± 3.60 | | | <0.001 |

*(Continued)*

**TABLE 2 |** Continued

| Subjective effects | Time (h) | Mean ± *SD* | ANOVA | | Comparison to baseline |
|---|---|---|---|---|---|
| | | | *F* | *p*-Value | Dunett's test |
| LSD | 2 | 6.44 ± 2.28 | 7.580 | 0.002 | 0.002 |
| | 6 | 5.94 ± 2.95 | | | 0.012 |
| BG | 2 | 5.50 ± 2.39 | 5.929 | 0.007 | 0.009 |
| | 6 | 5.44 ± 2.06 | | | 0.013 |
| A | 2 | 4.88 ± 1.86 | 86.834 | <0.001 | <0.001 |
| | 6 | 4.06 ± 1.44 | | | <0.001 |
| *VESSPA-SEE questionnaire* | | | | | |
| Sedation (S) | 2 | 5.63 ± 4.43 | 14.787 | <0.001 | <0.001 |
| | 6 | 5.06 ± 6.02 | | | <0.001 |
| Psychosomatic anxiety (ANX) | 2 | 3.19 ± 2.51 | 20.556 | <0.001 | <0.001 |
| | 6 | 2.81 ± 2.48 | | | <0.001 |
| Changes in perception (SP) | 2 | 3.69 ± 4.39 | 10.099 | <0.001 | 0.001 |
| | 6 | 3.56 ± 4.03 | | | 0.001 |
| Pleasure and sociability (SOC) | 2 | 12.13 ± 5.26 | 59.280 | <0.001 | <0.001 |
| | 6 | 9.88 ± 5.11 | | | <0.001 |
| Activity and energy (ACT) | 2 | 6.88 ± 4.18 | 20.797 | <0.001 | <0.001 |
| | 6 | 6.06 ± 4.73 | | | <0.001 |
| Psychotic symptoms (PS) | 2 | 0.81 ± 1.05 | 7.291 | 0.003 | 0.003 |
| | 6 | 0.75 ± 0.93 | | | 0.007 |

*Subjective effects measured by mm (VAS) and score (ARCI and VESSPA-SEE questionnaires). Time 2 h (effect at 2 h after administration). Time 6 h (maximum effects from 0 to 6 h).*

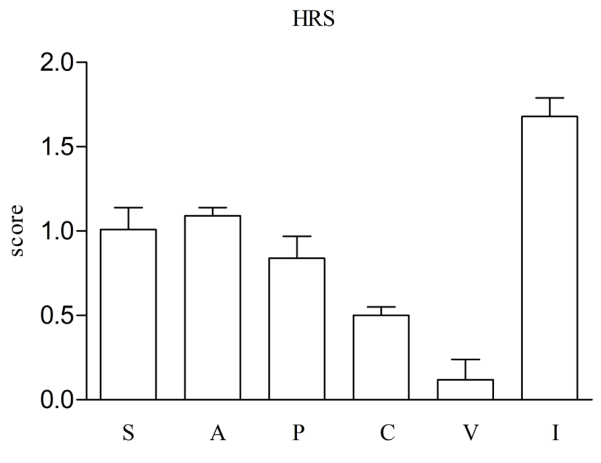


**FIGURE 2 |** Scores on The Hallucinogenic Rating Scale (HRS) questionnaire subscales ($n$ = 16, mean, standard error). Scores represent the maximum effects along 6 h after self-administration of 2C-B. S: somaesthesia; A: affect; P: perception, C: cognition; V: volition, I: intensity.

significant). From 3 to 4 h concentrations abruptly decreased returning slowly to baseline 16 h after administration.

## DISCUSSION

This study assessed the acute pharmacological effects of 2C-B in a non-controlled setting. The main finding is that 2C-B produces a constellation of psychedelic-psychostimulant like effects, a profile consistent with previous human data (Caudevilla-Gálligo et al., 2012; González et al., 2015). Our research provides unique results regarding 2C-B concentrations in oral fluid and cortisol.

Additionally, results show that the self-administration of 2C-B at the narrow dose range studied (10–20 mg) in healthy experienced users in a non-medical setting is relatively safe (González et al., 2015). In contrast to our previous publication (González et al., 2015), that focused on the effects of 20 mg on emotions, the present study included a more intensive evaluation of vital signs, and a more complete collection of oral fluid. These evaluations provided a picture of the time-course of the effects of 2C-B on physiological measures and permitted a comparison between the time-course of the effects and the concentrations of 2C-B in oral fluid. Again, our findings concur with the limited number of cases reporting severe acute toxicity related to 2C-B use.

In a non-controlled setting, the profile of physiological effects produced by 2C-B is characterized by moderate increases of blood pressure (SBP and DBP) and HR, but lower than those of MDMA, amphetamines, and related compounds administered in controlled conditions (Mas et al., 1999; Papaseit et al., 2016). The onset of cardiovascular effects occurred at 1 h assessment and maintained over a long-lasting period (4 h). At 6 h values returned toward pre-drug self-administration.

The subjective effect of 2C-B in this study consists of mixed euphoric, well-being reactions and alterations in mental functions closely related to psychostimulants such as MDMA, amphetamine, and mephedrone, and psychedelics such as ayahuasca, salvinorin A, and Salvia Divinorum. Globally, subjects under 2C-B effects reported euphoria, activation and a

psychedelic experience consisting of a temporary altered state of consciousness. Mood changes were more prominent that perceptual changes. Specifically, the mean VAS ratings of liking, good effects, and high (up to 78% of maximum possible VAS scores) were even greater than those determined in experimental conditions for MDMA and other related psychostimulants (Mas et al., 1999; Papaseit et al., 2016). In relation to the MBG subscale, considered a measure of drug-induced euphoria, 2C-B induced high scores which, when regarding to psychedelics, are indicative of subjective feelings of well-being and confidence. As previously postulated for other psychedelics, it is possible that euphoria may also be an essential component of the psychedelic experience after 2C-B use (Bouso et al., 2016). Interesting, 2C-B also resulted in increases in the LSD subscale and somatic VAS scales (drowsiness, dizziness, confusion). Despite the coexistence of dysphoric-somatic effects, the induced well-being and pleasant effects were clearly more important as reflected by the increases in rating scores. In contrast, fear and visual hallucinations were not experienced, and sedation was unremarkable. Is it noteworthy that alteration in perception ranged from changes in perceptions to hallucinations, although the latter were only experienced by 2 (hearing of sounds or voices, 10–36 mm score) and 3 subjects (seeing of lights or spots, 10–87 mm score). Such results differ from other psychedelics probably due to the relatively low-moderate doses self-administered in this study.

The HRS has been previously used to measure the hallucinatory effects of *N,N*-dimethyltryptamine (Strassman et al., 1994), ayahuasca (Riba et al., 2001b, 2004), psilocybin (Griffiths et al., 2006), salvinorin A (Johnson et al., 2011), and MDMA (Tancer and Johanson, 2007) among others. Our results showed the highest scores for intensity, volition and affect subscales. The scores for some subscales in the present study were lower (somaesthesia, perception, cognition, and intensity) or similar (affect and volition) than described in experimental studies administering other psychedelics as N,N-dimethyltryptamine (Strassman et al., 1994), ayahuasca (Riba et al., 2001b, 2004), psilocybin (Griffiths et al., 2006), and salvinorin A (Johnson et al., 2011). Interestingly, the present study found that the HRS scores were similar to those 2C-B ratings reported by recreational psychedelic users. Similar changes were observed in volition subscale whilst, in comparison, lower scores were obtained for intensity, somaesthesia, perception, affect, and cognition subscales (Caudevilla-Gálligo et al., 2012).

The psychedelic effects produced by 2C-B are varied and include somatic symptoms (dizziness, drowsiness, and confusion), perceptual symptoms (changes in distances, colors, shapes, lights, different body feelings, different surroundings, unreal body feelings, and unreal surroundings) and visual hallucinations Subjects under 2C-B effects reported a psychedelic experience consisting of euphoria and the activation, but not experience, of typical hallucinations. Results were similar to those described in previous works and surveys, all symptoms were resolved by 6 h (Caudevilla-Gálligo et al., 2012; Nugteren-van Lonkhuyzen et al., 2015).

In a similar manner to other NSPs and some psychedelics, the human pharmacokinetics of 2C-B has not yet been fully resolved. Analysis of oral fluid samples by LC–MS/MS concentrations ranged from 1.43 to 7.73 ng/ml, with an average peak concentration of 4.19 ng/ml observed between 1 and 3 h after administration. However, results of 2C-B pharmacokinetics indicate that 2C-B can be detected in oral fluid at very low concentrations up to 16–24 h after self-administration. Unfortunately, the interpretation of 2C-B concentrations in oral fluid is extremely difficult without data from plasma (not performed in this study neither in any study involving humans). By contrast, collection of oral fluid is easy and non-invasive, and was the method selected to obtain pharmacokinetic data in this study. Anecdotally, in two subjects involved in a road accident, 2C-B has been identified in blood at concentrations of 1.6 and 14 ng/ml together with amphetamine (Busardò et al., 2017).

After 2C-B administration, the increase in cortisol concentration was very small and no statistically significant. For other serotonergic psychedelic and psychedelic-like drugs including MDMA, ayahuasca, and psilocybin (Mas et al., 1999; Hasler et al., 2004; Dos Santos et al., 2011; Hysek et al., 2014; Seibert et al., 2014; Farré et al., 2015) a market increases in plasma cortisol concentrations have been detected.

Our work has several limitations which are mainly associated with its naturalistic observational design. Firstly, the study was open label without a lack of control/placebo, therefore an expectancy bias cannot be discarded. Secondly, data were obtained from a small sample of experienced psychedelic drug users, including both genders. Thirdly, a limited dose range was evaluated, the dose was selected by the participants according to their preferences and was relatively low-moderate (10–20 mg). Higher doses (>25 mg) are reported to cause unpleasant hallucinations and sympathomimetic effects such as tachycardia, hypertension and hyperthermia (Huang and Bai, 2011). Therefore, the observed acute effects may be useful in a similar subpopulation of polydrug-users but should be extrapolated with caution to the general population. Fourth, the exclusive reliance on subjective effects with few objective measures. Fifth, the setting used could influence the effects reported by participants. In addition, our findings may not apply to other routes of 2C-B administration. Finally, the effects obtained at 6 h are not a substitute for the assessment of the subjective effects in real peak effect time, ideally performed with a clinical trial design. Nonetheless, this study investigated the acute subjective effects using validated questionnaires with proven sensitivity for discriminating subjective effects, and validated analytic techniques for 2-CB and cortisol determinations.

## CONCLUSION

The results presented in this work constitute a preliminary approach to the acute physiological and subjective effects and pharmacokinetics of 2C-B. According to these preliminary results, oral fluid could be a suitable biologic matrix to detect 2C-B acute use. They suggest that oral 2C-B self-administration in experienced drug users, in a non-controlled setting, induces a constellation of psychedelic/psychostimulant

like effects commonly associated with drugs that have a greater influence on serotonin action.

Further experimental research under controlled conditions is needed to compare human pharmacology of 2C-B with other classical drugs.

## AUTHOR CONTRIBUTIONS

MF, MT, MV, and DG: conceptualized the study design; MF and DG: collected the data; RdlT and MP: analyzed the oral fluid; MV: analyzed the 2C-B contents; MF, EP, CP-M, DG, RdlT, MV, and MT: written the manuscript.

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# Acute Effects of 2C-E in Humans: An Observational Study

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2,5-Dimethoxy-4-ethylphenethylamine (2C-E) is psychedelic phenylethylamine, with a chemical structure similar to mescaline, used as new psychoactive substance (NPS). It inhibits norepinephrine and serotonin uptake and, more relevant, acts as a partial agonist of the serotonin 2A (5-$HT_{2A}$), 2B (5-$HT_{2B}$), and (5-$HT_{2C}$) receptors. Consumers have reported that 2C-E induces mild-moderate psychedelic effects, but its pharmacology in humans, including pharmacological effects and pharmacokinetics, have not yet studied. To assess the acute effects of 2C-E on physiological and subjective effects and evaluate its pharmacokinetics, an observational study was carried-out. Ten recreational users of psychedelics self-administered a single oral dose of 2C-E (6.5, 8, 10, 15, or 25 mg). Blood pressure and heart rate were evaluated at baseline, 2, 4, and 6 h post-administration. Three rating scales were administered to evaluate subjective effects: a set of Visual Analog Scales (VAS), the 49-item short form version of the Addiction Research Centre Inventory (ARCI), and the Evaluation of the Subjective Effects of Substances with Abuse Potential (VESSPA-SSE) at baseline, 2, 4, and 6 h after self-administration. To assess 2C-E concentrations oral fluid (saliva) was collected during 6 h. 2C-E induced primarily alterations in perceptions, hallucinations, and euphoric-mood. Saliva maximal concentrations were achieved 2 h after self-administration. Administration of oral 2C-E at recreational doses produces a group of psychedelic-like effects such to 2C-B and other serotonin-acting drugs.

**Keywords: 2C-E (2,5-Dimethoxy-4-ethylphenethylamine), novel psychoactive substances (NPS), psychedelic, phenylethylamines, psychostimulants**

## INTRODUCTION

Classical psychedelics (serotonergic psychedelics) have traditionally been defined as a class of psychoactive substances that induce in humans a wide range of complex physiological, behavioral and psychological effects through serotonin 5-HT2$_A$ receptors stimulation (Nichols, 2016). In the past few years, however, phenethylamine psychedelics have emerged as a class of new psychoactive substances (NPS) able to induce similar effects to those of controlled psychedelic substances (Vollenweider, 2001; Aarde and Taffe, 2017). 2C-compounds (2C-s) are ring-substituted phenylethylamines derived from the modification of the mescaline structure with two methoxy groups on the benzene ring (2nd and 5th positions) (Tracy et al., 2017). Although they are widely considered a family of substances with hallucinogenic/psychedelic and psychostimulant properties, information available on their pharmacology and toxicology in humans is very limited.

2,5-Dimethoxy-4-ethylphenethylamine [2C-E, or 2-(4-ethyl-2,5-dimethoxyphenyl) ethanamine] is colloquially known as "Aquarust," "Eternity," "Europe," and "Hummingbird" (Sutherland et al., 2016). Synthesized in 1977 by Alexander Shulgin it is one of the most potent 2C-compounds (Shulgin and Shulgin, 1990). 2C-E is structurally very closely related to other 2C-s and to other well-studied phenethylamine substitutes such as mescaline and MDMA (ecstasy). It first came out the club scene in the mid-1980s as a quick replacement for MDMA which had been banned in the United States. 2C-E then remerged on the psychedelic scene and lately has been present as part of the NPS phenomenon. In fact, 2C-E has been documented as being contained in pills sold as ecstasy in America and Europe (United Nations Office on Drugs and Crime [UNODC], 2014), and more recently in Colombia and other Latin American countries, where it is considered an NPS due to its new presence on the drug market (Observatorio de Drogas de Colombia [ODC], 2017).

Pharmacologically, 2C-E, in a similar manner to other 2C-compounds, inhibits the uptake of serotonin and norepinephrine by membrane transporters (SERT and NET, respectively), although with very low activity in relation to amphetamine (Nagai et al., 2007; Van Vrancken et al., 2013; Eshleman et al., 2014). 2C-E mainly acts as a partial agonist at the 5-HT2$_A$, 5-HT2$_B$, and 5HT2$_C$ receptors (related to its psychedelic effects) (Rickli et al., 2015). Also it binds mostly at the adrenergic α-2 receptor (Rickli et al., 2015).

Relatively little information is available regarding human 2C-E metabolism. Nevertheless, research has suggested that it follows similar metabolic pathways to 2C-Bwhich are carried out by O-demethylation and N-acetylation (Theobald et al., 2007).

With respect to epidemiological data on 2C consumption, the information available from web-based questionnaires and population-based surveys is particularly infrequent. In a self selected sample from the 2013 Global Drug Survey[1], including 2,282 participants in the United States, reporting attendance to nightclubs in the previous year, 46.4% described lifetime use of at least one of the 58 NPS assessed (age range 16–60 years). Among the psychedelic phenethylamines, consumption of 2C-compounds was the most commonly reported (21.7%), and 8.55% admitted taking 2C-E ($n$ = 195) (Palamar et al., 2016). In the latest Global Drug Survey there are no specific data regarding the prevalence of 2C-E (Global Drug Survey [GDS], 2018).

In Australia, national cross-sectional surveys among regular ecstasy users ($n$ = 693, year 2010) and regular psychostimulant users ($n$ = 1260, years 2012/2013) reported a 2 and 3% prevalence of 2C-E use in the previous 6 months, respectively (Bruno et al., 2012; Matthews et al., 2016). In 2014, a sample of Australian NPS users ($n$ = 800) described a 5.9% use in the previous 6 months (Sutherland et al., 2017).

In a survey done in Spain among 230 research chemical users a 25.7% had taken 2C-E in the previous year. It was the fifth most frequent substance consumed, and rarely used in combination with other psychostimulants or psychedelics (2C-E + MDMA 1.8%, 2C-E + amphetamine 0.9%, 2C-E + mephedrone 0.9%, and 2C-E + psilocybin 0.4%) (González et al., 2013). In a recent study in the United States, including 356,413 respondents to the 2008–2016 National Survey on Drug Use and Health, 0.12% reported lifetime novel psychedelic use. Of these, 30.1, 14.8, and 23.9%, reported lifetime use of 2C-B (2,5-dimethoxy-4-bromophenethylamine), 2C-E and 2C-I (2,5-dimethoxy-4-iodophenethylamine), respectively (Sexton et al., 2019).

The first description of 2C-E effects was published in *PiHKAL: A Chemical Love Story*, which considered the drug to be one of the "magical half-dozen" or more intense psychedelic phenethylamines (Shulgin and Shulgin, 1990). In recent years, 2C-E recreational users have reported its effects as being a combination of hallucinogenic and stimulating ones, like those of ecstasy and LSD. Like other psychedelics drugs and 2C compounds, 2C-E at low doses usually produces stimulant effects and increased auditory, visual and tactile sensations. At moderate doses it leads to mild hallucinations, and at high ones can cause the user to experience unpleasant hallucinations and sympathomimetic effects. In general, effects from 2C-E are reportedly more intense in comparison to 2C-B (Dean et al., 2013).

An average dose of 2C-E ranges from 10 to 20 mg (medium dose 15–25 mg, high dose 25–40 mg) although exceptionally elevated doses up to 100 mg have been reported (Dean et al., 2013)[2]. Recommendations for an initial dose are between 6 and 20 mg depending on the user's previous experience with similar drugs, whilst 3 mg is considerate a "microdose" which produces intense effects on cognitive processes and well-being without the typical ones on consciousness (Polito and Stevenson, 2019). As with most psychedelics, the effects of 2C-E are long-acting, lasting typically for 6–12 h, depending on the dose and individual.

To date, a dozen cases of acute intoxication (tachycardia, hypertension, agitation, delirium, and hallucinations) have been reported (Van Vrancken et al., 2013; Iwersen-Bergmann et al., 2019) and, although very rare, some deaths have been linked to 2C-E (Topeff et al., 2011; Sacks et al., 2012). Alarmingly, no human research has been conducted with 2C-E in spite of the relatively long history of its recreational use and the recent

[1]https://www.globaldrugsurvey.com/

[2]https://www.erowid.org/

resurgence of interest in psychedelic drugs. The aim of our study was to evaluate the pharmacological effects and pharmacokinetics of 2C-E in recreational users.

# MATERIALS AND METHODS

## Participants

Ten healthy subjects were selected (4 females and 6 males). Volunteers were recreative drug users who had experienced a 2C-series compound at least once in a lifetime. Exclusion criteria were a history of any serious medical or psychopathological disorder including substance use disorder (except nicotine), a previous serious adverse reaction with 2C-series, and chronic medicines use.

