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In silico toxicity as a tool for harm reduction: A study of new psychoactive amphetamines and cathinones in the context of criminal science

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A B S T R A C T

The emergence of new psychoactive substances (NPS) has raised many issues in the context of law enforcement and public drug policies. In this scenario, interdisciplinary studies are crucial to the decision-making process in the field of criminal science. Unfortunately, information about how NPS affect people's health is lacking even though knowledge about the toxic potential of these substances is essential: the more information about these drugs, the greater the possibility of avoiding damage within the scope of a harm reduction policy. Traditional analytical methods may be inaccessible in the field of forensic science because they are relatively expensive and time-consuming. In this sense, less costly and faster *in silico* methodologies can be useful strategies. In this work, we submitted computer-calculated toxicity values of various amphetamines and cathinones to an unsupervised multivariate analysis, namely Principal Component Analysis (PCA), and to the supervised techniques Soft Independent Modeling of Class Analogy and Partial Least Square-Discriminant Analysis (SIMCA and PLS-DA) to evaluate how these two NPS groups behave. We studied how theoretical and experimental values are correlated by PLS regression. Although experimental data was available for a small amount of molecules, correlation values reproduced literature values. The *in silico* method efficiently provided information about the drugs. On the basis of our findings, the technical information presented here can be used in decision-making regarding harm reduction policies and help to fulfill the objectives of criminal science.

1. Introduction

Criminal science relies on a multidisciplinary approach that seeks to gather the foundations of social, natural, and applied sciences to find new ways to prevent or to reduce crime [1]. Scientific data compilation and interpretation are important for decision-making within the judicial process. Understanding scientific procedures within the legal context may reduce the risk of misinterpretation, thus providing greater legal certainty [2–4].

The advent of the great wars led to prominent chemistry development as compared to other sciences. The synthesis of several substances and their respective derivatives by new routes became a constant activity. Advances in the late twentieth century drove the development of heroin, ketamines, and GHB substances, which later started being used for recreational purposes [5].

From a historical viewpoint, traditional drugs, such as opium, heroin, cocaine, and Cannabis, have been consumed on a large scale, which has caused us to associate drug abuse with problematic societies. The ban on traditional psychotropic substances in various countries has culminated in a new phenomenon: around 2010, new classes of compounds known as new psychoactive substances (NPS) emerged as an alternative to forbidden drugs. These new compounds were designed and formulated to escape the legal coverage of controlled substances and received diverse nomenclatures: *bath salts* [6], *legal highs* [7], and *research chemicals* [8], among others. In the vast majority of cases, these substances have labels stating that they are contraindicated for human consumption, which is an attempt to circumvent existing drug laws [5]. The illusory idea that these substances are safe comes from the fact that they end up achieving the legality status in many countries, which has contributed to increasing their popularity, especially among adolescents and young adults [9].

The speed at which these new drugs have been marketed has created numerous technical difficulties to the development of analytical procedures and risk assessment in a timely manner. From 2009 to 2016,

Abbreviations: 4-MEC, 4-Methylethcathinone; ADMET, Absorption, Distribution, Metabolism, Excretion, and Toxicity; BZP, Benzylpiperazine; DAT, Dopamine transporter; GHB, Gama-Hydroxybutyrate; IC50, Concentration needed to inhibit 50% of a biological process; LD50, Lethal Dose for 50% of the tested subjects; LogD, Distribution coefficient; LogP, Lipophilicity Coefficient; MDPBP, 3',4'-Methylenedioxy-α-pyrrolidinobutyrophenone; MDPV, 3,4-Methylenedioxypyrovalerone; NET, Norepinephrine transporter; NPS, New psychoactive substances; OECD, (Organization for Economic Co-operation and Development); PCA, Principal Component Analysis; pFPP, *para*-Fluorophenylpiperazine; PLS-DA, Partial Least Square-Discriminant Analysis; SERT, Serotonin transporter; SIMCA, Soft Independent Modeling of Class Analogy; TFMPP, 3-Trifluoromethylphenylpiperazine

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a total of 739 different NPS were registered and reported in 106 countries and territories [10]. Growing NPS variety has resulted in little information being available to examine their short- and long-term effects and risks to consumers [5]. In addition, information about NPS composition and purity is still scarce in the scientific literature [9].