Participants were recruited by word-of-mouth and snowball sampling through the harm reduction, non-governmental organization, Energy Control (ABD). The study protocol was submitted and approved by the Clinical Research Ethics Committee (CEIC Parc de Salut Mar, Barcelona, Spain, ref. 2016/6700/I). It was conducted according to the Declaration of Helsinki recommendations. All the participants were correctly and fully informed, both orally and in writing, of the purpose, methods and means of the study. All of them indicated their agreement to participate and signed an informed consent prior inclusion. Participants received monetary compensation for their participation.

## Design and Treatments

The design was a non-controlled prospective observational study with minimal intervention in subjects who self-administrated 2C-E orally. Most evaluations and procedures were similar to a previous naturalistic observational study evaluating acute effects of 2C-B (Papaseit et al., 2018). Each participant participated in one session. Treatment consisted of oral self-administration of one 2C-E capsule, that they brought to the testing site themselves, which they had obtained from an unknown source. Although no information was available about the synthesis of the drug, similar capsules tested by Energy Control, a harm reduction organization that provides a Drug Checking Service for users, showed that the capsules contained 2C-E at 95% purity with no toxic adulterants. The 2C-B pill content was previously analyzed by means of gas chromatography associated with mass spectrometry (GC/MS). The method used permits to check for most common drugs of abuse including most of the NPSs and to know the exact purity of 2C-E in the powder to prepare dosing by a precision scale (Papaseit et al., 2018). The dose of 2C-E self-administrated was selected by the participants based presumably on their previous experience. The mean 2C-E dose was 11.95 $\pm$ 5.30 mg [1 female ingested 6.5 mg, 1 female 8 mg, 5 males 10 mg, 2 subjects (1 male and 1 female) 15 mg, and 1 female 25 mg]. In order to standardize dosing for statistical analysis and to evaluate dose-response relationship, we grouped doses in two intervals: 6.5–10 and 15–25 mg (taken by 7 and 3 subjects, respectively). All the selected doses were well tolerated.

### Procedures

Prior to study session, the participants were submitted to a general medical examination and a psychiatric diagnostic examination. They received training with respect to questionnaires and procedures employed in the study. Upon arrival, they were questioned about any event that could affect their participation. They were asked to refrain from any drug use 2 days prior to the session. Participants were not allowed to consume alcohol or beverages containing caffeine the previous 24 h. Sessions took place on two different days (5 participants each day and administration were separated by various minutes among participants) at a private club with ambient music and participants could talk, read, or play table games during the session and interact in exception to the evaluation times. Also, they were instructed not to talk about the effects of the substance during the session. Assessments were performed by at baseline (pre-dose) and 2, 4, and 6 h after 2C-E self-administration. The experiment was conducted from 15:00 to 22:00 h. Urine spot samples were collected prior administration to exclude prior substance drug use (benzodiazepines, barbiturates, morphine, cocaine, amphetamines, methamphetamine, MDMA, marijuana, phencyclidine) with Instant-View, Multipanel 10 Test Drug Screen Alfa Scientific Designs Inc., Poway, CA, United States. Self-administration of 2C-E took place around 16.00 h. The sequence of procedures at each time point of the session was: physiological measures, oral fluid collection, and subjective effects questionnaires. A psychiatry was present during the entire session. Adverse effects were assessed during study session.

### Physiological Effects

Non-invasive systolic and diastolic blood pressure (SBP and DBP), and heart rate (HR) were determined with an Omron® monitor at baseline and 2, 4, and 6 h after administration. Oral temperature was measured simultaneously.

### Subjective Effects

Subjective effects of 2C-E were reported at baseline and at 2, 4, and 6 h after self-administration. They were measured using a set of Visual Analog Scales (VAS), the 49-item Addiction Research Centre Inventory (ARCI) short form, and the Evaluation of the Subjective Effects of Substances with Abuse Potential (VESSPA-SSE) questionnaires. VAS (100 mm, from "not at all" to "extremely") were used to rate intensity; stimulated; high; good effects; liking; content; changes in colors; changes in shapes; changes in lights; hallucinations-seeing of lights or spots; hallucinations-seeing animals, things, insects or people; changes in hearing; hallucinations-hearings of sounds or voices; different body feeling; unreal body feeling; changes in distances; different surroundings; unreal surroundings; confusion; fear; depression or sadness; drowsiness; dizziness; bad effects; headache; nausea; vertigo; breathing difficulty and face flushing (González et al., 2015; Papaseit et al., 2016, 2018).

The ARCI 49-item short form is a validated instrument that includes five subscales related to drug sedation (pentobarbital-chlorpromazine-alcohol group, PCAG), euphoria (morphine-benzedrine group, MBG), dysphoria and somatic symptoms (lysergic acid diethylamide group, LSD), intellectual efficiency and energy (benzedrine group, BG) and d-amphetamine-like effects (A) (Lamas et al., 1994; Papaseit et al., 2016; Martínez-Riera et al., 2019).

The VESSPA-SE is a questionnaire that measures changes in subjective effects caused by different drugs including stimulants and psychedelics and includes six subscales: sedation (S), psychosomatic anxiety (ANX), changes in perception (CP), pleasure and sociability (SOC), activity and energy (ACT), and psychotic symptoms (PS) (González et al., 2015; Papaseit et al., 2016).

### Oral Fluid Concentrations of 2C-E

To assess 2C-E concentrations in oral fluid (saliva), it was collected with Salivette® tubes at baseline, 2, 4, and 6 h after self-administration. After collection samples were centrifuged and frozen at -20°C until analysis. 2C-E concentrations were analyzed by a modified and validated liquid chromatography–mass spectrometry method LC-MS/MS) (Papaseit et al., 2018).

## Statistical Analysis

For physiological (SBP, DBP, HR, and T) and subjective effects (VAS, ARCI, and VESSPA), differences with respect to baseline were calculated. Maximum effects ($E_{max}$) were determined and the area under the curve of the effects ($AUC_{0-6\,h}$) were calculated using the trapezoidal rule.

For 2C-E oral fluid concentrations, the maximum concentration ($C_{max}$), the time needed to reach the maximum concentration ($T_{max}$) and the $AUC_{0-6\ h}$ were determined using the Pharmacokinetic Functions for Microsoft Excel (Joel Usansky, Atul Desai, and Diane Tang-Liu, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA, United States).

Although it is remarkably that the participant that selected the lowest dose (6.5 mg) presented higher acute effects and oral fluid concentrations in comparison to others, this subject was included in all the analysis.

A one-way analysis of variance (ANOVA) test including all doses as a factor was used for $E_{max}$ and $AUC_{0-6}$. When the dose factor was statistically significant, a *post hoc* analysis for the two defined groups were done using a Student *T*-test (lower dose group: 6.5–10 mg, $n = 7$; higher dose group: 10–25 mg, $n = 3$).

To evaluate the effects along time and to study the effects of the substance in comparison to baseline, a one-way repeated measures ANOVA, with time as factor (baseline, 2, 4, and 6 h), was done to evaluate the time-course of effects (for all doses). When the time condition was statistically significant, a Dunnett multiple comparison *post hoc* test was conducted to compare the different time points with baseline (0–2 h, 0–4 h, 0–6 h).

All statistical tests were conducted using PAWS Statistics version 18 (SPSS Inc., Chicago, IL, United States). A $p < 0.05$ value was considered statistically significant.

# RESULTS

## Participants

All ten selected subjects participated in the study (4 females and 6 males). Demographics were a mean age of 27 ± 4 years (range 24–37), mean weight of 64.60 ± 8.77 kg (range 58–78), and mean body mass index (BMI) of 20.26 ± 2.55 kg/m$^2$ (range 16–24). The mean weight-adjusted dose of 2C-E was 0.19 ± 0.09 mg/kg (range 0.13–0.43). All subjects had previous recreative experience with 2Cs, psychedelics/hallucinogens, cocaine, MDMA, amphetamines, and cannabis. Seven of them were current tobacco smokers (range 0.5–7 cigarettes/day) and all consumed alcohol daily (mean 1.4 units/day). All drugs of abuse urine tests were negative at baseline. As explained in the statistical analysis for dose-response analysis we grouped doses in two groups (6.5, 8–10, and 15–25 mg), **Figures 1–3** are showed as the two doses groups. **Supplementary Figures S1–S3** presented individual data in order to show the elevated variability of the acute effects and concentrations.

## Physiological Effects

Effects of 2C-E on physiological signs are summarized in **Table 1** and **Figure 1**, and **Supplementary Figure S1** (individual data). 2C-E produced a non-significant increase in SBP, DBP, HR and T. For HR significant differences were detected in the comparison of baseline and 4 hand 6 h after administration. Regarding T, only statistically significant differences were detected at 2 and 4 h. No dose-response relationship was observed.

## Subjective Effects

The subjective effects induced by 2C-E are presented in **Table 2** and **Figure 2**, and **Supplementary Figure S2** (individual data). In summary, 2C-E significantly increased scores for most of the outcomes measured with VAS. Some effects were related to dose, as higher doses produced more intense effects. The substance produced more intensity of effects in comparison to baseline for most variables.

For VAS scales related to euphoria-stimulation the highest scores were observed for "intensity," "stimulated," "high," "good effects," "liking," and "content." When compared to baseline, significant differences were detected at 2 and 4 h, except for "stimulated" (4 h) and "liking" (2, 4, and 6 h). No dose-response was observed when comparing both groups of doses.

For VAS scales measuring changes in perceptions, statistically significant differences in $E_{max}$ and $AUC_{0-6}$ were detected for all VAS except in "different body feelings." When compared to baseline, significant differences were found in VAS for "changes in colors" (2 h), "changes in lights" (4 h), "different body feeling" (h, 4 h), and "different surroundings" at 4 h and 6 h. A dose-response was observed in all VAS except for "changes in hearing," "changes in distances," and "different body feeling."

With respect to scales measuring hallucinations, the highest scores were found for "hallucination-seeing of lights/spots" ($E_{max}$ 21.00 mm) whilst modest and low scores were observed for "hallucination-seeing animals, things, insects or people" ($E_{max}$ 6.20 mm, no significant) and "hallucination-hearing of sounds or voices" ($E_{max}$ 2.20 mm, significant). Significant effects, baseline differences and dose-response were observed for "hallucinations-seeing of light and spots" (6 h) and "hallucination-hearing of sounds or voices."

In addition, 2C-E induced "confusion," "drowsiness," and "breathing difficulty." Differences from baseline were observed for "drowsiness," "dizziness," "bad effects," and "nausea." No dose-response was observed except for "breathing difficulty."

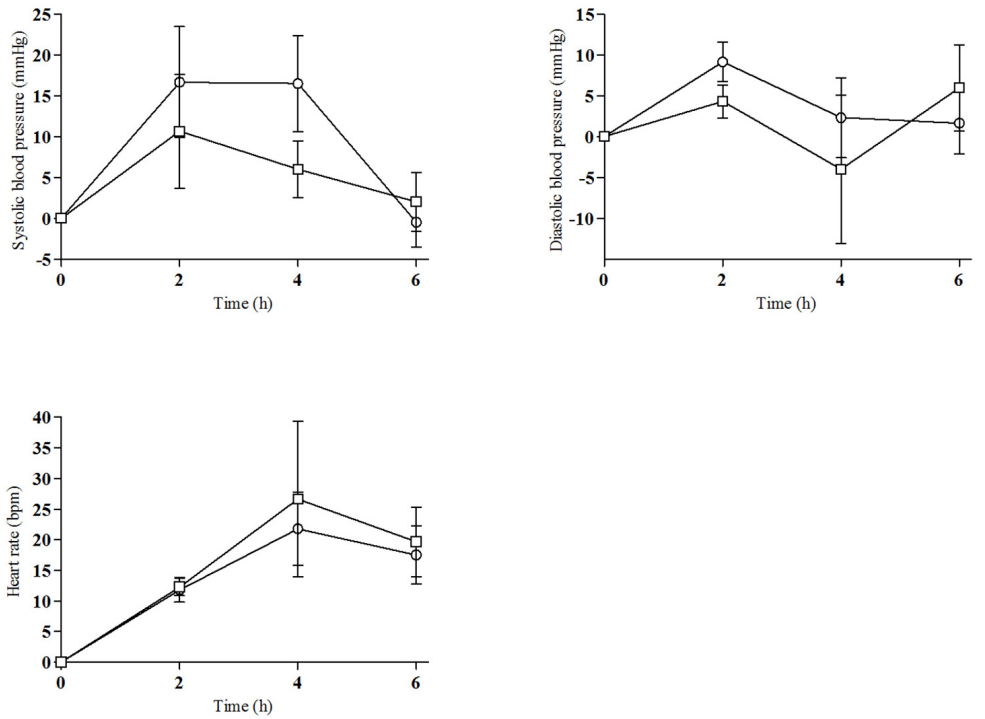


**FIGURE 1 |** Time course of changes from baseline for physiological effects [○, 6.5–10 mg of 2C-E (*n* = 7), □, 15–25 mg of 2C-E (*n* = 3); mean, standard error].

In relation to ARCI questionnaire, significant increases in the scores of all subscales were detected, however, differences in dose were not statistically significant. Similarly, differences from baseline were observed for all subscales at different times. No dose-response was observed.

With respect to the VESSPA, significant changes were shown in Sedation, Change in perception and Psychotic symptoms, with significant differences from baseline in all except Psychotic symptoms. Dose-response relationship were detected for Changes in Perception and Psychotic symptoms.

Most of the effects dissipated after 6 h, and all subjects returned to their usual routine. Two of them presented residual mild visual hallucinations (lights) at 6 h which disappeared 1–2 h later.

## Oral Fluid Concentrations

The oral fluid concentration-time curve for 2C-E are shown in **Figure 3**, and **Supplementary Figure S3** (individual data). Concentrations of 2C-E increased rapidly, reaching a peak 2 h after ingestion. Concentrations rapidly decrease from 2 to 6 h after ingestion. Mean maximum concentration ($C_{max}$) values of 5.8 ± 6.4 ng/mL (range 0.93–21.54) were obtained at a $T_{max}$ of 2 h following drug administration. The $AUC_{0-6}$ was 18 ± 18 ng·h/mL (range 3.69–57.70). Plasma concentrations varied considerably among doses and subjects. No significant differences between the two grouped doses were found for $C_{max}$ or $AUC_{0-6}$ (**Table 2**). All ten subjects presented positive concentrations of 2C-E at 4 h; only 5, however, had 2C-E concentrations in saliva at 6 h.

# DISCUSSION

To the best of our knowledge, this is the first study to assess the acute behavioral (subjective) and physiological effects and oral fluid concentrations of 2C-E after the administration of known doses (6.5–25 mg) in humans. The main finding is that 2C-E induced primarily a group of psychedelic-like effects, a profile consistent with prior data from surveys and poisonings symptoms (Matthews et al., 2016). Moreover, our study provides unique results about concentrations of 2C-E in oral fluid.

In our non-controlled setting, 2C-E only partially mimicked the prototypical sympathomimetic-like effects of other psychedelic and psychostimulant drugs (Schmid et al., 2015; Dolder et al., 2017) and 2C-B (Papaseit et al., 2018). The physiological actions induced by 2C-E included a mild-moderate increase of HR, without changes in blood pressure. The effects were lower than those produced by 2C-B (Papaseit et al., 2018) and by MDMA, mephedrone or other amphetamines administered in dose-controlled conditions (Farré et al., 2015; Papaseit et al., 2016). It is possible that the wide range of doses in the present study (from 6.5 to 25 mg) did not permit differences to be observed in blood pressure when compared to 2C-B (in a narrow range from 10 to 20 mg) (Papaseit et al., 2018). For 2C-E the maximal cardiac effect was observed at the 2 h assessment, maintained over 2–4 h, and returned to baseline at 6 h post-administration.

In this study, 2C-E produced mixed euphoria, pleasure and well-being feelings, and alterations in mental functions like psychedelics such as 2C-B (González et al., 2015;

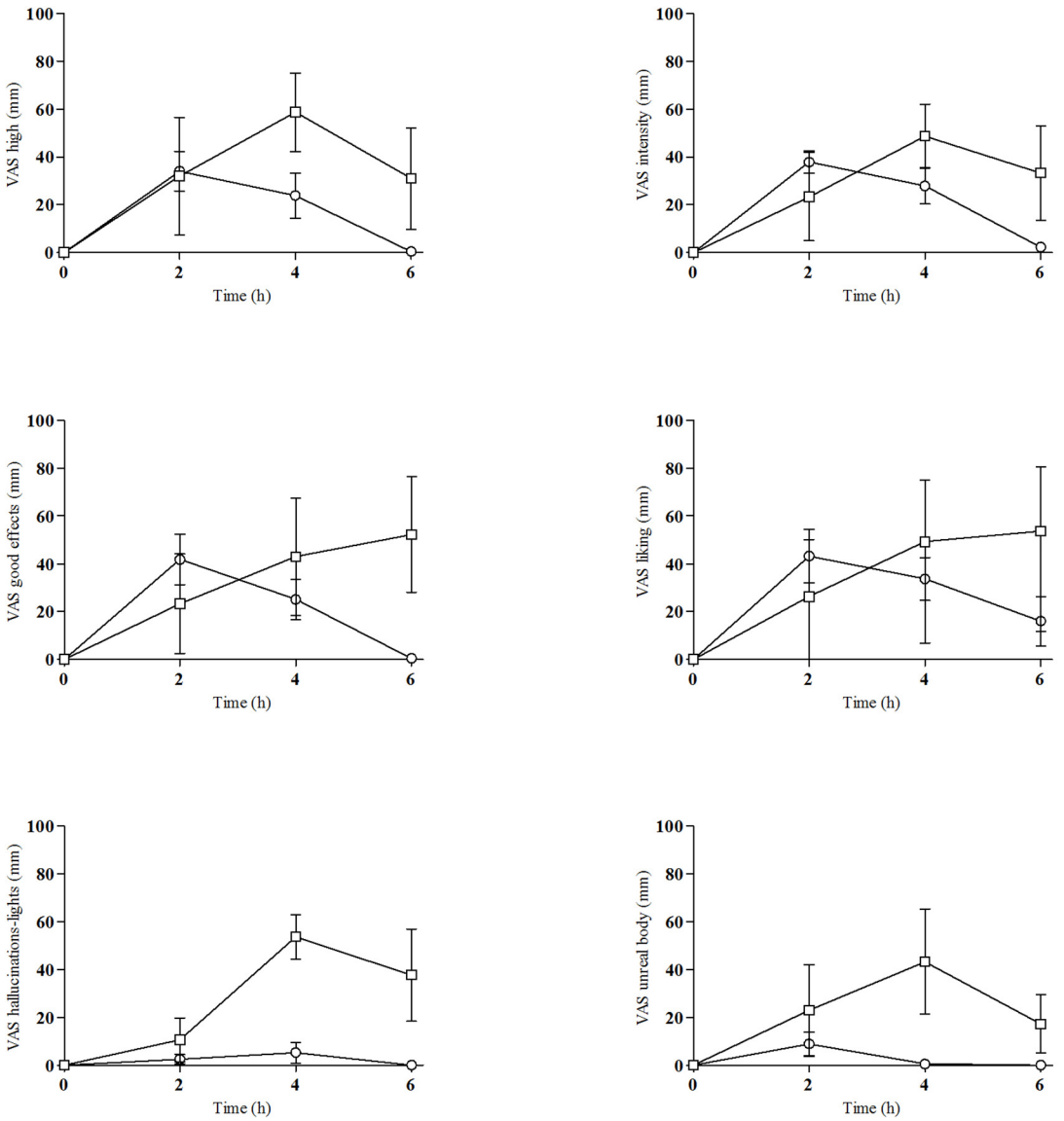


**FIGURE 2 |** Time course of changes from baseline for subjective effects [○, 6.5–10 mg of 2C-E (*n* = 7); □, 15–25 mg of 2C-E (*n* = 3); mean, standard error].