Motivations for NPS consumption are often based on various factors, including consumer's preference for stimulant or psychedelic effects, NPS superior quality and purity as compared to traditional psychotropic substances, NPS low probability of short- and long-term damage, and NPS positive peer or internet ratings [11]. Within a homogeneous group of drugs, the monetary value is the main reason why users choose to consume NPS [12].

Regular consumers take NPS on the grounds that these substances allow them to circumvent legal sanctions while still obtaining the same effects they would achieve with prohibited drugs. Furthermore, users who are under supervision or probation can circumvent drug tests. NPS also satisfy consumers in search for new altered states and help them to bypass drug providers that potentiate or adulterate controlled substances [5].

Many countries have a system that bans NPS analogous or generic structures instead of offering a list of individual drugs with their usual chemical names. This measure was adopted in an attempt to reduce the speed at which NPS enter these countries and to accelerate decision-making [5].

Governments and their respective regulatory agencies have sought strategies to respond to NPS emergence. Most have taken emergency precautionary measures and chosen to ban similar and/or generic structures of some known NPS [13].

Maximum information on psychoactive substances must be known. This information can be used to support consumption restriction policies and to implement harm reduction projects. Several harm reduction agencies are exploring mechanisms to study the effects of illicit drugs by allowing their users to make informed decisions [5,14,15].

By definition, harm reduction works to prevent risks and possible harmful effects rather than specifically prevent drug use in general [16]. Historically, harm reduction specifically geared to psychoactive substances consumption has been related to the Rolleston Report of 1926. This document concluded that doctors could prescribe opiates for users who were in a situation of dependency as a means to stabilize their lives. Although the first harm reduction records date back to the late 1920s, this practice was only recognized as a health strategy for drug users in the 1980s. After this recognition, the strategy was implemented in a number of prevention programs [17].

On the basis of the Rolleston Report, developing and planning NPS could bring two main potential gains. First, NPS cause the recreational effects sought by users, which is what really drives the market for psychoactive drugs but is not generally considered by policymakers. Second, substitution of traditional psychoactive drugs for NPS could be less harmful or have minor side effects. However, this type of substitution relationship entails a long and delicate process [5]. Understanding why individuals use NPS helps to tailor harm reduction proposals to each target group more effectively. The different motivations for drug use seem to match usage trends over time. The demand for NPS that started being consumed for reasons like availability or legality decreased over time, whereas the demand for NPS that started being taken due to cash value, effect duration, or greater/better purity increased over time [12].

Bioactive compounds can have their potency and/or toxicity estimated by biological assays. Such assays help to select new chemical compounds for therapeutic purposes and to evaluate and to reassess their efficacies. Toxicity is defined as any predicted or identified adverse effects of compounds of interest. These adverse effects are expressed by means of limits indicated by parameters. There may be quantitative parameters, such as LD50 (lethal dose for 50% of the tested subjects), and qualitative parameters that indicate whether a substance is toxic or not. Parameters that point to low, moderate, or high toxicity also exist. All these parameters have the same objective: to characterize the harmful effects of substances on different species (humans, animals, and plants) or the environment [18–21].

Monoamine transporters absorb neurotransmitter molecules released into the extracellular space. They represent established targets for many pharmacological agents that affect the brain function, including psychostimulants, antidepressants, and neurotoxins [22,23]. Three main transporters exist: DAT, NET, and SERT. Dopamine (DA), noradrenaline (or norepinephrine, NT), and serotonin (SER) are the endogenous ligands after which the transporters are named, respectively. Although they are substrates of their cognate transporter, both DAT and NET can carry DA and noradrenaline [23–25]. Drug interaction with DAT, NET, and SERT can be classified as transporter blocker reuptake and high presynaptic release [26]. Amphetamines and cathinones disrupt the transporter function and increase extracellular monoamine concentrations, inducing endogenous ligand extravasation and governing dopamine signaling strength during drug abuse and therapeutic treatments [27–29].

This work aims to study amphetamine- and cathinone-derived NPS toxicity by comparing the homologous structures of amphetamines and cathinones. In the literature, IC50NET, IC50DAT, and IC50SERT values were obtained by using the same experimental method [30–33]; *i.e.*, by evaluating the responses of human embryonic kidney 293 cells (HEK 293). This technique expresses values obtained in human NET, SERT, and DAT with similar tendency to the tendency verified for brain transporters [30,34].

Given that information about NPS toxicity is scarce and spread, we will evaluate how useful *in silico* methods are to obtain information about the toxicological behavior of these drugs. The idea is to address how harm reduction policies can benefit from the important information these methods provide regarding the NPS toxicological potential.

NPS sold in the non-regulated market do not follow the same manufacturing process, so each tablet will have a distinct formulation. Experimental trials conducted and reported in the literature have demonstrated different lethality values for the same substance. Experimental trials sometimes have large error margin. Theoretical prediction could indicate the potentiality of each of these substances, for which few data are available. Moreover, each way the problem is experimentally approached can give a different answer. The limit of detection varies between devices, so their responses and composition determination fluctuate significantly.

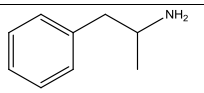
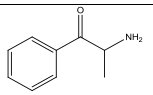
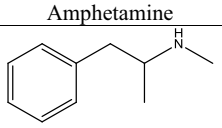
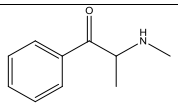
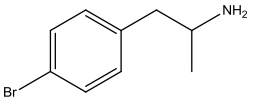
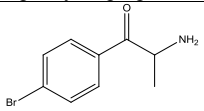
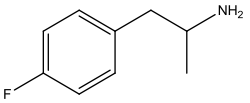
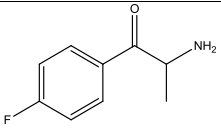
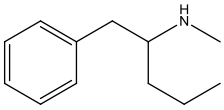
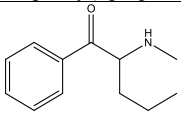
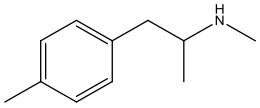
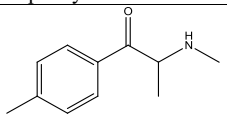
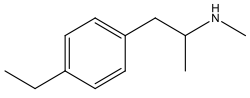
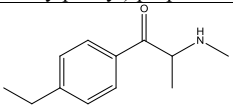
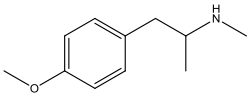
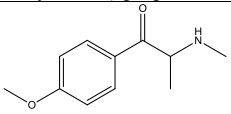
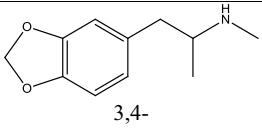
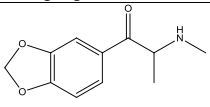
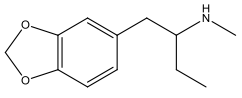
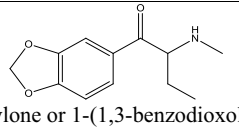
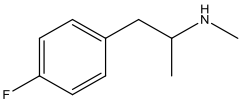
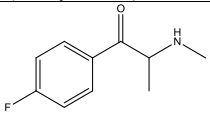
The popularity of these substances poses a risk to the health of individuals who consume them and challenges clinical and forensic laboratories. Most NPS are easy to access, and lack of knowledge about their risks contributes to their popularity and dangerousness. The effect of long-term NPS consumption remains unknown. Establishing toxic and lethal concentrations is difficult, and concentrations found in living and dead individuals overlap. In many cases, very low NPS concentrations can culminate in intoxication and death [35].

Drug toxicity assessment has evolved so as to dismiss the need for tests in animal tests, which has consequently led to the search for more ethical and less expensive alternatives. Alternative approaches can help to reduce experiments in animals and are defined as complementary methods [36]. Experimentally, LD50 determinations have mainly been substituted for acute toxicity correlation with basal cytotoxicity [22,23].