Papaseit et al., 2018), psilocybin (Griffiths et al., 2006), salvinorin A (Johnson et al., 2011) and ayahuasca (Riba et al., 2001, 2004) and psychostimulants such as MDMA (Papaseit et al., 2016), amphetamine (Cami et al., 2000), and mephedrone (Papaseit et al., 2016). Under 2C-E influence participants reported euphoria, stimulation, and altered state of consciousness due to the psychedelic experience. Changes in mood were more pronounced than perceptual ones. As an example, the mean VAS ratings of "high," "good effects," and "liking" reached up to 50% of the maximum possible VAS scores, but they were still lower than those observed in experimental dose-controlled conditions for 2C-B, MDMA, and other stimulants as mephedrone (Mas et al., 1999; Farré et al., 2015; Papaseit et al., 2016, 2018). It is possible that euphoria could be an important issue of the psychedelic experience after 2C-B or 2C-E use, as previously postulated for other psychedelics (Bouso et al., 2016). It is noteworthy that 2C-E increased some somatic VAS scales (drowsiness, dizziness, and confusion) in a similar manner to 2C-B.

Moreover, alteration in perception varied from changes in perceptions to hallucinations, that were experienced by 5 volunteers (3 only visual and 2 visual and auditory hallucinations). Of these, 5 subjects reported visual (seeing of lights or spots, 14–72 mm), 1 subject visual (seeing things/people, 50 mm) and 2 participants auditory (hearing sounds/voices, 8–14 mm score), effects. Results differ in intensity from other psychedelics probably because in this study subjects self-administered low to moderate doses of the substance. Additionally, 2C-E produced higher increases in sociability

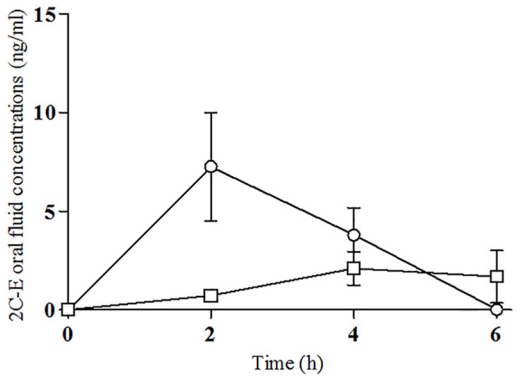


**FIGURE 3 |** Time course of 2C-E concentrations in oral fluid [Δ, 6.5 mg of 2C-E ($n$ = 1); ∘, 8–10 mg of 2C-E ($n$ = 6), □, 15–25 mg of 2C-E ($n$ = 3); mean, standard error].

(VESPA SOC subscale) and augmented ratings on change perceptions, effects widely related to MDMA and LSD (Papaseit et al., 2016; Dolder et al., 2017; Puxty et al., 2017). Overall, the subjective effects induced by 2C-E appear to be closely related to psychedelic drugs indicating that it produces mind-altering and hallucinogenic effects which could be primarily mediated by the $5HT_{2A}$ receptor.

In a similar manner to 2C-B, the sole 2C-compound with previous observational data in humans after dose-controlled administration, 2C-E induced modest sympathomimetic effects, similar feelings of well-being, euphoria, and changes in perception although with more profound hallucinations (Caudevilla-Gálligo et al., 2012; González et al., 2015; Papaseit et al., 2018).

As expected, in our study 2C-E produced the prototypical effects of psychedelic substances that include visual hallucinations, perceptual changes, somatic symptoms, and activation of euphoria. Although it also induced headache, confusion, and breathing difficulty, no severe adverse reactions were observed. Our results show that in a recreational setting, self-administration of low-moderate doses of 2C-E by healthy experienced users is well tolerated and relatively safe. The results are consistent with a relatively low number of severe acute toxicity cases associated to 2C-E use (Iwersen-Bergmann et al., 2019).

The pharmacokinetics of 2C-E in humans has not yet been fully known. Our results on oral fluid concentrations of 2C-E are the first data in humans to be reported. 2C-E concentrations ranged from 0.93 to 21.54 ng/mL, with an average peak concentration of 5.8 ± 6.4 ng/mL observed at 2 h after administration. Oral fluid 2C-E showed a similar time course with effect outcomes. Nevertheless, because the study included

**TABLE 1 |** Summary of result on the physiological effects observed after self-administration of 2C-E.

| Effects | Parameter | ANOVA | | | Comparison to baseline | T-Student | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Doses (6.5–25 mg) ($n$ = 10) | | | Doses (6.5–25 mg) ($n$ = 10) | 6.5–10 mg ($n$ = 7) | 15–25 mg ($n$ = 3) | $T$-value | $p$-value |
| | | Mean ± SD | $F$ | $p$-value | Dunnett's test | Mean ± SD | Mean ± SD | | |
| **Physiological effects** | | | | | | | | | |
| Systolic blood pressure | $E_{max}$ | 15 ± 23 | 0.047 | 0.995 | | 15 ± 28 | 15 ± 5.8 | ND | ND |
| | $AUC_{0-6}$ | 41 ± 74 | 0.050 | 0.994 | | 43 ± 89 | 35 ± 22 | ND | ND |
| | T-C | | | | NS | | | | |
| Diastolic blood pressure | $E_{max}$ | 1.6 ± 20 | 0.840 | 0.554 | | 2 ± 22 | 0.7 ± 20 | ND | ND |
| | $AUC_{0-6}$ | −2.1 ± 63 | 0.873 | 0.539 | | −5.9 ± 74 | 6.7 ± 39 | ND | ND |
| | T-C | | | | NS | | | | |
| Heart rate | $E_{max}$ | 18 ± 19 | 2.883 | 0.138 | | 12 ± 17 | 33 ± 19 | ND | ND |
| | $AUC_{0-6}$ | 58 ± 56 | 4.799 | 0.058 | | 41 ± 57 | 98 ± 34 | ND | ND |
| | T-C | | | | b, c | | | | |
| Temperature | $E_{max}$ | 0.5 ± 0.2 | 2.366 | 0.185 | | 0.1 ± 0.2 | 0.3 ± 0.2 | ND | ND |
| | $AUC_{0-6}$ | 0.3 ± 0.5 | 1.122 | 0.440 | | 0.2 ± 0.5 | 0.6 ± 0.6 | ND | ND |
| | T-C | | | | b | | | | |

*Emax = peak effects 0–6 h (differences from baseline). $AUC_{0-6}$ = area under the curve from 0 to 6 h. Units: mmHg (systolic blood pressure and diastolic blood pressure), beats per minute (heart rate), °C (temperature). For $E_{max}$ and $AUC_{0-6}$ a one-way ANOVA was used to examine the effect of all doses. A $p < 0.05$ was considered statistically significant. Only if a statistical difference were detected an unpaired T-Student was used to examine differences between the grouped doses (6.5–10 mg vs. 15–25 mg). A $p < 0.05$ was considered statistically significant. ND, not done. For T-C a one-way ANOVA and a post hoc Dunnett's test for multiple comparisons was used. Statistical differences between are presented as "a" $p < 0.05$, "**a**" $p < 0.01$ (times 0–2 h), "b" $p < 0.05$, "**b**" $p < 0.01$ (times 0–4 h), "c" $p < 0.05$, "**c**" $p < 0.01$ (times 0– 6 h).*

**TABLE 2 |** Summary of result on the subjective effects and saliva concentrations observed after self-administration of 2C-E.

| Effects | Parameter | ANOVA | | | Comparison to baseline | T-Student | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Doses (6.5–25 mg) ($n$ = 10) | | | Doses (6.5–25 mg) ($n$ = 10) | 6.5–10 mg ($n$ = 7) | 15–25 mg ($n$ = 3) | $T$-value | $p$-value |
| | | Mean ± SD | $F$ | $p$-value | Dunnett's test | Mean ± SD | Mean ± SD | | |
| **Visual analog scale (VAS)** | | | | | | | | | |
| Intensity | $E_{max}$ | 46 ± 17 | 1.045 | 0.468 | | 43 ± 11 | 55 ± 27 | ND | ND |
| | $AUC_{0-6}$ | 147 ± 68 | 5.464 | 0.045 | | 134 ± 52 | 177 ± 104 | −0.916 | 0.387 |
| | T-C | | | | **a**, **b** | | | | |
| Stimulated | $E_{max}$ | 37 ± 28 | 1.423 | 0.349 | | 29 ± 25 | 55 ± 31 | ND | ND |
| | $AUC_{0-6}$ | 114 ± 104 | 3.666 | 0.093 | | 86 ± 87 | 179 ± 130 | ND | ND |
| | T-C | | | | **b** | | | | |
| High | $E_{max}$ | 48 ± 23 | 1.924 | 0.245 | | 48 ± 21 | 54 ± 44 | ND | ND |
| | $AUC_{0-6}$ | 145 ± 99 | 6.003 | 0.038 | | 134 ± 74 | 185 ± 189 | ND | ND |
| | T-C | | | | **a**, **b** | | | | |
| Good effects | $E_{max}$ | 50 ± 27 | 0.839 | 0.555 | | 72 ± 86 | 62 ± 30 | ND | ND |
| | $AUC_{0-6}$ | 150 ± 110 | 3.875 | 0.085 | | 116 ± 74 | 212 ± 133 | ND | ND |
| | T-C | | | | **a,** b | | | | |
| Liking | $E_{max}$ | 51 ± 30 | 0.751 | 0.598 | | 49 ± 24 | 55 ± 48 | ND | ND |
| | $AUC_{0-6}$ | 181 ± 134 | 1.691 | 0.287 | | 170 ± 113 | 205 ± 203 | ND | ND |
| | T-C | | | | **a**, **b**, **c** | | | | |
| Content | $E_{max}$ | 47 ± 30 | 1.048 | 0.467 | | 44 ± 25 | 53 ± 47 | ND | ND |
| | $AUC_{0-6}$ | 145 ± 110 | 1.784 | 0.269 | | 130 ± 92 | 180 ± 161 | ND | ND |
| | T-C | | | | a, b | | | | |
| Changes in colors | $E_{max}$ | 32 ± 21 | 6.786 | 0.030 | | 23 ± 7.9 | 52 ± 32 | −2.426 | 0.041 |
| | $AUC_{0-6}$ | 102 ± 111 | 51.871 | < 0.001 | | 55 ± 16 | 209 ± 173 | −2.545 | 0.034 |
| | T-C | | | | **a**, b | | | | |
| Changes in shapes | $E_{max}$ | 27 ± 27 | 3.717 | 0.091 | | 15 ± 16 | 53 ± 32 | ND | ND |
| | $AUC_{0-6}$ | 73 ± 91 | 14.974 | 0.005 | | 34 ± 35 | 165 ± 128 | −2.665 | 0.029 |
| | T-C | | | | NS | | | | |
| Changes in lights | $E_{max}$ | 35 ± 28 | 9.468 | 0.015 | | 23 ± 18 | 64 ± 32 | −2.665 | 0.029 |
| | $AUC_{0-6}$ | 99 ± 90 | 34.980 | 0.001 | | 59 ± 39 | 193 ± 114 | −2.930 | 0.019 |
| | T-C | | | | **c** | | | | |
| Hallucinations-seeing of lights or spots | $E_{max}$ | 21 ± 26 | 8.564 | 0.018 | | 6.6 ± 12 | 55 ± 16 | −5.388 | 0.001 |
| | $AUC_{0-6}$ | 61 ± 88 | 13.026 | 0.007 | | 16 ± 28 | 166 ± 92 | −4.220 | 0.003 |
| | T-C | | | | c | | | | |
| Hallucinations-seeing animals, things, insects, or people | $E_{max}$ | 6.2 ± 16 | 1.002 | 0.485 | | 1.4 ± 3.8 | 17 ± 28 | ND | ND |
| | $AUC_{0-6}$ | 11 ± 26 | 0.987 | 0.491 | | 2.9 ± 7.6 | 29 ± 46 | ND | ND |
| | T-C | | | | NS | | | | |
| Changes in hearing | $E_{max}$ | 4.1 ± 7.4 | 15.425 | 0.005 | | 4.0 ± 8.5 | 4.3 ± 5.1 | −0.062 | 0.952 |
| | $AUC_{0-6}$ | 12 ± 23 | 19.891 | 0.003 | | 12 ± 27 | 11 ± 14 | 0.080 | 0.938 |
| | T-C | | | | NS | | | | |
| Hallucinations-hearings of sounds or voices | $E_{max}$ | 2.2 ± 4.9 | 13.444 | 0.007 | | 0.0 ± 0.0 | 7.3 ± 7.0 | −3.026 | 0.016 |
| | $AUC_{0-6}$ | 4.9 ± 11 | 29.642 | 0.001 | | 0.0 ± 0.0 | 16 ± 15 | −3.189 | 0.013 |
| | T-C | | | | NS | | | | |

*(Continued)*

**TABLE 2 |** Continued

| Effects | Parameter | ANOVA | | | Comparison to baseline | T-Student | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Doses (6.5–25 mg) (*n* = 10) | | | Doses (6.5–25 mg) (*n* = 10) | 6.5–10 mg (*n* = 7) | 15–25 mg (*n* = 3) | *T*-value | *p*-value |
| | | Mean ± SD | *F* | *p*-value | Dunnett's test | Mean ± SD | Mean ± SD | | |
| Different body feeling | $E_{max}$ | 46 ± 23 | 1.559 | 0.315 | | 46 ± 20 | 46 ± 33 | ND | ND |
| | $AUC_{0-6}$ | 135 ± 78 | 3.792 | 0.088 | | 120 ± 46 | 169 ± 133 | ND | ND |
| | T-C | | | | **a**, **b** | | | | |
| Unreal body feeling | $E_{max}$ | 20 ± 26 | 6.413 | 0.033 | | 9.4 ± 13 | 43 ± 38 | −2.231 | 0.056 |
| | $AUC_{0-6}$ | 58 ± 101 | 26.999 | 0.001 | | 19 ± 26 | 150 ± 161 | −2.273 | 0.053 |
| | T-C | | | | NS | | | | |
| Changes in distances | $E_{max}$ | 22 ± 30 | 1.286 | 0.387 | | 13 ± 25 | 44 ± 34 | ND | ND |
| | $AUC_{0-6}$ | 60 ± 98 | 5.499 | 0.045 | | 26 ± 50 | 139 ± 149 | −1.899 | 0.094 |
| | T-C | | | | NS | | | | |
| Different surroundings | $E_{max}$ | 29 ± 29 | 2.311 | 0.191 | | 17 ± 18 | 56 ± 32 | ND | ND |
| | $AUC_{0-6}$ | 82 ± 100 | 8.625 | 0.018 | | 37 ± 38 | 187 ± 129 | −3.001 | 0.017 |
| | T-C | | | | b, c | | | | |
| Unreal surroundings | $E_{max}$ | 13 ± 27 | 14.432 | 0.006 | | 0.0 ± 0.0 | 43 ± 36 | −3.428 | 0.009 |
| | $AUC_{0-6}$ | 45 ± 102 | 29.938 | 0.001 | | 0.0 ± 0.0 | 150 ± 153 | −2.843 | 0.022 |
| | T-C | | | | NS | | | | |
| Confusion | $E_{max}$ | 15 ± 22 | 1.891 | 0.250 | | 0.0 ± 0.0 | 2.3 ± 2.08 | ND | ND |
| | $AUC_{0-6}$ | 35 ± 49 | 6.297 | 0.034 | | 9 ± 12 | 30 ± 37 | −1.461 | 0.182 |
| | T-C | | | | NS | | | | |
| Fear | $E_{max}$ | 3.1 ± 5.2 | 0.802 | 0.573 | | 1.1 ± 3.0 | 7.7 ± 7.1 | ND | ND |
| | $AUC_{0-6}$ | 6.7 ± 12 | 0.785 | 0.581 | | 2.3 ± 6.1 | 17 ± 16 | ND | ND |
| | T-C | | | | NS | | | | |
| Depression or sadness | $E_{max}$ | 3.0 ± 5.3 | 3.774 | 0.089 | | 1.3 ± 3.0 | 7.0 ± 8.2 | ND | ND |
| | $AUC_{0-6}$ | 7.0 ± 12 | 2.437 | 0.178 | | 2.6 ± 6.0 | 17 ± 16 | ND | ND |
| | T-C | | | | NS | | | | |
| Drowsiness | $E_{max}$ | 22 ± 28 | 10.050 | 0.013 | | 15 ± 18 | 38 ± 44 | −1.221 | 0.257 |
| | $AUC_{0-6}$ | 66 ± 89 | 17.533 | 0.004 | | 48 ± 64 | 106 ± 140 | −0.933 | 0.378 |
| | T-C | | | | **a** | | | | |
| Dizziness | $E_{max}$ | 15 ± 21 | 1.916 | 0.246 | | 9.9 ± 16 | 27 ± 30 | ND | ND |
| | $AUC_{0-6}$ | 44 ± 71 | 4.783 | 0.058 | | 22 ± 36 | 97 ± 114 | ND | ND |
| | T-C | | | | a | | | | |
| Bad effects | $E_{max}$ | 8.4 ± 10 | 2.761 | 0.147 | | 9.3 ± 12 | 8.7 ± 4.5 | ND | ND |
| | $AUC_{0-6}$ | 23 ± 29 | 1.938 | 0.243 | | 22 ± 33 | 26 ± 20 | ND | ND |
| | T-C | | | | a | | | | |
| Headache | $E_{max}$ | 14 ± 17 | 1.509 | 0.327 | | 8.3 ± 12 | 26 ± 22 | ND | ND |
| | $AUC_{0-6}$ | 28 ± 33 | 3.647 | 0.094 | | 25 ± 39 | 32 ± 22 | ND | ND |
| | T-C | | | | NS | | | | |
| Nausea | $E_{max}$ | 11 ± 10 | 0.262 | 0.891 | | 11 ± 11 | 12 ± 7.3 | ND | ND |
| | $AUC_{0-6}$ | 32 ± 30 | 0.761 | 0.593 | | 28 ± 31 | 40 ± 30 | ND | ND |
| | T-C | | | | a | | | | |
| Vertigo | $E_{max}$ | 12 ± 20 | 0.316 | 0.857 | | 8.7 ± 18 | 19 ± 26 | ND | ND |
| | $AUC_{0-6}$ | 20 ± 32 | 0.143 | 0.959 | | 17 ± 37 | 25 ± 23 | ND | ND |
| | T-C | | | | NS | | | | |