Determining cytotoxicity in cell cultures may be an important complementary method to obtain more information about the general basal toxicity of a substance to an organism during the course of an acute toxicity experiment [36].

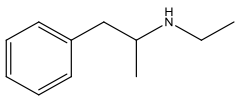
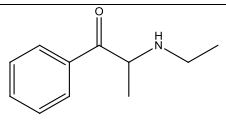
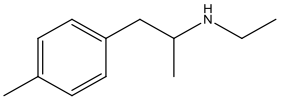
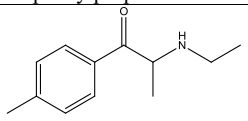
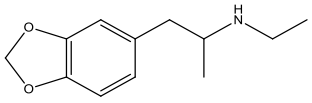
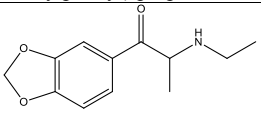
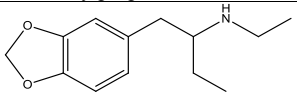
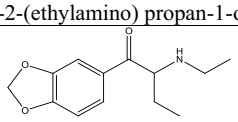
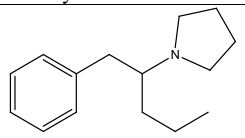
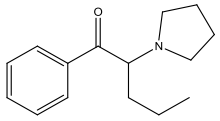
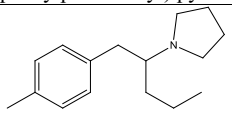
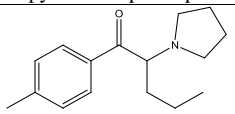
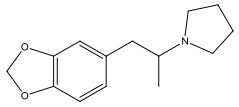
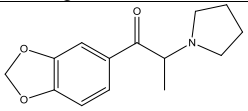
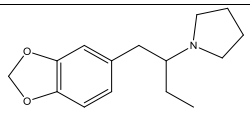
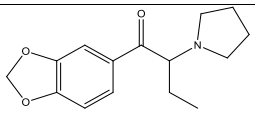
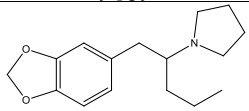
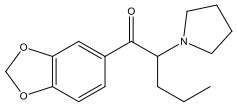
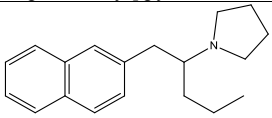
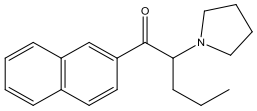
The ACuteTox program is an example of these efforts. This program targets the use of tests that do not involve animals, like *in vitro* cytotoxicity studies, to estimate acute toxicities. Because effective concentrations *in vitro* are irrelevant for concentrations that may cause toxicity at the target site in target organs *in vivo*, deviations from a simple linear relationship between effective concentrations *in vitro* and

Table 1
Amphetamines and cathinones with similar structures.

a01	 Amphetamine	**c01**	 cathinone
a02	 Methamphetamine	**c02**	 methcathinone or 2-methylamino-1-phenyl-1-propanone
a03	 1-(4-bromophenyl) propan-2-amine	**c03**	 4-bromocathinone or 2-amino-1-(4-bromophenyl)-propanone
a04	 4-fluoroamphetamine or 1-(4-fluorophenyl) propan-2-amine	**c04**	 4-fluorocathinone or 2-amino-1-(4-fluorophenyl)-propanone
a05	 *N*-methyl-1-phenylpentan-2-amine	**c05**	 pentedrone or 2-(methylamino)-1-phenylbutan-1-one
a06	 *N*-methyl-1-(4-methylphenyl) propan-2-amine	**c06**	 mephedrone or 2-(methylamino)-1-(4-methylphenyl) propan-1-one
a07	 1-(4-ethylphenyl)-*N*-methylpropan-2-amine	**c07**	 4-EMC or 1-(4-ethylphenyl)-2-(methylamino) propan-1-one
a08	 1-(4-methoxyphenyl)-*N*-methylpropan-2-amine	**c08**	 methedrone or 1-(4-methoxyphenyl)-2-(methylamino) propan-1-one
a09	 3,4-methylenedioxymethamphetamine (MDMA)	**c09**	 methylone or 1-(1,3-benzodioxol-5-yl)-2-(methylamino)propan-1-one
a10	 MDBD or 1-(2*H*-1,3-benzodioxol-5-yl)-*N*-methylbutan-2-amine	**c10**	 butylone or 1-(1,3-benzodioxol-5-yl)-2-(methylamino) butan-1-one
a11	 4-FMA or 1-(4-fluorophenyl)-*N*-methylpropan-2-amine	**c11**	 flephedrone or 1-(4-fluorophenyl)-2-methylaminopropan-1-one