*(Continued)*

**TABLE 2 |** Continued

| Effects | Parameter | ANOVA | | | Comparison to baseline | T-Student | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Doses (6.5–25 mg) (*n* = 10) | | | Doses (6.5–25 mg) (*n* = 10) | 6.5–10 mg (*n* = 7) | 15–25 mg (*n* = 3) | *T*-value | *p*-value |
| | | Mean ± SD | *F* | *p*-value | Dunnett's test | Mean ± SD | Mean ± SD | | |
| Breathing difficulty | $E_{max}$ | 2.7 ± 6.5 | 90.601 | < 0.001 | | 0.3 ± 0.8 | 8.3 ± 11 | −2.103 | 0.069 |
| | $AUC_{0-6}$ | 10 ± 27 | 319.150 | < 0.001 | | 0.6 ± 1.6 | 32 ± 47 | −1.910 | 0.093 |
| | T-C | | | | NS | | | | |
| Face flushing | $E_{max}$ | 13 ± 20 | 0.374 | 0.819 | | 16 ± 17 | 27 ± 29 | ND | ND |
| | $AUC_{0-6}$ | 20 ± 20 | 0.883 | 0.535 | | 53 ± 59 | 72 ± 90 | ND | ND |
| | T-C | | | | NS | | | | |
| **Addiction research center inventory (ARCI)** | | | | | | | | | |
| PCAG (sedation) | $E_{max}$ | 3.1 ± 4.6 | 0.443 | 0.775 | | 3.1 ± 4.3 | 3.0 ± 6.1 | ND | ND |
| | $AUC_{0-6}$ | 14 ± 13 | 1.101 | 0.447 | | 12 ± 13 | 18 ± 14 | ND | ND |
| | T-C | | | | **a** | | | | |
| MBG (euphoria) | $E_{max}$ | 4.4 ± 4.4 | 0.904 | 0.526 | | 3.1 ± 3.5 | 7.3 ± 5.7 | ND | ND |
| | $AUC_{0-6}$ | 16 ± 19 | 1.549 | 0.318 | | 11 ± 14 | 28 ± 28 | ND | ND |
| | T-C | | | | b, **c** | | | | |
| LSD (dysphoria and somatic symptoms) | $E_{max}$ | 4.5 ± 2.7 | 1.469 | 0.337 | | 3.6 ± 1.0 | 6.7 ± 2.5 | ND | ND |
| | $AUC_{0-6}$ | 12 ± 9.8 | 3.802 | 0.088 | | 7.4 ± 6.3 | 23 ± 7.55 | ND | ND |
| | T-C | | | | **a**, **b** | | | | |
| BG (intellectual efficieny and energy) | $E_{max}$ | 1.5 ± 2.2 | 0.330 | 0.847 | | 1.1 ± 2.0 | 2.3 ± 3.1 | ND | ND |
| | $AUC_{0-6}$ | 4.1 ± 6.6 | 0.419 | 0.790 | | 4.0 ± 5.6 | 4.3 ± 10 | ND | ND |
| | T-C | | | | b | | | | |
| A (amphetamine-like effects) | $E_{max}$ | 4.2 ± 1.9 | 0.755 | 0.596 | | 3.7 ± 1.2 | 5.3 ± 2.9 | ND | ND |
| | $AUC_{0-6}$ | 14 ± 8.1 | 0.658 | 0.647 | | 13 ± 5.9 | 19 ± 12 | ND | ND |
| | T-C | | | | **a**, **b**, c | | | | |
| **Evaluation of subjective effects of substances with abuse potential (VESSPA-SEE)** | | | | | | | | | |
| S (sedation) | $E_{max}$ | 6.7 ± 3.3 | 9.231 | 0.016 | | 5.8 ± 3.5 | 8.7 ± 2.08 | −1.275 | 0.238 |
| | $AUC_{0-6}$ | 19 ± 11 | 3.051 | 0.126 | | 16 ± 11 | 24 ± 12 | ND | ND |
| | T-C | | | | a | | | | |
| ANX (psychosomatic anxiety) | $E_{max}$ | 4.0 ± 2.9 | 1.996 | 0.234 | | 3.3 ± 3.1 | 5.7 ± 1.5 | ND | ND |
| | $AUC_{0-6}$ | 13 ± 10 | 3.178 | 0.118 | | 11 ± 10 | 19 ± 8.7 | ND | ND |
| | T-C | | | | a, **b** | | | | |
| CP (changes in perception) | $E_{max}$ | 4.2 ± 4.7 | 8.452 | 0.019 | | 1.7 ± 1.2 | 10 ± 4.6 | −4.736 | 0.001 |
| | $AUC_{0-6}$ | 13 ± 17 | 17.663 | 0.004 | | 4.3 ± 3.9 | 33 ± 18 | −4.311 | 0.003 |
| | T-C | | | | **b** | | | | |
| SOC (pleasure and sociability) | $E_{max}$ | 8.2 ± 7.7 | 2.389 | 0.183 | | 5.9 ± 5.2 | 13 ± 11 | ND | ND |
| | $AUC_{0-6}$ | 26 ± 29 | 3.212 | 0.116 | | 18 ± 20 | 47 ± 40 | ND | ND |
| | T-C | | | | b | | | | |
| ACT (activity and energy) | $E_{max}$ | 6.0 ± 6.3 | 1.205 | 0.412 | | 3.9 ± 4.4 | 11 ± 7.9 | ND | ND |
| | $AUC_{0-6}$ | 18 ± 20 | 1.362 | 0.365 | | 11 ± 12 | 35 ± 27 | ND | ND |
| | T-C | | | | **b** | | | | |

*(Continued)*

**TABLE 2 |** Continued

| Effects | Parameter | ANOVA | | | Comparison to baseline | T-Student | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Doses (6.5–25 mg) (*n* = 10) | | | Doses (6.5–25 mg) (*n* = 10) | 6.5–10 mg (*n* = 7) | 15–25 mg (*n* = 3) | *T*-value | *p*-value |
| | | Mean ± SD | *F* | *p*-value | Dunnett's test | Mean ± SD | Mean ± SD | | |
| PS (psychotic symptoms) | $E_{max}$ | 3.1 ± 4.1 | 3.753 | 0.090 | | 1.2 ± 1.1 | 7.3 ± 5.7 | −2.919 | 0.019 |
| | $AUC_{0-6}$ | 11 ± 18 | 15.680 | 0.005 | | 3.1 ± 3.0 | 28 ± 17 | −2.418 | 0.042 |
| | T-C | | | | NS | | | | |
| **Oral fluid concentrations** | | | | | | | | | |
| 2C-E | $C_{max}$ | 5.8 ± 6.4 | 0.491 | 0.745 | | 7.3 ± 7.2 | 2.4 ± 1.7 | ND | ND |
| | $AUC_{0-6}$ | 18 ± 18 | 0.532 | 0.720 | | 22 ± 21 | 7.3 ± 4.7 | ND | ND |
| | T-C | | | | **a** | | | | |

*$E_{max}$ = peak effects 0–6 h. Emax = peak effects 0–6 h (differences from baseline). $AUC_{0-6}$ = area under the curve from 0 to 6 h. Units: mm [visual analog scale (VAS)], and score [Addiction Research Center Inventory (ARCI), Evaluation of Subjective Effects of Substances with Abuse Potential questionnaire (VESSPA-SEE)] and expressed as mean. $C_{max}$ = maximal concentrations 0–6 h (differences from baseline) measured by ng/mL. For Emax and $AUC_{0-6}$ a one-way ANOVA was used to examine the effect of all doses. A $p < 0.05$ was considered statistically significant. Only if a statistical difference were detected an unpaired T-Student was used to examine differences between the grouped doses (6.5–10 mg vs. 15–25 mg). A $p < 0.05$ was considered statistically significant. ND, not done. For T-C a one-way ANOVA and a post hoc Dunnett's test for multiple comparisons was used. Statistical differences between are presented as "a" $p < 0.05$, "**a**" $p < 0.01$ (times 0–2 h), "b" $p < 0.05$, "**b**" $p < 0.01$ (times 0–4 h), "c" $p < 0.05$, "**c**" $p < 0.01$ (times 0–6 h).*

five different 2C-E doses in a limited number of subjects, a dose-concentration relationship was not observed. We do not have an explanation for the high variability observed, with higher concentrations after lower doses. Problems in the collection of the samples or an erratic distribution of 2C-E in saliva could be possible causes. Concentrations in oral fluid were present in all subjects until 4 h, and5 of them were positive at 6 h post-administration. Oral fluid, in contrast to plasma, is a suitable, non-invasive, and easy biological matrix to collect in a non-controlled setting. Nevertheless, the interpretation of oral fluid 2C-E concentrations without data from plasma is extremely difficult (not obtained in this study or any other).

Our study has several limitations mainly associated with its design as naturalistic-observational. An expectancy bias could appear due to the non-placebo-controlled design. Because participants selected the dose according to their preferences, it resulted in low-moderate doses (ranging from 6.5 to 25 mg), and some doses were only used by one participant. A limited number of subjects could be responsible for a lack of power in some measures. Our findings may not refer to other 2C-E routes of administration. Moreover, the recreational setting could have influenced the effects reported by participants. The limited number of time-point measures did not permit to know the real peak effect/concentration times that will need more intensive evaluations. However, it should be noted that there are a number of strengths: the participation of female subjects, the dose selection by the subjects according to their preferences (6.5–25 mg representing real-life quantities), effects previously experienced with the same or similar psychedelic substances, the recreational scenario, and the use of validated rating scales, questionnaires, and analytic techniques. We cannot discard that a more controlled dose-response study using defined drug doses equal for all subjects would produce a different picture. Future studies should be carried out in controlled conditions and with a larger sample. In addition, it should be noted that 2C-E profiles may vary considerably due to the dose administered and the interindividual differences in pharmacodynamic-pharmacokinetics.

## CONCLUSION

The results of this non-controlled, observational study in a real-life setting of recreational use provide useful preliminary data of the acute pharmacodynamic effects and pharmacokinetics in oral fluid of 2C-E. Taken together, the current findings suggest that self-administered oral 2C-E induced a constellation of alterations in perceptions, hallucinations, and euphoric-mood which displayed marked similarities to psychedelic experience. Even at low-moderate doses, notable perceptual changes and hallucinations were the most prominent 2C-E effects. High interindividual variability among doses was observed. Participants with self-administered higher doses were more susceptible to experiencing the most intense subjective effects. Based on these preliminary data, oral fluid can be an appropriate, non-invasive, biologic matrix to detect acute 2C-E use.

It can be concluded that further research in humans is needed to compare the effects of 2C-E with other classical and new psychedelic substances.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the senior author (MF, magi.farre@uab.cat).

## ETHICS STATEMENT

The protocol was approved by the local Research Ethics Committee (CEIC-Parc de Salut Mar, Barcelona, Spain). The study was conducted in accordance with the Declaration of Helsinki and Spanish laws concerning clinical research. The participants provided their written informed consent previous to participate in this study.

## AUTHOR CONTRIBUTIONS

MF, RT, MV, MG, MT, ES, JR, and EO conceptualized the study design. MF, EO, MG, ES, and MV collected the data. EO and OP analyzed the oral fluid. MV analyzed the 2C-E contents. EP and CP-M analyzed the data. EP, EO, CP-M, MT, MG, MV, OP, ES, JR, RT, and MF wrote, revised, and approved the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.00233/full#supplementary-material

**FIGURE S1** | Time course of individual changes from baseline for selected physiological effects ($n$ = 10; mean, standard error).

**FIGURE S2** | Time course of individual changes from baseline for selected subjective effects ($n$ = 10; mean, standard error).

**FIGURE S3** | Time course of individual 2C-E concentrations in oral fluid ($n$ = 10; mean, standard error).

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Acute Pharmacological Effects of Oral and Intranasal Mephedrone: An Observational Study in Humans

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**Abstract:** Mephedrone (4-methylmethcathinone) is a synthetic cathinone with psychostimulant properties which remains one of the most popular new psychoactive substances (NPS). It is frequently used orally and/or intranasally. To date, no studies have evaluated the acute effects and pharmacokinetics after self-administration of mephedrone orally (ingestion) and intranasally (insufflation) in naturalistic conditions. An observational study was conducted to assess and compare the acute pharmacological effects, as well as the oral fluid (saliva) concentrations of mephedrone self-administered orally and intranasally. Ten healthy experienced drug users (4 females and 6 males) self-administered a single dose of mephedrone, orally ($n$ = 5, 100–200 mg; mean 150 mg) or intranasally ($n$ = 5, 50–100 mg, mean 70 mg). Vital signs (blood pressure, heart rate, and cutaneous temperature) were measured at baseline (0), 1, 2, and 4 h after self-administration. Each participant completed subjective effects questionnaires: A set of Visual Analogue Scales (VAS), the 49-item Addiction Research Centre Inventory (ARCI), and Evaluation of the Subjective Effects of Substances with Abuse Potential (VESSPA-SSE) at baseline, 1, 2, and 4 h after self-administration. Oral fluid and urine were collected during 4 h. Both routes of mephedrone self-administration enhanced ratings of euphoria and well-being effects and increased cardiovascular effects in humans. Although it was at times assessed that the oral route produced greater and larger effects than the intranasal one, concentrations of mephedrone in oral fluid and also the total amount of mephedrone and metabolites in urine showed that concentrations of mephedrone are considerably higher when self-administered intranasally in comparison to orally. Controlled clinical trials are needed to confirm our observational results.

**Keywords:** mephedrone (4-methylmethcathinone); novel psychoactive substances (NPS); psychostimulants; cathinones bath salts; oral administration; intranasal administration



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## 1. Introduction

Mephedrone (4-methylmethcathinone) is considered to be the most popular synthetic cathinone drug, resembling the designer drug 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) [1]. It is an amphetamine with an additional beta-ketone group [2,3]. Mephedrone acts as a releaser of monoamines similar to MDMA, but with greater relative potency to release dopamine versus serotonin compared with MDMA [4,5], indicating

more stimulant-like properties. After emerging at the new psychoactive substances (NPS) drug market, mephedrone has remained present among certain recreational drug/NPS users and particularly among chemsex participants [6,7].

Mephedrone is most commonly available in powder form, but it is also available as tablets and capsules. Similarly to other psychostimulant drugs, mephedrone can be consumed via different routes. The predominant patterns of use are oral ingestion and nasal insufflation (snorting), although there are also reports of use by rectal insertion and intravenous/intramuscular injection. Because of the common desire to recapture the pleasurable initial high, the use of different routes and re-doses are frequent [8,9]. Users sometimes reported mixing oral and nasal routes, and re-dosing during single-use sessions in which the total doses per session typically reached 0.5–2 g, usually taken in every one or two hours [10]. In this respect, in regular mephedrone recreational users, mephedrone induces some undesirable sub-acute effects such as negative mood, fatigue, and physical symptoms [11]. Additionally, numerous fatal cases and non-fatal mephedrone intoxication cases attributable to high-dose use of mephedrone and to poly-drug use have been documented and attributed to potential interindividual differences in pharmacokinetics–pharmacodynamics [12–16]. Mephedrone and mephedrone metabolites have been detected in human plasma, urine, hair, and nails [17–21]. Until now, pre-clinical self-administration models using mephedrone intravenously and orally have evidenced that mephedrone produces psychomotor speed improvement and abuse liability, both typical psychostimulant properties [22–24]. Different metabolic disposition studies including human specimens suggest that mephedrone is metabolized in part by the CYP2D6 isoenzyme [25–28].

Despite the non-depreciable recreational use of mephedrone over the last years, there is limited scientific knowledge about its acute pharmacological effects and pharmacokinetics in humans [26–34] and anecdotal data related to the route of administration. Although as mentioned, mephedrone is frequently used via oral and/or intranasal routes and/or mixing them, no studies have evaluated the acute pharmacological effects of mephedrone in humans comparing both routes of administration. To date, the only three experimental studies conducted with humans have focused primarily on the physiological and subjective effects produced after oral mephedrone, a route of administration least often associated with abuse presumably due to its slow onset of effects [29–31]. After controlled administration, the onset of peak effects ($E_{max}$) produced by oral mephedrone occur about 0.5–0.75 h after [29].

In comparison, recreational users reported that the maximum effects produced by intranasal mephedrone occur within 5 min [34], similarly to other drugs also used intranasally [35–37]. Recently, an experimental study in humans was performed after controlled intranasal administration of mephedrone (100 mg nasally insufflated) in healthy volunteers describing the profile of pharmacokinetics of mephedrone and its enantiomers, but no data about its acute effects were included in the results published [32,33].

To date, there have been no comparisons of mephedrone using different common routes of administration despite the recreational use of mephedrone. The main objective of the present study was to compare the acute effects after self-administration of oral (ingestion) and intranasal (insufflation) mephedrone in observational naturalistic conditions.

## 2. Results

Table 1 presents a summary of the physiological and subjective effects where at least one statistical difference in peak effect ($E_{max}$) and/or $AUC_{0–4\,h}$ were found and includes time-course (T-C) points that showed significant differences.

**Table 1.** Summary of statistically significant results on the physiological and subjective effects observed after self-administration of oral ($n$ = 5) and intranasal ($n$ = 5) mephedrone.

| Effects | Parameter | Mean ± SD | | *T*-Student | | ANOVA | | T-Cpoints |
|---|---|---|---|---|---|---|---|---|
| | | Oral | Intranasal | *t* | *p*-Value | *F* | *p*-Value | |
| Temperature | $E_{max}$ | 0.4 ± 0.6 | −0.2 ± 0.2 | 2.477 | 0.038 | | | |
| | $AUC_{0–4}$ | 0.8 ± 1.1 | −0.5 ± 0.4 | 2.271 | 0.071 | | | |
| | T-C | | | | | 3.356 | 0.036 | |
| Intensity | $E_{max}$ | 48 ± 13 | 25 ± 17 | 2.376 | 0.045 | | | |
| | $AUC_{0–4}$ | 114 ± 57 | 37 ± 30 | 2.700 | 0.027 | | | |
| | T-C | | | | | 3.940 | 0.020 | b |
| Stimulated | $E_{max}$ | 56 ± 17 | 22 ± 19 | 2.976 | 0.018 | | | |
| | $AUC_{0–4}$ | 141 ± 56 | 32 ± 32 | 3.775 | 0.005 | | | |
| | T-C | | | | | 6.828 | 0.002 | a, **b** |
| High | $E_{max}$ | 65 ± 15 | 25 ± 17 | 3.952 | 0.004 | | | |
| | $AUC_{0–4}$ | 156 ± 60 | 33 ± 24 | 4.238 | 0.003 | | | |
| | T-C | | | | | 8.645 | <0.001 | a, **b** |
| Good effects | $E_{max}$ | 79 ± 24 | 26 ± 14 | 4.168 | 0.003 | | | |
| | $AUC_{0–4}$ | 217 ± 101 | 32 ± 22 | 3.954 | 0.004 | | | |
| | T-C | | | | | 7.120 | 0.001 | **a**, **b** |
| Liking | $E_{max}$ | 83 ± 21 | 35 ± 15 | 4.110 | 0.003 | | | |
| | $AUC_{0–4}$ | 246 ± 94 | 62 ± 34 | 4.114 | 0.003 | | | |
| | T-C | | | | | 7.330 | 0.001 | **a**, **b**, c |
| Content | $E_{max}$ | 79 ± 25 | 28 ± 16 | 3.737 | 0.006 | | | |
| | $AUC_{0–4}$ | 238 ± 108 | 45 ± 37 | 3.766 | 0.005 | | | |
| | T-C | | | | | 8.210 | 0.001 | **a**, **b**, c |
| ARCI-MBG | $E_{max}$ | 12 ± 1.7 | 6.4 ± 2.4 | 4.575 | 0.002 | | | |
| | $AUC_{0–4}$ | 333 ± 14 | 12 ± 6.7 | 3.048 | 0.016 | | | |
| | T-C | | | | | 1.448 | 0.254 | |
| VESSPA-SOC | $E_{max}$ | 17 ± 6.0 | 6.6 ± 4.4 | 2.999 | 0.017 | | | |
| | $AUC_{0–4}$ | 46 ± 24 | 8.7 ± 8.7 | 3.237 | 0.012 | | | |
| | T-C | | | | | 8.901 | <0.001 | b |
| VESSPA-ACT | $E_{max}$ | 13 ± 4.0 | 5.6 ± 1.5 | 3.679 | 0.006 | | | |
| | $AUC_{0–4}$ | 35 ± 16 | 7.7 ± 4.9 | 3.589 | 0.007 | | | |
| | T-C | | | | | 8.266 | 0.027 | **a**, **b**, c |
| VESSPA-PS | $E_{max}$ | 1.8 ± 1.1 | 0.4 ± 0.9 | 2.214 | 0.058 | | | |
| | $AUC_{0–4}$ | 2.6 ± 1.7 | 0.4 ± 0.9 | 2.549 | 0.034 | | | |
| | T-C | | | | | 1.567 | 0.223 | |

Emax = peak effects 0–4 h (differences from baseline); $AUC_{0–4}$ = Area under the curve 0–4 h; T-C = temporal course 0–4 h. $E_{max}$ measured by °C (T (temperature)) mm (visual analog scale (VAS)), and score (Addiction Research Center Inventory (ARCI), Evaluation of Subjective Effects of Substances with Abuse Potential questionnaire (VESSPA-SEE)), and expressed as mean and standard deviation. For $E_{max}$ and $AUC_{0–4}$, a Student's *t*-Test for independent sample was used (see Statistical Analysis). A $p$-value < 0.05 was considered statistically significant. For T-C, a one-way analysis of variance (ANOVA) was used (see Statistical analysis). Statistical differences between oral and intranasal are presented as "a" $p < 0.05$, "**a**" $p < 0.01$ (time 1 h), "b" $p < 0.05$, "**b**" $p < 0.01$ (time 2 h), "c" $p < 0.05$, "**c**" $p < 0.01$ (time 4 h). Background color displays empty cells.

Supplementary Figure S1 presented individual data of systolic blood pressure (SBP) in order to show the elevated variability of the acute effects.

Supplementary Table S1 shows significant T-C statistical differences of each route of administration in comparison to placebo. All subjects tolerated study procedures well.

There were neither significant adverse effects including hallucinations, psychotic episodes, nor any other psychiatric symptoms for oral or intranasal mephedrone self-administration during the experimental session.

### *2.1. Physiological Effects*

Regarding physiological effects, both oral and intranasal mephedrone self-administration produced an increase in SBP, DBP, HR, and T (see Table 1, Supplementary Table S1 and Supplementary Figure S1). Comparisons of the two routes of administration revealed no significant differences for $E_{max}$, $AUC_{0–4\,h}$, and T-C in vital signs except for cutaneous T ($E_{max}$). T-C comparison to baseline revealed significant differences at 1 and 2 h after oral self-administration for SBP, DBP, and HR, whilst after intranasal self-administration differences were found only at 1 h in comparison to baseline for DBP, HR, and T.

### *2.2. Subjective Effects*

Both oral and intranasal mephedrone increased subjective drug effects (VAS, ARCI, and VESSPA-SEE) (see Table 1, Supplementary Table S1 and Figure 1). The comparison of the two routes of administration showed significant differences for stimulant-like and pleasurable effects for both $E_{max}$ and $AUC_{0–4\,h}$. T-C comparison between oral and intranasal mephedrone showed significant statistical differences for stimulated, high, good effects, liking, and content feelings at 1, 2, and/or 4 h after administration.

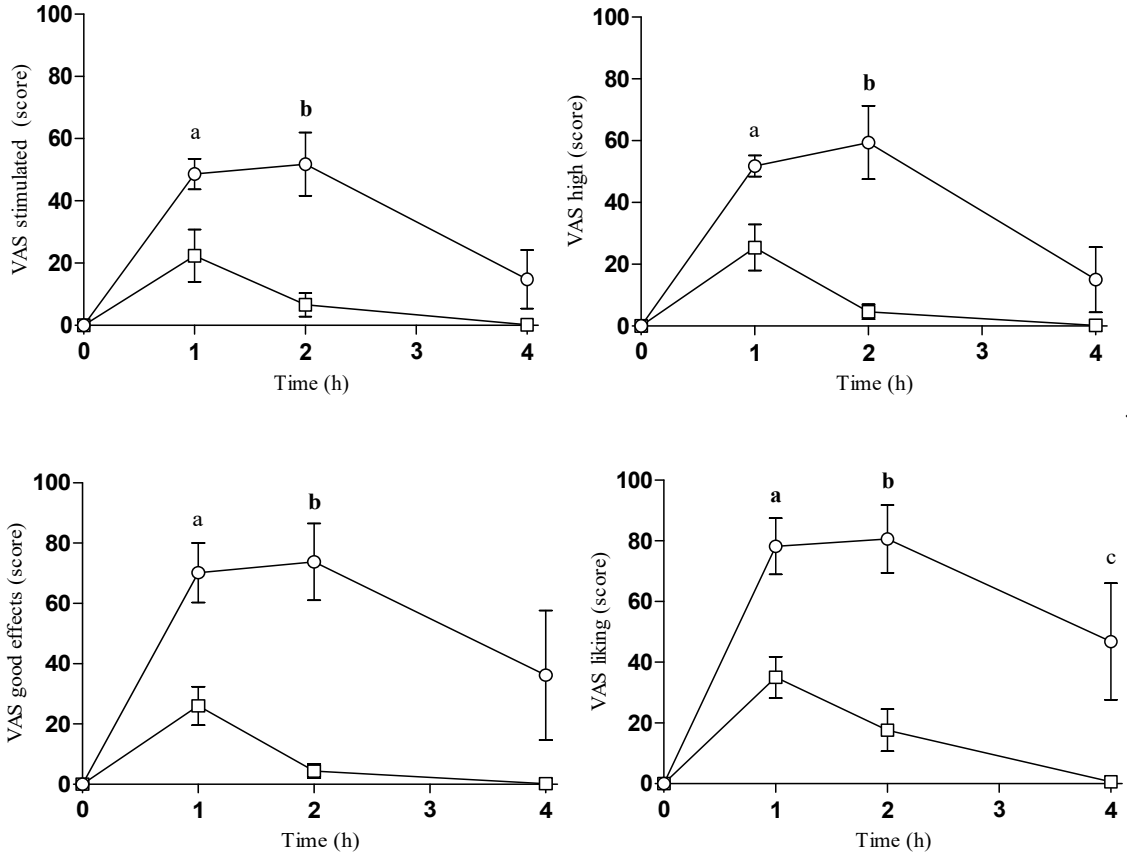


**Figure 1.** *Cont.*

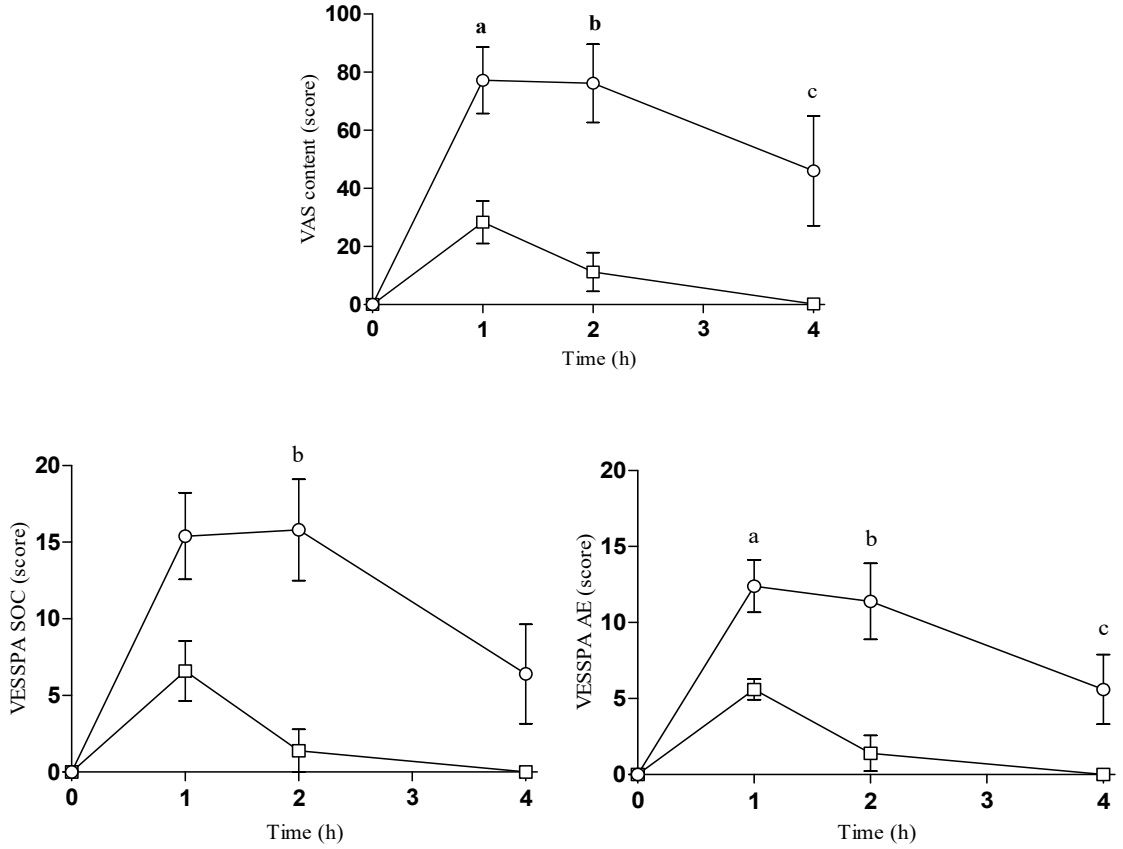


**Figure 1.** Summary of the course of physiological and subjective effects of mephedrone after oral and intranasal self-administration (○ oral mephedrone ($n$ = 5); □ intranasal mephedrone ($n$ = 5)). Statistical differences between oral and intranasal are presented as "a" $p < 0.05$, "**a**" $p < 0.01$ (time 1 h), "b" $p < 0.05$, "**b**" $p < 0.01$ (time 2 h), "c" $p < 0.05$, "**c**" $p < 0.01$ (time 4 h). See text for abbreviations.

After oral mephedrone, T-C comparison to baseline showed significant differences for intensity, stimulates, high and good effects at 1 and 2 h, whilst for liking and content, differences were detected in all times evaluated. In contrast, after intranasal self-administration T-C comparison to baseline only showed significant differences for intensity, stimulates, high, good effects, and content at 1 h and for liking at 1 and 2 h, respectively.

Both oral and intranasal mephedrone produced mild changes in perceptions, but not hallucinations, although no statistically significant differences were detected among routes of administration except for different body feeling ($AUC_{0–4\,h}$).

With respect to the ARCI questionnaire, mephedrone self-administered orally and intranasally produced an increase in all the subscales evaluated. The most marked increases were observed in scores for the MBG (euphoria), BG (intellectual efficiency and energy), and A (amphetamine) subscales. When comparing both routes of administration, statistical differences were detected only for the MBG subscale in $E_{max}$ and $AUC_{0–4\,h}$.

In comparison to baseline, statistical differences were shown in several T-C points after oral self-administration at 1 h and 2 h for MBG and BG subscales, and at 1, 2, and 4 h for A subscales, and also after intranasal self-administration at 1 and 2 h for PCAG, BG, and A subscales, and at 1h for the MBG subscale.

Regarding the VESSPA-SEE questionnaire, mephedrone increased all the subscales regardless of the route of administration except for the CP (changes in perception) subscale for intranasal mephedrone. Comparing both routes, statistical differences were observed in $E_{max}$ and $AUC_{0–4\,h}$ for the SOC (pleasure and sociability) and ACT (activity and energy) subscales and only in $AUC_{0–4\,h}$ for PS (psychotic symptoms) subscale. Whilst for T-C, statistical differences were found only in ACT scores in all points.

T-C comparison at baseline revealed significant differences at 1 h for the PS subscale, at 1 and 2 h for ANX and SOC subscales, and at 1, 2, and 4 h for ATC subscales after oral self-administration, and at 1h for SOC and ACT subscales, and at 1 and 2 h for ANX subscales after intranasal self-administration.

### *2.3. Oral Fluid Concentrations of Mephedrone*

The oral fluid (saliva) T-C concentrations curve for mephedrone is shown in Figure 2 and Supplementary Figure S2 (individual data).

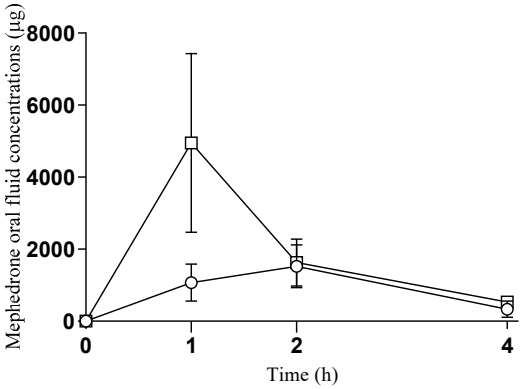


**Figure 2.** Time-course of mephedrone oral fluid concentrations after oral and intranasal self-administration (○ oral mephedrone ($n$ = 5); □ intranasal mephedrone ($n$ = 5)).

After self-administration of oral mephedrone, concentrations of mephedrone in oral fluid increased rapidly, reaching a peak 2 h after ingestion, and decreased at 4 h. Mean maximum concentration ($C_{max}$) values of 1571 ± 1367 ng/mL (range 18–2999 ng/mL) were obtained at a $T_{max}$ of 2 h following drug administration. The $AUC_{0–4\,h}$ was 3686 ± 3443 ng·h/mL (range 61–7593 ng·h/mL). At 4 h, all subjects presented mephedrone concentrations except for one subject that had no detectable concentrations.

After self-administration of intranasal mephedrone, oral fluid concentrations of mephedrone increased rapidly, reaching a peak 1 h after ingestion, and then rapidly decreased at 4 h. $C_{max}$ values of 4950 ± 5545 ng/mL (range 1091–14,525 ng/mL) were obtained at a $T_{max}$ of 1 h following drug administration. At 4 h, mephedrone concentration was 9 times lower (4–41 times) in comparison to $C_{max}$. The $AUC_{0–4\,h}$ was 7917 ± 7717 ng·h/mL (range 1633–20,918 ng·h/mL). Oral fluid mephedrone concentrations varied considerably among oral and intranasal doses and subjects. No significant differences between oral and intranasal mephedrone were found for $C_{max}$, $AUC_{0–4\,h}$, and $T_{max}$ (Table 2).

**Table 2.** Oral fluid pharmacokinetics parameters of oral ($n$ = 5) and intranasal ($n$ = 5) mephedrone.

| Pharmacokinetic Parameters | $C_{max}$ (ng/mL) | $AUC_{0–4}$ (ng/mL $h^{-1}$) | $T_{max}$ (h) |
|---|---|---|---|
| Oral | 1571 ± 1367 | 3684 ± 3443 | 2 (1–2) |
| Intranasal | 4950 ± 5545 | 7917 ± 7717 | 1 (1–1) |
| $p$-value | 0.296 | 0.373 | 0.083 |

Abbreviations: AUC: Area under the curve. SD: Standard deviation. $T_{max}$ is shown as median (range) values.

### *2.4. Urinary Concentrations of Mephedrone and Metabolites*

Recovery of mephedrone and its metabolites nor-mephedrone, dihydro-mephedrone, 4-carboxy-mephedrone, and succinyl-nor-mephedrone in urine in the 0–4 h period post self-administration is shown in Figure 3. The profile of metabolites recovered in urine was similar for all doses tested, and for the oral doses it was congruent with previous data published [15].

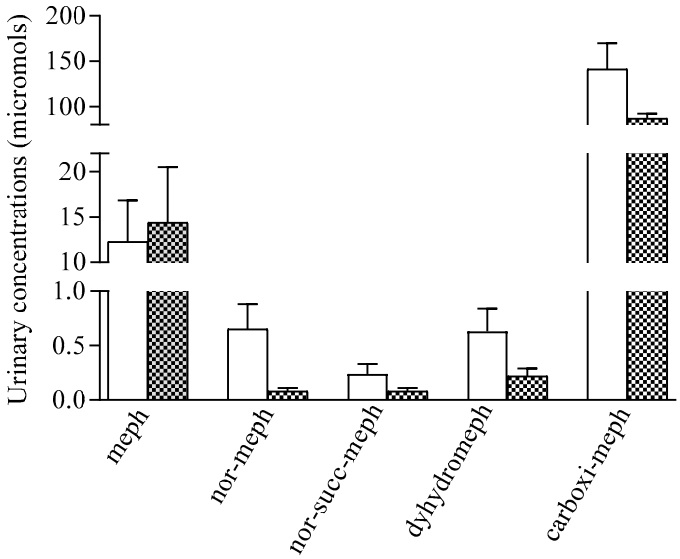


**Figure 3.** Urinary concentrations of mephedrone after oral and intranasal self-administration (unfilled bar: Oral mephedrone ($n$ = 5); filled bar: Intranasal mephedrone ($n$ = 5)).

## 3. Discussion

The overall purpose of this study was to describe the acute effects of oral and intranasal mephedrone in naturalistic conditions and to compare the two most important routes of its administration.

The present findings show that mephedrone self-administered in observational naturalistic conditions induced acute effects that are similar to those produced under experimental conditions [29,30]. Consistent with these results, mephedrone produced similar effects on the majority of physiological and subjective measures. Furthermore, both routes of mephedrone administration (oral and intranasal self-administration) enhanced ratings of euphoria and increased cardiovascular effects.

With respect to the pharmacological effects after oral self-administration of mephedrone, the magnitude and maximum intensity of the pharmacological effects are in accordance with those observed under controlled conditions. Overall, peak effects were observed between 1–2 h and returned to baseline 3–4 h after drug administration [29,30]. In relation to intranasal mephedrone, as mentioned initially, there is no previously published pharmacodynamic data to compare with. In general terms, the intranasal self-administration of mephedrone produces acute pharmacological effects similar to those produced by oral mephedrone. The most remarkable result of this study showed that, at times assessed (1 and/or 2 h), mephedrone oral self-administration in comparison to intranasal self-administration produced greater and larger effects on some subjective measures (e.g., ratings of VAS and several subscales of ARCI and VESSPA). Nonetheless, as would be expected, mephedrone, similarly to other psychostimulant drugs that are also usually used by the intranasal route (insufflation), dilated the vascular-rich areas of the intranasal cavity and pulmonary network, thus increasing the absorptive surface area and allowing for more rapid entry of the drug into the bloodstream, producing fast and reinforcing effects [37]. This well-known factor could justify the fact that the punctuation of subjects who self-administered intranasal mephedrone was lower than those who self-administered orally, because the first evaluation (at 1 h) was conducted once the maximum subjective effects were induced (several minutes after self-administration, which was not assessed).

According to previous published results of mephedrone pharmacokinetics in plasma after controlled intranasal administration, mephedrone showed rapid absorption with a mean $T_{max}$ of 0.88 $\pm$ 0.35 h [32]. Besides, this $T_{max}$ in plasma was slightly shorter in comparison to the plasma $T_{max}$ of 1.2 h after controlled oral administration of 200 mg of mephedrone by our research group [29]. Again, these data point to faster acute pharmacological effects of intranasal mephedrone compared to oral mephedrone.

Additionally, both results obtained from concentrations of mephedrone in oral fluid and from the total amount of mephedrone and metabolites in urine confirm that concentrations of mephedrone are considerably higher after intranasal self-administration in comparison to oral self-administration. As expected, mean oral fluid concentrations of mephedrone at 1 h post administration was 4.6 times higher after intranasal than oral administration (4950 ng/mL versus 1070 ng/mL, respectively), achieving by both routes similar concentrations at 2 h.

In urine, again mephedrone concentrations were higher after intranasal than oral administration of mephedrone. In relation to mephedrone metabolites after oral self-administration, all metabolites were detected with a similar profile of recovery in comparison with a previous study [17], whilst there are no previous data for intranasal mephedrone.

The relevance of our results for intoxication cases is limited because usually concentrations in different biological samples have documented great variability. To date, urine concentrations have been analyzed in several intoxications and fatality cases of mephedrone, whilst there is no data about oral fluid ones. Urinary concentrations reported in clinical trials or mild intoxications are in the range of concentrations measured in our study [11,17,38].

Finally, there were a number of limitations presented by this study design. The main limitations associated with the study are the naturalistic-observational design, that doses varied across subjects and were different in subjects using intranasal vs. oral route and the number of time-point measures and their time interval. This last limitation is particularly important for the evaluation of fast acute effects. It did not permit us to accurately know the real maximal or peak effect/concentration times particularly for intranasal mephedrone, which will need more frequent and earlier evaluations. Other limitations to consider are the non-placebo-controlled design, because participants selected the dose and the route of administration according to their preferences and previous experiences (expectancy bias), a limited number of subjects (lack of statistical power in some measures). Furthermore, the effects reported by participants could have influenced the recreational setting. Finally, we did not collect data on genetic polymorphism of CYP2D6 that can influence the pharmacokinetics and effects of the substance.

However, the strengths should be remarked on: The participation of female subjects, the dose selection by the participants according to their preferences, the inclusion of two different routes of administration, effects previously experienced with the same or similar psychoactive substances, the recreational scenario, and the use of validated methodology using in controlled studies (rating scales, questionnaires) and analytic techniques.

Preliminary data from this observational study have pointed out for the first time that mephedrone profiles in real conditions may vary considerably depending on the route of administration due to the dose administered and the interindividual differences in pharmacodynamic-pharmacokinetics. Thus, it is not possible to make valid conclusions and comparisons, and controlled clinical trials are needed to confirm our observational results.

## 4. Materials and Methods

### *4.1. Participants*

Ten healthy subjects were selected (4 females and 6 males). Participants were recreative drug users who had experience with amphetamines, ecstasy, mephedrone, and/or cathinones at least once in their lifetimes without experiencing previous serious adverse reactions.