(*continued on next page*)

Table 1 (*continued*)

a12	 *N*-ethyl-1-phenylpropan-2-amine	**c12**	 ethcathinone or 2-(ethylamino)-1-phenylpropan-1-one
a13	 *N*-ethyl-1-(4-methylphenyl) propan-2-amine	**c13**	 4-MEC or 2-(ethylamino)-1-(4-methylphenyl) propan-1-one
a14	 1-(2*H*-1,3-benzodioxol-5-yl)-*N*-ethylpropan-2-amine	**c14**	 ethylone or 1-(1,3-benzodioxol-5-yl)-2-(ethylamino) propan-1-one
a15	 1-(2*H*-1,3-benzodioxol-5-yl) -*N*-ethylbutan-2-amine	**c15**	 1-(1,3-benzodioxol-5-yl)-2-(ethylamino) butan-1-one
a16	 1-(1-phenylpentan-2-yl) pyrrolidine	**c16**	 alpha-pyrrolidinopentiophenone
a17	 1-[1-(4-methylphenyl) pentan-2-yl] pyrrolidine	**c17**	 pyrovalerone or 1-(4-methylphenyl)-2-(pyrrolidin-1-yl) pentan-1-one
a18	 1-[1-(2*H*-1,3-benzodioxol-5-yl) propan-2-yl] pyrrolidine	**c18**	 MDPPP or 1-(1,3-benzodioxol-5-yl)-2-(pyrrolidin-1-yl) propan-1-one
a19	 1-[1-(2*H*-1,3-benzodioxol-5-yl) butan-2-yl] pyrrolidine	**c19**	 MDPBP or 1-(1,3-benzodioxol-5-yl)-2-(1-pyrrolidinyl)-1-butanone
a20	 1-[1-(2*H*-1,3-benzodioxol-5-yl) pentan-2-yl] pyrrolidine	**c20**	 MDPV or 1-(1,3-benzodioxol-5-yl)-2-(pyrrolidin-1-yl) pentan-1-one
a21	 1-[1-(naphthalen-2-yl) pentan-2-yl] pyrrolidine	**c21**	 naphyrone or 1-(naphthalen-2-yl)-2-(pyrrolidin-1-yl) pentan-1-one

toxic doses *in vivo* can arise [37–39]. Some papers have used lethality indexes (LI = IC50/LD50) to predict how LD50 and IC50 are correlated [36,40]. There are many literature reports on the use of cytotoxicity values to estimate acute toxicity [36,40–58].

In vitro and *in vivo* research may require costly and time-consuming techniques. Therefore, the search for faster and less costly study alternatives to obtain reliable results about the effect of unknown substances is necessary. The study of new medicines is an example of the long time that a complete experimental study demands. Several medicines have had to be withdrawn from the market because pre- and post-clinical trials have failed to predict their toxicity. According to the literature, it typically takes about 10 to 12 years for a drug to be developed, tried, and made commercially available, and costs involved in this process reach values of up to US$ 500 million [5]. Therefore, determining possible toxicities as soon as possible would be very convenient and useful and would be of the greatest interest to pharmaceutical companies. In this context, methods based on computer simulations, also called *in silico* methods, have been employed to obtain a reliable estimate of toxicity even before a new drug is synthesized [12].

In silico methods aim to complement existing *in vitro* and *in vivo* toxicity tests, thereby minimizing the need for animal testing (which is one of the main discussions in ethics committees), reducing the cost and time of relevant toxicity tests, and improving toxicity prediction and safety assessments. Moreover, computational methods can estimate whether not yet synthesized chemicals are toxic [21].

In silico methods encompass a vast amount of computational tools such as shared databases on chemicals, toxicity, and chemical properties, software to generate molecular descriptors, tools to simulate biological systems and molecular dynamics, and statistical packages to create forecasting models, among others. However, there are problems in using these methods. First, toxic effects cover a large quantity of adverse effects that may not be mimicked in computers. Second, data on these drugs and on how they affect humans are scarce. Finally, available *in silico* methods may not be sufficiently accurate [59,60].

Despite these limitations, we believe that *in silico* methods are good enough to provide more information about undetected or unknown substances. These data can be used to enforce law and to implement harm reduction policies. In both cases, these procedures are intimately linked to the assurance of human rights. The more information available, the more reliable the analysis. Considering the rate at which NPS proliferate, obtaining information about their risks to public health is essential because little knowledge about NPS chemical and toxicological behavior exists, which is a challenge for drug policymakers due to uncertainties about use-case damage. Toxicity information in a more expeditious way can help us to compile data and to give feedback that can aid us in meeting criminal science goals [61,62].

2. Studied system

Table 1 lists the molecules we have studied. More specifically, Table 1 lists 42 molecules corresponding to 21 pairs of amphetamines and cathinones with homologous structures. We compiled these molecules and some experimental values from the literature [9,33,63,64]. On the basis of the original molecules, we extended the group by considering the homologous structure in each case. Our intention was to encompass a structurally similar group. We have already studied the same system in terms of IR detection [65]. The idea is to obtain information about the detection and toxicological behavior of these drugs. However, this study can be extended to new molecules because they are *in silico* studies.

3. Methodology

3.1. Computational procedure

The computational procedure was divided into three parts:

3.1.1. In silico values calculation

Toxicity values were calculated with the software Advanced Chemistry Development ACD/I-Lab[1] 2.0 [66,67], SwissADME [68], Way2Drug GUSAR Online [69], ProToxII [70], and Toxicity Estimation Software Tool (TEST) [71].

The main proposal of the ACD/I-Lab Software is to reduce intensive experimental testing and literature searches. Concerning functionalities, it can predict ADME toxicities among other physicochemical properties [66,67].

The following variables were calculated:

LogP (ACD/I-Lab) **–** Hydrophobicity is very important in different scientific areas and indicates membrane permeability, interaction with biological receptors and enzymes, toxicity, and biological potency. If reliable hydrophobicity values are available for a number of compounds, it is possible to estimate many drug properties [72]. SwissADME [68] predicts LogP by different methods:

- LogP by iLOGP: it is a method based on the free energies of solvation in n-octanol and water, calculated by the Generalized-Born and solvent accessible surface area (GB/SA) model. Its performance has been validated for external sets of drugs or drugs [68,73].
- LogP by XLOGP3: it is an atomistic method that includes corrective factors and knowledge-based library [74].
- LogP by WLOGP: it uses the fragmentary system of Wildman and Crippenis to compose the purely atomistic method [75].
- LogP by MLOGP: it is based on a topological model containing a linear relation with 13 molecular descriptors [76].
- LogP by SILICOS-IT: it is a hybrid method that consists of mixing information from 27 fragments and seven topological descriptors [77].

LD50 – LD50 expresses the degree of acute toxicity of chemicals. It corresponds to doses that are likely to kill 50% of the animals in a batch used for experimentation [78]. Here, LogLD50 was used for Mice (ACD/I-Lab 2.0 [66,67]) and Rats.