Exclusion criteria included a history of any serious medical or psychopathological disorder including substance use disorder (except for nicotine), a previous serious adverse reaction with users of amphetamines, ecstasy (MDMA), mephedrone, and cathinones, and use of chronic medication. Participants were recruited by word-of-mouth and snowball sampling through the harm reduction, non-governmental organization Energy Control (ABD). The protocol was approved by the Clinical Research Ethics Committee. The study was conducted in accordance with the Declaration of Helsinki recommendations. All the participants were fully informed, both orally and in writing, about the study characteristics.

All of them indicated their agreement to participate and signed an informed consent prior inclusion. Subjects were financially compensated for their participation.

### *4.2. Design and Treatments*

The study was conducted according to a non-controlled prospective observational study with minimal intervention in subjects who self-administered mephedrone orally or intranasally. Similarly to previous naturalistic observational studies evaluating acute effects of other NPS, the methodology including evaluations and procedures were similar [39,40]. Each subject participated in one session. Treatment consisted of oral or intranasal self-administration of mephedrone that they brought to the testing site themselves, which they had obtained from an unknown source. Although no information was available about the synthesis of the drug, similar capsules tested by Energy Control, a harm reduction organization that provides a Drug Checking Service for users, showed that the substance contained mephedrone at 95% purity with no toxic adulterants. A gas chromatography associated with mass spectrometry (GC/MS) was previously used by the mephedrone analysis. The method used permits to check for most common drugs of abuse including most of the NPSs and to know the exact purity of mephedrone in the powder to prepare dosing by a precision scale [29]. The dose of oral and intranasal mephedrone self-administered was selected by the participants based presumably on their previous experience. Five subjects self-administered one dose of mephedrone orally, the mean mephedrone dose was 150 mg (100–200 mg) (1 female ingested 100 mg, 2 females and 1 male ingested 150 mg, 1 male ingested 200 mg), and five subjects self-administered one dose of mephedrone intranasally, the mean mephedrone dose was 70 mg (50–100 mg) (3 males insufflated 50 mg and 2 males 100 mg). All the selected doses were well tolerated, and no serious adverse events were observed. No local tissue damage to the nostrils or any other potential acute medical complication after snorting was reported.

### *4.3. Procedures*

Prior to the study session, the participants underwent a general medical examination and a psychiatric interview. They received training with respect to questionnaires used in the study. Upon arrival, they were questioned about any event that could affect their participation and any drug use 2 days prior to the session. Participants were not allowed to consume alcohol or beverages containing caffeine the previous 24 h. The session took place on the same day at a private club with ambient music and participants could talk, read, or play table games during the session and interact in exception to the evaluation times. Moreover, they were instructed not to talk about the effects of the substance during the session. Assessments were performed at baseline (pre-dose) and 1, 2, and 4 h after oral or intranasal self-administration of mephedrone. The experiment was conducted from 15:00 to 21:00 h. Earlier assessment (<1 h) could not be carried out due to the setting of consumption. Urine spot samples were collected at baseline (pre-dose) to exclude prior substance drug use (benzodiazepines, barbiturates, morphine, cocaine, amphetamines, methamphetamine, MDMA, marijuana, phencyclidine) with Instant-View, Multipanel 10 Test Drug Screen (Alfa Scientific Designs Inc., Poway, CA, USA). Self-administration of mephedrone took place around 16.00 h. At each time point of the session, the sequence of procedures was: Physiological measures, oral fluid collection, subjective effects questionnaires, and urine recollection. During entire study session, a psychiatrist was present and adverse effects were assessed.

### *4.4. Physiological Effects*

Physiological effects including non-invasive systolic blood pressure (SPB), diastolic blood pressure (DBP), and heart rate (HR) were determined with an Omron monitor at baseline and 1, 2, and 4 h after administration. Cutaneous temperature was measured simultaneously.

### *4.5. Subjective Effects*

Subjective effects of mephedrone were measured at baseline and at 1, 2, and 4 h after self-administration using different scales and questionnaires. A set of Visual Analog Scales (VAS) (100 mm, from "not at all" to "extremely") were used to measure rate intensity; stimulated; high; good effects; liking; content; changes in colors; changes in shapes; changes in lights; hallucinations—seeing of lights or spots; hallucinations—seeing animals, things, insects, or people; changes in hearing; hallucinations—hearing sounds or voices; different body feeling; unreal body feeling; changes in distances; different surroundings; unreal surroundings; confusion; fear; depression or sadness; drowsiness; dizziness; bad effects; headache; nausea; vertigo; breathing difficulty; and face flushing [29,39–42]. The 49-item Addiction Research Centre Inventory (ARCI) short form, a validated instrument that includes subscales related to drug sedation (pentobarbital chlorpromazine-alcohol group, PCAG), euphoria (morphine-benzedrine group, MBG), dysphoria and somatic symptoms (lysergic acid diethylamide group, LSD), intellectual efficiency and energy (benzedrine group, BG), and d-amphetamine like effects (A) that evaluate subjective effects produced by psychoactive drugs [38,39]. The Evaluation of the Subjective Effects of Substances with Abuse Potential (VESSPA-SE) questionnaire that includes subscales related to sedation (S), psychosomatic anxiety (ANX), changes in perception (CP), pleasure and sociability (SOC), activity and energy (ACT), and psychotic symptoms (PS) that measures changes in subjective effects caused by different drugs including stimulants and psychedelics [29,43].

### *4.6. Urinary Concentrations of Mephedrone and Metabolites*

Urine samples were collected at baseline (0 h) and during the entire session (0–4 h). Urine was stored at −20 °C until analysis. Urinary samples were analyzed following a previously reported validated method based on liquid chromatography tandem–mass spectrometry (LC-MS/MS). Mephedrone and its main metabolites, nor-mephedrone, dihydro-mephedrone, nor-succinyl-mephedrone and carboxy-mephedrone were quantified [44,45].

### *4.7. Oral Fluid Concentrations of Mephedrone*

Oral fluid samples were collected with Salivette® tubes at 0 h (baseline), 2, and 4 h after mephedrone self-administration. After collection, samples were centrifuged and frozen at −20 °C until analysis. Mephedrone concentrations were analyzed by a validated LC-MS/MS [39,40]. A Mephedrone chromatogram (one participant that self-administrated orally 150 mg of mephedrone and one participant that self-administrated 50 mg intranasal) and chromatograms of the internal standard used for the previous samples (Mephedrone-d3) were available in Supplementary Figure S2 and linearity parameters of the oral fluid methodology in Supplementary Figure S3.

The oral fluid (saliva) T-C concentrations curve for mephedrone is shown in Figure 2 and Supplementary Figure S4 (individual data).

### *4.8. Statistical Analysis*

Differences with respect to baseline were calculated for physiological (SBP, DBP, HR, and T) and subjective effects (VAS, ARCI, and VESSPA). Maximum effects ($E_{max}$) were determined and the area under the curve of the effects ($AUC_{0–4\,h}$) was calculated using the trapezoidal rule by the Pharmacokinetic Functions for Microsoft Excel (Joel Usansky, Atul Desai, and Diane Tang-Liu, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA, USA).

To study possible differences between doses, a one-way analysis of variance (ANOVA) test including all doses for each route of administration as a factor was used for $E_{max}$ and $AUC_{0–4\,h}$.The results showed <15% of statistically significant differences among doses for each route of administration. Therefore, it was decided to consider all doses for each route of administration globally, and Student's *t*-Test for paired sample was conducted for $E_{max}$ and $AUC_{0–4\,h.}$

To compare the time course (T-C) of effects of mephedrone between the two routes of administration, a one-factor repeated measures ANOVA (baseline, 1, 2, and 4 h) was performed. Additionally, to evaluate the mephedrone effects along time of each route of administration a Dunnett multiple comparison post hoc test was conducted to compare the different time points with baseline (times 0–1 h, 0–2 h and 0–4 h) for each route of administration.

Differences in time to reach peak effects ($T_{max}$) values were assessed using a Non-Parametric Test (Wilcoxon test).

Statistically analyses were performed using PAWS Statistics version 18 (SPSS Inc., Chicago, IL, USA). Statistically significance was defined as $p < 0.05$.

For mephedrone oral fluid concentrations, the maximum concentration ($C_{max}$), the time needed to reach the maximum concentration ($T_{max}$) and the $AUC_{0–4\,h}$ was calculated using the Pharmacokinetic Functions for Microsoft Excel (Joel Usansky, Atul Desai, and Diane Tang-Liu, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA, USA).

For mephedrone and metabolites urine concentrations, the amount of drug recovered in urine was calculated.

## 5. Conclusions

The study examined for the first time the acute effects of oral and intranasal mephedrone in observational naturalistic conditions. Preliminary data demonstrate that the route of administration of mephedrone could yield some appreciable differences in the acute effects attributed to mephedrone in a sample of young-adult recreational drug users. It is important to remark that each of the routes of administration carries unique and acute medical associated risks, and clinicians should be prepared to educate patients about the acute risks associated not only with mephedrone use, but also with its route of administration.

In conclusion, these results confirm that oral and intranasal mephedrone produced in natural conditions reinforcing and well-being effects in humans.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/1424-8247/14/2/100/s1, Figure S1: (a). Individual data of systolic blood pressure (SBP) after oral self-administration ($n$ = 5); (b) Individual data of systolic blood pressure (SBP) after intranasal self-administration ($n$ = 5), Figure S2: (a) Mephedrone chromatogram of a participant that self-administrated orally 150 mg of mephedrone (right) and one subject that self-administrated 50 mg intranasal (left) from and oral fluid sample (b) Chromatograms of the internal standard used for the previous samples (Mephedrone-d3), Figure S3: Linearity parameters of the oral fluid methodology, Figure S4: (a) Individual data of time-course of mephedrone oral fluid concentrations after oral self-administration of mephedrone ($n$ = 5); (b) Individual data of time-course of mephedrone oral fluid concentrations after intranasal self-administration of mephedrone ($n$ = 5), Table S1: Summary of time course result on the physiological and subjective effects observed after self-administration of oral ($n$ = 5) and intranasal ($n$ = 5) mephedrone.

**Author Contributions:** Conceptualization, E.O., M.T., M.V., R.d.l.T., and M.F.; formal analysis, E.P., E.O., C.P.-M., M.V., R.d.l.T., and M.F.; investigation, E.P., E.O., M.G., M.V., and M.F.; writing—original draft, E.P., C.P.-M., and M.F.; writing—review & editing, E.P., E.O., C.P.-M., M.T., F.F., M.G., M.V., R.d.l.T., and M.F. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of PARC DE SALUT MAR (protocol code 2016/6700 and date of approval on 10 May 2016).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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# A Comparison of Acute Pharmacological Effects of Methylone and MDMA Administration in Humans and Oral Fluid Concentrations as Biomarkers of Exposure

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**Simple Summary:** Methylone is a synthetic cathinone that is usually used as a substitute for conventional psychostimulants, such as MDMA. Chemically, methylone is considered the β-keto analogue of MDMA, with which it presumably shares similar pharmacological effects. To date, the available data about the human pharmacology of methylone in humans are very scarce and are mainly derived from user experiences, published in internet forums or intoxication reports. Thus, an observational–naturalistic study was conducted to evaluate the acute pharmacological effects and determine biomarkers of exposure in oral fluid of methylone after oral self-administration in comparison to MDMA. Methylone induced the prototypical psychostimulant and empathogenic effects commonly associated with MDMA, although they were of lower intensity. Oral fluid concentrations of methylone can be considered a suitable biomarker of acute exposure, and oral fluid has been proven to be a useful biological matrix of detection.

**Abstract:** Considered the β-keto analogue of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), 3,4-Methylenedioxymethcathinone (methylone) is a synthetic cathinone. Over the years, methylone has been used as a substitute for conventional psychostimulants, such as MDMA. To date, little is known about the human pharmacology of methylone; the only available information has been provided by surveys or published intoxication reports. In the present observational–naturalistic study, we evaluate the acute subjective and physiological effects of methylone after oral self-administration in comparison to MDMA in healthy poly-drug users. Fourteen participants (10 males, 4 females) selected their single oral doses of methylone from 100 to 300 mg ($n$ = 8, mean dose 187.5 mg) or MDMA from 75 to 100 mg ($n$ = 6, mean dose 87.5 mg) based on their experience. Study variables were assessed at 0, 1, 2, and 4 h (h) and included vital signs (non-invasive blood pressure, heart rate, cutaneous temperature) and subjective effects using visual analogue scales (VAS), the 49-item Addiction Research Centre Inventory (ARCI) short form, and the Evaluation of the Subjective Effects

of Substances with Abuse Potential (VESSPA-SSE) questionnaire. Additionally, oral fluid concentrations of methylone and MDMA were determined. Acute pharmacological effects produced by methylone followed the prototypical psychostimulant and empathogenic profile associated with MDMA, although they were less intense. Methylone concentrations in oral fluid can be considered a useful biomarker to detect acute exposure in oral fluid. Oral fluid concentrations of MDMA and methylone peaked at 2 h and concentrations of MDMA were in the range of those previously described in controlled studies. Our results demonstrate that the potential abuse liability of methylone is similar to that of MDMA in recreational subjects.

**Keywords:** methylone (3,4-methylenedioxymethcathinone); MDMA (3,4-methylenedioxymethamphetamine); new psychoactive substances (NPS); synthetic cathinones; bath salts; psychostimulants

## 1. Introduction

Over the last few years, new psychoactive substances (NPS) have become a trend among substance users seeking non-illegal alternatives to classical illicit drugs. For this reason, these substances are also known as "legal highs", although, in the market, they are also advertised as bath salts, plant foods, or fertilizer, and are labelled as "not for human consumption" to bypass regulations [1,2]. For the first time, in 2019, synthetic cathinones were one of the most frequently reported groups of NPS to the European Union Early Warning System according to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) [3]. These synthetic substances are chemically related to cathinone, a compound with psychostimulant effects, found in the khat plant (*Catha edulis*) [4,5]. In the last decade, numerous new synthetic cathinone derivatives have emerged given the high dynamism of the NPS market. Some of the most well-known derivatives are methylone (3,4-methylenedioxymethcathinone), mephedrone (4-methylmethcathinone), and MDPV (3,4-methylenedioxypyrovalerone).

Methylone, also known as MDMC, M1, and bk-MDMA, is a ring-substitute β-keto analogue of the well-known 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) [6]. Since its first appearance as a "room odorizer" in smartshops, methylone gained popularity as a substitute for the traditional MDMA [7]. Methylone resembles MDMA in its mechanism of action, as methylone acts on the monoaminergic system, inducing the reversal or inhibiting the activity of monoamine reuptake transporters. These actions on the monoaminergic system result in increased extracellular brain levels of monoamines, such as dopamine, norepinephrine, and, mainly, serotonin [8–12]. In this area, there are some discrepancies regarding the potency of its effects on the monoaminergic transporters. Results from an in vitro study suggested that methylone had a selectivity comparable to that of mephedrone and MDMA but displayed a lower potency on transporter-mediated release [8]. Another study concluded that methylone was the most potent serotonin and dopamine uptake inhibitor compared to mephedrone and butylone [13]. Methylone and other cathinones, such as mephedrone, butylone, and ethylone, act as nonselective monoamine uptake inhibitors, similar to cocaine, and have effects on serotonin release similar to MDMA [14]. In general, methylone acts on monoaminergic transporters with potency and selectivity comparable to that of MDMA [8]. Methylone and other cathinones also can activate 5-HT2A receptors and increase extracellular dopamine [13].

Methylone is metabolized in the liver, principally by the enzymatic activity of CYP2D6, located in cytochrome P450, with a limited contribution of CYP1A2, CYP2B6, and CYP2C19 [15]. Similarly to MDMA, its metabolism results in the formation of O-demethylenated metabolites (HHMC, 3,4-methylenedioxy-N-methylcathinone; HMMC, 4-hydroxy-3-methoxy-Nmethylcathinone) and an N-demethylated metabolite (MDC, 3,4-methylenedioxycathinone) [16,17]. The activity of methylone seems to be related to brain concentrations of methylone and MDC but not its hydroxylated metabolites [18].

Whereas MDMA is the fourth most commonly used recreative substance worldwide according to the UNODC, information about methylone prevalence is scarce. Globally, an estimate of 20.5 million people reported use of MDMA in the last year in 2018 [19]. In the European Union, approximately 2.7 million people aged 15–64 were estimated to have used MDMA in the previous year [3]. On the other hand, the use of methylone as an adulterant and its rebranding as other psychostimulant substances hinder the determination of its prevalence of use, since this unintentional use is not reflected in surveys [20,21]. Most of the information regarding the prevalence of the use of methylone comes from seizure data or reported intoxications. According to the National Forensic Laboratory Information System (NFLIS) data, methylone was the most reported synthetic cathinone (33.35%) in the USA between 2013 and 2015 [22].

Similarly to other synthetic cathinones, methylone can be administered via different routes, including oral, intranasal (insufflation), intravenous, sublingual, and rectal administration. The most common route is oral consumption of tablets or pills containing methylone. In accordance with recreative drug user reports, doses up to 100 mg are considered to be low, doses from 100 mg to 200 mg are moderate, and doses above 200 mg are considered to be high. After oral administration of methylone, users described the onset of effects at 15–60 min after administration, with peak effects occurring at 60–90 min and a total duration of effects of 3–5 h [23]. A frequent pattern of use, also similar to other cathinones, is to firstly administer a large dose followed by smaller re-doses in order to extend the effects [23,24].

According to user reports, methylone also displays a similar but milder range of effects compared to MDMA that encompasses stimulation, calm euphoria, a sense of well-being and happiness, alertness, reduced fatigue, heightened empathy, and entactogenic effects (sense of oneness) [25,26]. Among the published cases of intoxication involving methylone [27–32], a patient that visited the emergency department after using 1.0–1.5 g of methylone presented vomiting, palpitations, agitation, sweating, paresthesia, muscle twitching, tremors, and vertigo [33]. Other adverse effects associated with methylone intoxication are hyperthermia, anxiety, seizures, psychosis, hallucinations, and suicidal ideation [6].

Oral fluid concentrations of amphetamine derivatives are considered a suitable biomarker for the detection of their acute use, and they have been useful in cases of intoxication and driving under the influence of substances [34,35]. In the case of MDMA, there is evidence of a correlation between the oral fluid and blood concentrations [36,37]. However, there are no previous reports evaluating the possible usefulness of methylone oral fluid concentrations as a biomarker of acute exposure.

To date, little is known about the pharmacokinetic and pharmacodynamic profile of methylone in humans. The purpose of our observational study was to assess the acute pharmacological effects and oral fluid concentrations of methylone as a biomarker of exposure in recreational users after oral administration in a naturalistic environment. The subjective and physiological effects and oral fluid concentrations of methylone are compared to those of its non-β-analogue MDMA, which was also administered in similar conditions.

## 2. Materials and Methods

### *2.1. Participants*

Fourteen healthy volunteers were included (10 males and 4 females). The subjects were recreative drug users that had previous experience with MDMA and/or synthetic cathinones at least once in their lifetime. Exclusion criteria included a history of any serious medical or mental disorder, including substance use disorder (except nicotine), serious adverse reactions to MDMA and/or synthetic cathinones, and use of chronic medication.

Participants were recruited by word-of-mouth through Energy Control. The protocol of this study was approved by the Parc de Salut Mar Clinical Research Ethics Committee (ref. 2016/6700/I). The study was conducted in accordance with the Declaration of Helsinki and Spanish legislation. All participants were fully informed of the purpose and procedures

of the study and they were provided with a written informed consent form before enrolling in the study.

### 2.2. Design and Treatments

The study was designed as a non-controlled prospective observational study in a naturalistic setting with methylone and MDMA self-administration. The methodology, including procedures and evaluations, coincides with previous observational–naturalistic studies aimed at evaluating the acute effects of other NPS [38–40]. Six subjects (5 M, 1 F) self-administered MDMA and 8 subjects (5 M, 3 F) self-administered methylone. Each subject participated only in one session. Participants brought their own substance obtained from an unknown source, which was tested by Energy Control, a harm reduction organization that provides a drug checking service to drug users. Pills containing methylone and MDMA were analyzed by gas chromatography associated with mass spectrometry (GC/MS), a technique that traces the presence of the most frequent drugs of abuse, such as MDMA, cocaine, heroin, amphetamine and methamphetamine, LSD, and multiple NPS (methylone, mephedrone, and other synthetic cathinones, synthetic cannabinoids, tryptamines, among others). The testing of the pills showed a more than 95% purity of methylone and MDMA, as well as the absence of toxic components or adulterants [38–40].

In both sessions, participants selected the dose of methylone or MDMA according to their previous drug use experience. Doses of methylone could be selected from a range (75–300 mg) that was previously defined according to the consulted literature [23]. The WHO Expert Committee on Drug Dependence (Thirty-Sixth Meeting, Geneva, Switzerland, 16–20 June 2014) established that 60 mg of methylone was the threshold dose and doses over 250 mg were often related to very strong activity [23]. Some users have reported uses of 300 mg of methylone as a common dose [www.erowid.org] (accessed on 15 June 2021). The mean of the selected doses of MDMA was 87.50 mg (3 subjects ingested 75 mg (2 males and 1 female), 3 males 100 mg). In the other study session, methylone doses ranged from 100 to 300 mg, with a mean of 187.50 mg. Based on their dose selection, 1 male ingested 100 mg, 2 subjects 150 mg (1 male and 1 female), 4 subjects 200 mg (2 males and 2 females), and 1 male 300 mg.

### 2.3. Procedures

Sessions were conducted in a private club closed to the public for the study, where participants were summoned at 15:00 h and stayed until the end of the session at 20:00 h. Upon arrival, urine samples were collected to detect the presence of any conventional drug (benzodiazepines, barbiturates, morphine, cocaine, amphetamines, methamphetamine, MDMA, marijuana, phencyclidine) with Instant-View, Multipanel 10 Test Drug Screen (Alfa Scientific Designs Inc., Poway, CA, USA). Subjects were not allowed to use any recreational drug 2 days prior to the session or consume alcohol and caffeinated beverages in the previous 24 h. Participants received instructions and training on the procedures and questionnaires used throughout the sessions.

The sessions were conducted in a naturalistic setting. Participants were allowed to talk, read, listen to music, or play games, except during the evaluation times. However, they were asked to refrain from talking about the effects of the substance. All the subjects were evaluated simultaneously at baseline, 1, 2, and 4 h after administration of methylone or MDMA, which occurred approximately at 16:00 h. At each time point, evaluations were followed in a specific order: physiological effects, oral fluid collection, and subjective effects scales and questionnaires.

### 2.4. Physiological Effects

Non-invasive systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured with subjects in the sitting position, using an automatic Omron monitor at baseline, 1, 2, and 4 h (h) after self-administration. Cutaneous temperature was determined at the same time points.

### 2.5. Subjective Effects

Subjective effects were evaluated at baseline, 1, 2, and 4 h after self-administration using visual analogue scales (VAS), the Addiction Research Center Inventory (ARCI), and the Evaluation of Subjective Effects of Substances with Abuse Potential questionnaire (VESSPA-SSE).

Visual analogue scales (100 mm, from "not at all" to "extremely") contained 30 items that subjects were asked to rate, such as: intensity, stimulated, high, good effects, liking, happiness, drunkenness, changes in colors, changes in shapes, changes in lights, hallucinations (seeing lights or spots), hallucinations (seeing of animals), changes in hearing, hallucinations (hearing sounds or voices), different or changed body feeling, unreal body feeling, changes in distances, different surroundings, unreal surroundings, confusion, fear, depression or sadness, drowsiness, dizziness, bad effects, headache, sickness, vertigo, shortness of breath, and face flushing [38–40].

The standardized ARCI 49-item short form is a true/false questionnaire used to evaluate the subjective effects of drugs of abuse. This inventory includes five subscales that assess pentobarbital–chlorpromazine–alcohol-like effects (PCAG, sedation), morphine–benzedrine-like effects (MBG, euphoria), lysergic acid diethylamide-like effects (LSD, dysphoria), benzedrine-like effects (BG, intellectual efficiency), and amphetamine-like effects (A, increased energy) [38–40].

The VESSPA-SSE is a questionnaire sensitive to subjective effects related to stimulants such as MDMA that includes six subscales: sedation (S), psychosomatic anxiety (ANX), changes in perception (CP), pleasure and sociability (SOC), activity and energy (ACT), and psychotic symptoms (PS) [38–40].

### 2.6. Oral Concentrations

In both sessions, oral fluid samples were collected with Salivette to determine methylone and MDMA concentrations in oral fluid at baseline, 1 h, 2 h, and 4 h. All samples were centrifuged after collection and stored frozen at −20 °C until analysis. Methylone and MDMA oral fluid concentrations were quantified via liquid chromatography tandem–mass spectrometry (LC–MS/MS) [38–40].

### 2.7. Statistical Analysis

The determination of the sample size was based on the methodology of bioequivalence studies, which resulted in 5–6 subjects needed, considering an alpha risk of 0.05, a power of 80%, with a difference of at least 35% between MDMA to methylone in the intensity/high effect and with 20% of variability.

Vital signs (SBP, DBP, and HR) and subjective effects (VAS, ARCI, and VESSPA-SSE) were baseline-adjusted. Maximum effects and the time in which maximum effects appeared were determined, and the area under the curve ($AUC_{0–4h}$) was calculated with the trapezoidal rule.

For oral fluid concentrations of MDMA and methylone, only a descriptive analysis was presented showing main pharmacokinetics data such as the maximum concentration (Cmax), the time required to reach maximum concentrations (Tmax), and $AUC_{0–4h}$. These parameters were calculated using the Pharmacokinetic Functions for Microsoft Excel (Joel Usansky, Atul Desai, and Diane Tang-Liu, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA, USA).

The following stages in the statistical analysis comprised 4 tests. Firstly, a two-way ANOVA with dose and gender as factors was conducted to determine whether the difference in doses or gender had an impact on the acute effects of methylone or MDMA. In the case of methylone, out of all variables analyzed, only 17 out of 131 variables that corresponded to effects with low scores were found to be significant. For MDMA, none of the variables were significant. Thus, given that any of the main effects associated with methylone or MDMA showed significant differences related to dose and gender, all

participants were grouped independently of these factors considering just one group of methylone and MDMA.

Secondly, the comparison of Emax and $AUC_{0–4h}$ values of physiological and subjective effects between MDMA and methylone was performed with an independent samples *t*-test. Tmax values were compared with the non-parametric Mann–Whitney *U*-test.

Thirdly, to find possible significant changes from baseline, a Dunnett multiple comparison test was conducted to compare each time point with baseline in both drug conditions (0–1 h, 0–2 h, 0–4 h).

Finally, to compare the time-course of all the pharmacological effects between methylone and MDMA, they were evaluated with a two-way ANOVA test with time and drug condition as factors. When these results were significant, a Tukey post-hoc test compared the differences in each time point between conditions.

Statistical analysis was carried out using PASW Statistics version 18 (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant when the resulting *p* value was <0.05.

## 3. Results

Table 1 provides a summary with the statistically significant results (Emax, Tmax, $AUC_{0–4h}$) of the physiological and subjective effects after methylone and MDMA self-administration. Oral fluid concentrations of both substances are presented in Table 2.

**Table 1.** Summary of the statistically significant results on physiological and subjective effects after methylone ($n$ = 8) and MDMA ($n$ = 6) self-administration. Only variables with some statistically significant differences in any of the parameters (Emax, Tmax, $AUC_{0–4h}$) and Dunnett's test are presented.

| | Parameters | Mean ± SD | | T-Student | Dunnet's Test | |
|---|---|---|---|---|---|---|
| | | Methylone | MDMA | *p* Value | Methylone | MDMA |
| Physiological effects | | | | | | |
| SBP (mmHg) | Emax | 31.25 ± 14.77 | 46.83 ± 20.83 | 0.126 | a, b, c | a, b |
| | Tmax | 1.5 (1.0–4.0) | 2.0 (1.0–2.0) | 0.659 | | |
| | $AUC_{0–4h}$ | 80.81 ± 42.89 | 107.67 ± 44.34 | 0.275 | | |
| DBP (mmHg) | Emax | 19.63 ± 13.96 | 32.17 ± 11.29 | 0.097 | a, b, c | a, b |
| | Tmax | 2.0 (1.0–4.0) | 2.0 (1.0–2.0) | 0.150 | | |
| | $AUC_{0–4h}$ | 55.19 ± 31.02 | 78.92 ± 30.33 | 0.178 | | |
| HR (bpm) | Emax | 20.50 ± 19.78 | 10.67 ± 18.22 | 0.360 | a | NS |
| | Tmax | 3.0 (1.0–4.0) | 3.0 (1.0–4.0) | 0.436 | | |
| | $AUC_{0–4h}$ | 52.06 ± 39.16 | 23.83 ± 38.73 | 0.205 | | |
| Subjective effects | | | | | | |
| VAS intensity (mm) | Emax | 20.0 ± 16.48 | 47 ± 11.19 | **0.005** | a, b | a, b |
| | Tmax | 2.0 (1.0–2.0) | 1.5 (1.0–2.0) | 0.411 | | |
| | $AUC_{0–4h}$ | 45.88 ± 43.66 | 111.33 ± 36.34 | **0.012** | | |
| VAS stimulated (mm) | Emax | 22.50 ± 18.81 | 50.17 ± 17.12 | **0.015** | b | a, b |
| | Tmax | 2.0 (1.0–4.0) | 1.5 (1.0–4.0) | 0.160 | | |
| | $AUC_{0–4h}$ | 50.56 ± 47.38 | 113.17 ± 40.92 | **0.024** | | |
| VAS high (mm) | Emax | 24.0 ± 21.28 | 60.17 ± 14.96 | **0.004** | a, b | a, b |
| | Tmax | 2.0 (1.0–4.0) | 1.5 (1.0–2.0) | **0.032** | | |
| | $AUC_{0–4h}$ | 55.06 ± 53.54 | 136.92 ± 41.05 | **0.009** | | |
| VAS good effects (mm) | Emax | 35.63 ± 30.63 | 67.83 ± 16.51 | **0.039** | a, b | a, b |
| | Tmax | 2.0 (1.0–2.0) | 1.5 (1.0–2.0) | 0.548 | | |
| | $AUC_{0–4h}$ | 74.06 ± 67.08 | 167.67 ± 54.01 | **0.016** | | |

**Table 1.** *Cont.*

| | Parameters | Mean ± SD | | T-Student | Dunnet's Test | |
|---|---|---|---|---|---|---|
| | | Methylone | MDMA | *p* Value | Methylone | MDMA |
| VAS content (mm) | Emax | 35.25 ± 30.38 | 74.50 ± 18.96 | **0.017** | | |
| | Tmax | 1.5 (1.0–2.0) | 1.0 (1.0–2.0) | 0.133 | a, b | a, b, c |
| | $AUC_{0–4h}$ | 80.06 ± 82.59 | 186.25 ± 52.39 | **0.018** | | |
| VAS change in lights (mm) | Emax | 4.88 ± 4.82 | 20.00 ± 25.11 | 0.118 | | |
| | Tmax | 1.0 (0.0–2.0) | 1.5 (0.0–4.0) | 0.491 | a, b | NS |
| | $AUC_{0–4h}$ | 10.88 ± 11.85 | 40.00 ± 53.35 | 0.156 | | |
| VAS different body feeling (mm) | Emax | 22.25 ± 20.60 | 50.33 ± 22.59 | **0.032** | | |
| | Tmax | 2.0 (0.0–4.0) | 1.0 (1.0–2.0) | 0.258 | NS | a |
| | $AUC_{0–4h}$ | 44.13 ± 43.86 | 95.25 ± 53.40 | 0.072 | | |
| VAS different surrounding (mm) | Emax | 4.38 ± 7.50 | 17.33 ± 17.68 | 0.085 | | |
| | Tmax | 0.5 (0.0–4.0) | 1.0 (0.0–1.0) | 0.581 | NS | a |
| | $AUC_{0–4h}$ | 5.69 ± 10.39 | 27.33 ± 39.71 | 0.161 | | |
| VAS dizziness (mm) | Emax | 2.13 ± 2.53 | 13.00 ± 9.84 | **0.010** | | |
| | Tmax | 0.5 (0.0–4.0) | 1.0 (0.0–4.0) | **0.034** | a | NS |
| | $AUC_{0–4h}$ | 2.63 ± 3.02 | 20.17 ± 20.68 | **0.034** | | |
| VAS headache (mm) | Emax | 20.25 ± 28.93 | 5.83 ± 7.68 | 0.261 | | |
| | Tmax | 3.0 (0.0–4.0) | 1.0 (0.0–4.0) | 0.629 | c | NS |
| | $AUC_{0–4h}$ | 23.63 ± 28.94 | 10.58 ± 18.86 | 0.357 | | |
| VAS face flushing (mm) | Emax | 31.25 ± 21.91 | 45.00 ± 22.74 | 0.275 | | |
| | Tmax | 2.0 (1.0–4.0) | 1.5 (1.0–4.0) | 0.406 | b | a |
| | $AUC_{0–4h}$ | 64.50 ± 56.75 | 89.33 ± 58.31 | 0.439 | | |
| ARCI PCAG (score) | Emax | −1.25 ± 1.58 | −0.83 ± 3.54 | 0.771 | | |
| | Tmax | 1.0 (1.0–4.0) | 1.0 (1.0–2.0) | 0.105 | a | NS |
| | $AUC_{0–4h}$ | −2.75 ± 3.73 | −1.58 ± 8.39 | 0.730 | | |
| ARCI MBG (score) | Emax | 7.5 ± 5.13 | 8.0 ± 3.35 | 0.839 | | |
| | Tmax | 2.0 (1.0–2.0) | 1.0 (1.0–2.0) | **0.011** | a, b | a, b |
| | $AUC_{0–4h}$ | 16.63 ± 13.21 | 20.83 ±10.05 | 0.528 | | |
| ARCI BG (score) | Emax | 4.63 ± 3.34 | 4.67 ± 2.25 | 0.979 | | |
| | Tmax | 2.0 (1.0–4.0) | 1.0 (1.0–2.0) | **0.030** | a, b | a, b |
| | $AUC_{0–4h}$ | 10.06 ± 7.83 | 9.83 ± 6.38 | 0.954 | | |
| ARCI A (score) | Emax | 5.38 ± 3.29 | 5.83 ± 1.17 | 0.752 | | |
| | Tmax | 1.5 (1.0–4.0) | 1.0 (1.0–2.0) | 0.106 | a, b | a, b, c |
| | $AUC_{0–4h}$ | 12.56 ± 7.77 | 15.75 ± 2.79 | 0.360 | | |
| VESSPA S (score) | Emax | 3.38 ± 2.50 | 2.83 ± 2.23 | 0.683 | | |
| | Tmax | 4.0 (1.0–4.0) | 1.0 (1.0–1.0) | 0.061 | b, c | a, c |
| | $AUC_{0–4h}$ | 7.06 ± 6.45 | 5.92 ± 6.09 | 0.742 | | |
| VESSPA ANX (score) | Emax | 7.50 ± 4.69 | 6.67 ± 4.37 | 0.741 | | |
| | Tmax | 3.0 (1.0–4.0) | 1.0 (1.0–2.0) | **0.019** | b, c | a, b |
| | $AUC_{0–4h}$ | 17.94 ± 11.17 | 16.50 ± 8.82 | 0.800 | | |
| VESSPA SOC (score) | Emax | 12.25 ± 8.31 | 14.50 ± 5.96 | 0.585 | | |
| | Tmax | 2.0 (1.0–2.0) | 1.0 (1.0–2.0) | 0.298 | a, b | a, b |
| | $AUC_{0–4h}$ | 31.31 ± 25.40 | 38.67 ± 17.84 | 0.557 | | |
| VESSPA ACT (score) | Emax | 9.75 ± 6.25 | 13.33 ± 4.84 | 0.268 | | |
| | Tmax | 1.0 (1.0–2.0) | 1.5 (1.0–2.0) | 0.877 | a, b | a, b |
| | $AUC_{0–4h}$ | 24.44 ± 17.87 | 32.58 ± 15.71 | 0.393 | | |
| VESSPA PS (score) | Emax | 2.63 ± 2.20 | 1.33 ± 1.21 | 0.221 | | |
| | Tmax | 1.0 (0.0–2.0) | 2.0 (0.0–2.0) | 0.362 | b | NS |
| | $AUC_{0–4h}$ | 5.63 ± 5.58 | 2.25 ± 2.36 | 0.192 | | |

Abbreviations: Area under the curve (AUC), visual analogue scales (VAS), Addiction Research Center Inventory (ARCI) (PCAG (sedation), MBG (euphoria), BG (intellectual efficiency), and A (increased energy)), Evaluation of Subjective Effects of Substances with Abuse Potential questionnaire (VESSPA-SSE) (sedation (S), psychosomatic anxiety (ANX), pleasure and sociability (SOC), activity and energy (ACT), and psychotic symptoms (PS)), not significant (NS). Results of Cmax and $AUC_{0–4h}$ are presented as mean ± standard deviation, Tmax is shown as median (min–max). To compare the T-C with baseline values, a post-hoc Dunnett's test for multiple comparisons was performed. Statistical differences between conditions are indicated as "a" (times 0–1 h), "b" (times 0–2 h), "c" (times 0–4 h). Significant T-Student $p < 0.05$ values are marked in bold.

**Table 2.** Concentrations of methylone ($n$ = 8) and MDMA ($n$ = 6) in oral fluid.

| Oral Fluid Concentrations | Methylone | MDMA |
|---|---|---|
| Cmax | 15,514.00 ± 9748.86 | 2936.37 ± 2761.57 |
| Tmax | 2.0 (2.0–2.0) | 2.0 (2.0–4.0) |
| $AUC_{0–4\,h}$ | 40,623.79 ± 20,001.70 | 6586.44 ± 5229.92 |

Abbreviations: Area under the curve (AUC). Results of Cmax (ng/mL) and $AUC_{0–4h}$ (ng/mL·h) are presented as mean ± standard deviation, Tmax (h) is shown as median (min–max).

### *3.1. Participants*

In total, 14 subjects (10 males, 4 females) were selected to participate in the study. Eight subjects (5 males, 3 females) were included for the self-administration of methylone. Participants had a mean age of 30 ± 5 years (range 23–37), weighed 64.88 ± 9.20 kg (range 54.0–78.0), and had a mean body mass index (BMI) of 22.24 ± 3.50 kg/m$^2$ (range 16.48–26.03). The mean dose of methylone was 187.50 ± 58.25 mg (range 100–300), which, adjusted to weight, resulted in 2.97 ± 0.99 mg/kg (range 1.28–4.35). At the beginning of the session, urine samples were collected to determine previous drug use. All subjects tested negative in the urine drug tests.

Six participants (5 males, 1 female) were included for the self-administration of MDMA. Participants had a mean age of 29 ± 6 years (range 22–38), weighed a mean of 65.33 ± 8.45 kg (range 54.0–75.0), and had a mean BMI of 22.41 ± 3.17 kg/m$^2$ (range 16.48–25.65). The mean dose of MDMA was 87.50 ± 13.69 mg (range 75–100), which, adjusted to weight, resulted in 1.35 ± 0.19 mg/kg (range 1.00–1.54). Five subjects obtained negative results in the urine test and one subject tested positive for cannabis. This participant reported that their last cannabis use was 48 h prior to the session, as specified in our selection criteria.

In both cases, all selected participants reported previous experience with psychostimulants (including MDMA, amphetamines, NPS/synthetic cathinones, cocaine), cannabis, and hallucinogens. See Table S1 for history of drug use.

### *3.2. Physiological Effects*

With respect to physiological effects, both methylone and MDMA produced a statistically significant increase in SBP and DBP compared to baseline over the first 2 h, although this significant effect was prolonged to 4 h in the case of methylone (see Figure 1). Regarding HR, only methylone showed a significant increase in the first hour. Neither of the substances caused significant variations in temperature.

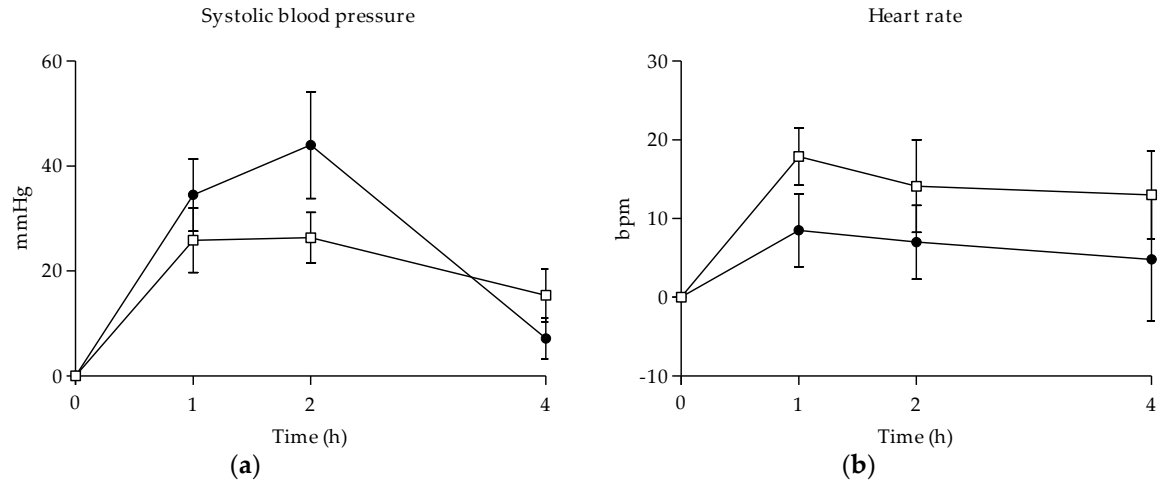



**Figure 1.** Time course of systolic blood pressure (**a**) and heart rate (**b**) after methylone and MDMA oral administration. (□, 100–300 mg methylone ($n$ = 8); ●, 75–100 mg MDMA ($n$ = 6); mean, standard error).

In addition, the maximum effects on SBP and BDP were higher after the administration of MDMA, whereas methylone showed higher maximal effects on HR (see Table 1). However, no significant differences between methylone and MDMA were detected in Emax, $AUC_{0–4h}$, and Tmax of the cardiovascular effects.

### *3.3. Subjective Effects*

Methylone and MDMA produced significant subjective effects, which were collected in VAS, ARCI, and VESSPA-SSE. Overall, subjects reported subjective effects starting at 1 h, with maximum values ranging from 1 h to 2 h; hence, most of these effects had almost disappeared at 4 h.

When compared to baseline, both substances caused significant changes in VAS measures, reflecting stimulant-like effects ("intensity", "stimulated", "high", "good effects", "liking", "content", "drunkenness"), changes in perception ("changes in lights" (methylone), "different or changed body feeling" (MDMA), "different surroundings" (MDMA)), and face flushing. Subjects also mentioned slight feelings of dizziness and headache after methylone and MDMA administration (see Table 1 and Figure 2) [38–40]. When comparing both conditions, marked differences were detected in maximum effects, $AUC_{0–4h}$, and at several T-C points in scales related to stimulant-like effects and body perception, with higher values after MDMA administration.

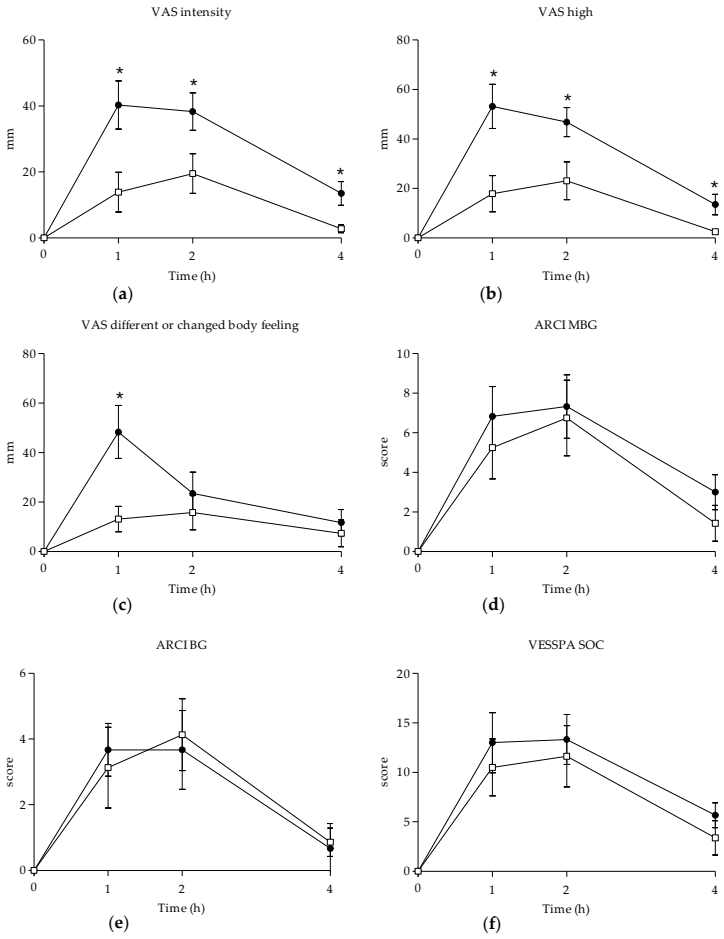



**Figure 2.** Summary of the time course of subjective effects collected through VAS (intensity (**a**), high (**b**), different or changed body feeling (**c**)), ARCI (MBG (euphoria) (**d**), BG (intellectual efficiency) (**e**)), and VESSPA-SSE (SOC (pleasure and sociability) (**f**)) questionnaires after methylone and MDMA oral administration. (□, 100–300 mg methylone ($n = 8$); ●, 75–100 mg MDMA ($n = 6$); mean, standard error). Statistical differences of $p < 0.05$ between conditions are indicated with "*". See text for abbreviations.

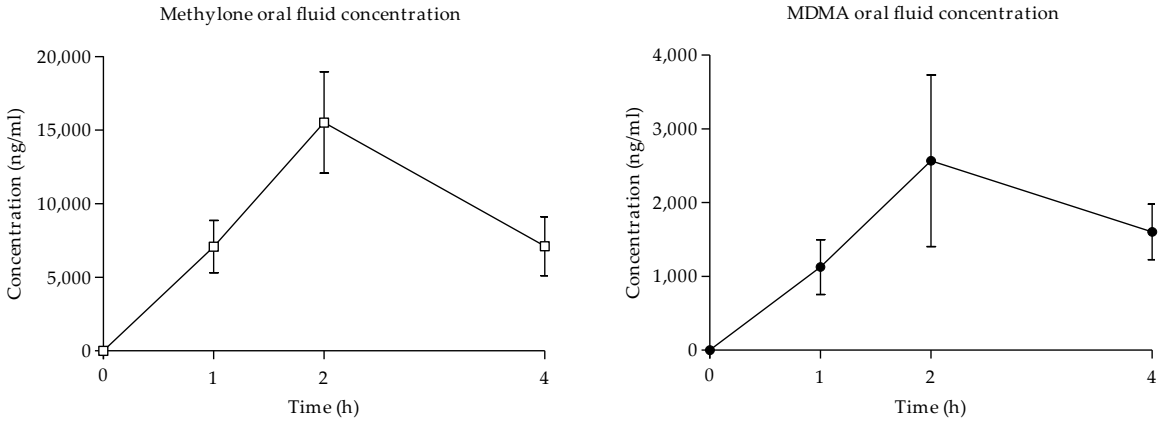
Regarding the ARCI questionnaire, significant differences from baseline were detected between substances for subscales MBG (euphoria), BG (intellectual efficiency and energy), and A (amphetamine). Subjects who self-administered methylone also reported significant changes in the PCAG subscale (sedation). When comparing methylone and MDMA, peak scores in MBG and BG subscales were very similar, although with significantly earlier onset (Tmax) after MDMA administration (see Table 1 and Figure 2). However, no statistical differences in maximal effects, $AUC_{0–4h}$, and T-C points in any of the subscales were observed.

In relation to VESSPA-SSE, methylone and MDMA produced significant changes compared to baseline in some subscales, such as S (sedation), ANX (anxiety), SOC (pleasure and sociability), and ACT (activity and energy). The most relevant effects caused by both conditions with the highest scores of maximum effects were SOC and ACT. However, no statistically significant differences were found in peak effects, $AUC_{0–4h}$, or T-C points between the two substances in any of the subscales (see Table 1 and Figure 2). The only significant difference was the time of maximum values for the ANX subscale, which showed an earlier onset for MDMA.

The selected doses of both substances were well-tolerated, and no serious adverse effects appeared.

### *3.4. Oral Fluid Concentrations*

Oral fluid concentrations of methylone increased rapidly until maximum concentrations were reached at 2 h, with a mean Cmax of 15,514.00 ± 9748.86 ng/mL. The $AUC_{0–4h}$ obtained from the concentrations was 40,623.79 ± 20,001.70 ng/mL·h. Concentrations of methylone started to rapidly decrease at 4 h (see Table 2 and Figure 3).




(**a**) (**b**)

**Figure 3.** Evolution of concentrations of methylone (**a**) and MDMA (**b**) in oral fluid over time (□, 100–300 mg methylone ($n$ = 8); •, 75–100 mg MDMA ($n$ = 6); mean, standard error).

In the case of MDMA, oral fluid concentrations increased until they reached their peak at 2 h after administration in all the subjects, with a mean Cmax of 2936.37 ± 2761.57 ng/mL. MDMA obtained an $AUC_{0–4h}$ of 6586.44 ± 5229.92 ng/mL·h. Concentrations of MDMA in oral fluid started to decrease at 4 h (see Table 2 and Figure 3).

In both cases, subjects ended the sessions with remaining concentrations of methylone or MDMA in oral fluid.

## 4. Discussion

To the best of our knowledge, this is the first observational study that evaluates the acute physiological and subjective effects of methylone in humans and compares its pharmacological profile with MDMA. Moreover, the other purpose of this study was to

determine oral fluid concentrations of methylone and see how they relate to the time course of the pharmacological effects.

Our main finding is that the oral administration of methylone in a naturalistic setting exhibits prototypical psychostimulant and empathogenic effects in healthy and experienced recreational drug users. Methylone and traditional MDMA showed similar pharmacological effects.

Methylone and MDMA produced perceptible increases in SBP and DBP, with higher effects after MDMA administration, although differences between them were not statistically significant. Interestingly, maximum effects in SBP occurred earlier in time after methylone intake (1.5 h), and this rise was also further extended in time (4 h) compared to MDMA, which caused higher effects at 2 h that returned to baseline values at the end of the session. Regarding HR, methylone induced a higher increase than MDMA at 1 h after administration. These findings are consistent with previous methylone intoxication reports which described tachycardia and hypertension as some of the clinical manifestations [41]. In the case of MDMA, cardiovascular effects are in line with previous studies under controlled conditions that reported marked increases in SBP, DBP, and HR [42–45].

As expected, methylone and MDMA displayed prototypical psychostimulant and empathogenic effects, extensively described for MDMA [42–49]. In general, subjective effects appeared in the first hour in both conditions and reached maximum values at 2 h after methylone administration, whereas most of these effects peaked earlier, at 1.5 h, in the case of MDMA administration. This finding slightly differs from user reports, which define maximum effects at 1 or 1.5 h after methylone administration [23]. Methylone produced increases in VAS related to stimulation and well-being (stimulation, high, content, good effects), although these effects were half as intense as those produced by MDMA. A possible explanation for these differences could be that the doses selected are not comparable, meaning that methylone was underdosed; however, the tested doses were similar to those most frequently selected by habitual users. For this reason, our data suggest that common doses of methylone produce similar subjective effects to MDMA, although they are milder. In relation to the effects related to perceptual alterations, subjects under methylone influence did not report marked differences, contrary to those participants that self-administered MDMA, who experienced significant changes in body feeling and surroundings.

The profile of physiological and subjective effects produced by methylone in our naturalistic setting was in line with preliminary data obtained from a dose-finding study administering oral doses of 50–150 mg of methylone in a controlled environment [50].

In relation to the effects obtained through ARCI and VESSPA-SSE, methylone showed a similar profile to MDMA and other psychostimulant substances such as amphetamines and mephedrone. Methylone scored predominantly in ARCI subscales related to euphoria (MBG), intellectual efficiency and energy (BG), and in amphetamine-like effects (A). In the same manner, VESSPA-SSE results reflect an increase in activity and energy (ACT) and pleasure and sociability (SOC). However, those stimulant effects usually sought by recreative users also coexisted with sedation and anxiety. Overall, coinciding with previous user reports, most of the subjective effects exhibited by methylone disappeared 4 h after administration [44].

As previously mentioned, there is no published information about the pharmacokinetic profile of methylone in humans to use as a comparison with our findings; the only data available come from studies in rodents [51]. According to the results of our analysis, in oral fluid, methylone reached peak concentrations of 15,514.00 $\pm$ 9748.86 ng/mL while MDMA obtained maximum levels of 2936.37 $\pm$ 2761.57 ng/mL. Overall, concentrations were not comparable given the great difference between conditions. In both sessions, all the subjects reached maximum levels of methylone and MDMA at 2 h after self-administration and decreased at 4 h.

Our results of the oral fluid concentrations of MDMA are similar to those previously published after the administration of 100 mg of MDMA [36,37] and 1–1.6 mg/kg [52].

Previous studies evaluating concentrations of MDMA in oral fluid and blood reported highly variable oral fluid to blood (OF/B) ratios, with concentrations notably higher in oral fluid. This ratio showed a maximum value of 18.1 ± 7.9 (range 10.3–32.3) at 1.5 h after a 100 mg dose of MDMA [36,37]. Other study evaluating oral fluid and plasma correlation reported a median overall OF/B of 5.2 (range 0.1–40.4) after administering the low dose (1.0 mg/kg) and a OF/B of 6.0 (range 0.4–52.3) following the high dose of MDMA (1.6 mg/kg) [52]. Although oral fluid and blood concentrations exhibited a statistically significant correlation, we cannot confirm that blood concentrations can be predicted from oral fluid concentrations due to the high variability of OF/B ratios [52]. Our MDMA oral fluid concentrations would theoretically correspond to the range of concentrations described in previous studies, obtaining a Cmax in plasma of approximately 205 ng/mL.

Currently, there is no study with synthetic cathinones that examines OF/B ratios. However, previous studies on the oral administration of mephedrone provide independent pharmacokinetic data of oral fluid concentrations and blood concentrations that allow us to estimate the OF/B ratios of mephedrone. After comparing the Cmax values of mephedrone collected from a controlled study and an observational study, OF/B ratios resulted in values of 49.43, 4.28, and 11.73 following oral mephedrone doses of 100, 150, and 200 mg, respectively [40,53]. Using data from a study that compared blood concentrations of mephedrone and MDMA, estimated OF/B ratios were found to be 22.28 after a 200 mg mephedrone dose and 22.40 after a 100 mg MDMA dose [40,44]. There are no previously published results about blood concentrations of methylone in experimental or observational studies. The OF/B ratio of methylone cannot be calculated from our results because of a lack of blood concentrations. When comparing the time course of oral fluid concentrations and acute effects, peak concentrations of methylone coincide with the maximum subjective effects, which also appeared at 2 h. Although it is still unclear whether oral fluid concentrations fully correlate with those in blood, this non-invasive sample collection was considered the most suitable for the design of the experiment as an observational study in a naturalistic environment. These results also demonstrate that oral fluid concentrations are a suitable, non-invasive, alternative biomarker that can be used to identify acute methylone use.

Additionally, this observational study also provided unique preliminary data about the acute effects and oral fluid concentrations of mephedrone, another synthetic cathinone closely related to methylone, administered by oral and intranasal routes [40]. Mephedrone effects were also in line with the typical profile of psychostimulants, although the maximum values of subjective effects after oral administration were higher compared to methylone and showed close similarity to those of MDMA. These results suggest that even though both synthetic cathinones and their non-β-analogue share a clinical profile, the intensity of their effects differs, given that those induced by methylone are milder compared to those of MDMA and mephedrone. The difference observed in methylone and MDMA's effects can be explained in part by their molecular activity. Methylone has exhibited some affinity for binding 5-HT2A receptors but at significantly lower potencies than MDMA. Methylone has been described as a partial agonist at the 5-HT1A receptor, with weak antagonist effects on 5-HT2C receptors, contrary to MDMA, which is considered a partial agonist rather than an antagonist [54,55].

Moreover, mephedrone also produced similar stimulant-like effects to MDMA in controlled conditions, but with a more rapid onset and shorter duration of effects [44]. This would be contrary to our results, which showed that the maximum effects induced by methylone appeared later compared to MDMA. However, further investigations of methylone administration in controlled conditions are required to confirm the findings obtained in this observational study.

This study has limitations typically associated with observational–naturalistic designs. The study was non-placebo-controlled (negative control) and open-label, since participants selected their doses according to their experience; hence, its design makes it susceptible to an expectancy bias. Moreover, the naturalistic environment could have influenced their subjective reports. Concentrations of methylone and MDMA were only analyzed

in oral fluid. Blood samples were not collected in order to maintain the naturalistic setting. Another limitation to consider was the low sample size, which decreased the statistical power of the study. Additionally, sessions were divided into a few time-point evaluations. More evaluations would have allowed us to define a more complete time course of pharmacological effects and oral fluid pharmacokinetics. Finally, subjects were not genotyped for the genetic polymorphism of CYP2D6 involved in methylone and MDMA metabolism, which could have had an impact on the outcomes of the study.

However, despite its limitations, this design of study is useful and provides valuable information about novel or emergent substances of which there are still no available data in humans. Moreover, the MDMA effects observed in this study are consistent with those described in previous experimental studies administering MDMA [44]. In the same way, this consistency between results obtained from a naturalistic and an experimental study was proven in the administration of mephedrone [40,44] or THC [56,57]. With this in mind, the following strengths of the study should also be considered. Firstly, the sample included participants of both genders. Moreover, subjects were free to select their doses based on their preference and previous experience. All the measurements were taken with validated methodology (questionnaires and rating scales) and determinations were made with validated analytic techniques. The pharmacological effects of methylone were compared with those of a well-known psychostimulant, such as MDMA, which was administered in similar conditions. Finally, the study was conducted in a naturalistic setting, so that the experience mimicked a more recreational scenario compared to controlled studies.

## 5. Conclusions

This observational–naturalistic study constitutes an initial preliminary approach to the determination of the acute effects and oral fluid concentrations of methylone after the oral administration of known doses of methylone. Our findings suggest that the pharmacological effects produced by methylone follow the prototypical psychostimulant and empathogenic profile associated with MDMA, including euphoria, stimulation, alteration of perception, and an increase in energy and sociability. Although the subjective effects were similar, those induced by methylone were less intense and peaked later in time compared to MDMA. Oral fluid concentrations of methylone changed in time following the same pattern as the time course of the acute effects, peaking at 2 h after administration. These results confirm that methylone can be considered a suitable biomarker of exposure and that oral fluid is, in the same way, a useful biological matrix to monitor and detect recent methylone use. Finally, our results suggest that the abuse liability and toxicity of methylone is similar to that of MDMA.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/biology10080788/s1, Table S1: Summary of sociodemographic and history of substances use data of participants self-administering methylone ($n$ = 8) and MDMA ($n$ = 6).

**Author Contributions:** M.F., M.T., R.d.l.T., M.V. and M.G. conceptualized the study design; M.F., E.P., E.O., M.V. and M.G. collected the data; R.d.l.T. and E.O. analyzed the oral fluid; M.V. and X.C. analyzed the methylone and MDMA contents; L.P., E.P. and C.P.-M. analyzed the data. L.P., E.P., E.O., C.P.-M., M.V., X.C., M.G., F.F., M.T., R.d.l.T. and M.F. wrote, revised and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki and Spanish legislation and approved by the Parc de Salut Mar Clinical Research Ethics Committee (ref. 2016/6700/I).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

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