Rat values were calculated by:

- Way2Drug GUSAR Online: this software uses **the** OECD (Organization for Economic Co-operation and Development) principles and QSAR (Quantitative Structure Activity Relationships) modeling achievements, consensus prediction, applicability domain assessment, internal and external model validation, and clear interpretation of the obtained results. Around ~10,000 chemical structures that cause acute toxicity in rats compose the training sets [69].
- Toxicity Estimation Software Tool (TEST): this tool uses QSARs methodologies to foresee the toxicity of a chemical. The trading set is around 7413 chemicals [71].

Finally, ProTox II is used as a training set with rodent values; there are approximately 40,000 compounds with LD50 values for mouse or rat experiments. There are cases in which several experiments or values in different species are used to compose LD50 [70].

The following quantities were calculated and are represented according to the legend:

- LogLD50 in mice after oral administration (LogLD50M-O)
- LogLD50 in mice after intravenous administration (LogLD50M-IV)
- LogLD50 in mice after intraperitoneal administration (LogLD50M-IP)
- LogLD50 in mice after subcutaneous administration (LogLD50M-SC)
- LogLD50 calculated by ProTox, which corresponds to oral values in

[1] When these values were obtained, software ACD/I-Lab was available for free.

rodents (LogLD50-Protox)
- LogLD50 in rats after oral administration (LogLD50R-O)
- LogLD50 in rats after intraperitoneal administration (LogLD50R-IP)
- LogLD50 in rats after intravenous administration (LogLD50R-IV)
- LogLD50 in rats after subcutaneous administration (LogLD50R-SC)
- LogLD50 calculated by Test: LogLD50R-OTest

LogD – The Distribution Coefficient describes the lipophilicity of a given substance since most of the drugs are not in their neutral state. This measure corresponds to the partition coefficient for all the possible species in octanol and all the possible species in water [72,79]. The following values were calculated by ACD/I-Lab 2.0 [66,67]:

- LogD, pH = 1.7 – Stomach: LogDS
- LogD, pH = 4.6 – Duoden: LogDD
- LogD, pH = 6.5 – Jejunum: LogDJ
- LogD, pH = 7.4 – Blood: LogDB
- LogD, pH = 8 – Colon: LogDC

3.1.2. Multivariate classification

To analyze results, multivariate classification was employed. Principal Component Analysis (PCA) was used as an unsupervised classification technique. In this case, the objective was to verify structural similarities between the studied molecules. To verify whether there were classes that could be previously assigned according to the group of studied molecules, the supervised classification techniques Soft Independent Modeling of Class Analogy (SIMCA) and Partial Least Square-Discriminant Analysis (PLS-DA) were applied [80–82]. In this case, the objective was to verify whether the classification of the studied groups was suitable according to the toxicity data.

3.1.3. Correspondence with experimental data

To evaluate how calculated variables fit with experimental values, Partial Least Square (PLS) regression was conducted for calculated *in silico* LD50 values against experimental values. All multivariate techniques were performed with Pirouette® Software, which is a tool that was designed to organize and to understand complex data. The software presents algorithmic requirements for organization, classification, visualization, regression models, and other functionalities [83].

Fig. 1 shows the scheme used for the computational procedure. All *in silico* values calculated for the studied system were used for both supervised and unsupervised classification; they were also compared with experimental data by means of multivariate tools implemented in Pirouette software [82–84].

4. Results

4.1. In silico values calculation

Tables 2–5 summarize values simulated with the software used in this work (ACD/I-Lab 2.0 [66,67] for Mice, Way2Drug GUSAR Online [69] for Rats, ProToxII [70], and Toxicity Estimation Software Tool (TEST) [71]). Letters **a** and **c** indicate amphetamines and cathinones, respectively. The first column corresponds to the number of each pair of amphetamines and cathinones listed in Table 1. The idea was to compare the values calculated for each pair of molecules, which differed only by the presence of a carbonyl. Blue values for each pair indicate the lower value for each variable.

Tables 2**and**3 present values for LogLD50 calculated with different software and for different species. We basically observed the same tendency, except for the intravenous values for Rats calculated by Way2Drug GUSAR Online [69].

Tables 4and5 show the values regarding lipophilicity. In all cases, amphetamines had higher LogP values and smaller LogD values, which meant that they are more lipophilic than cathinones.

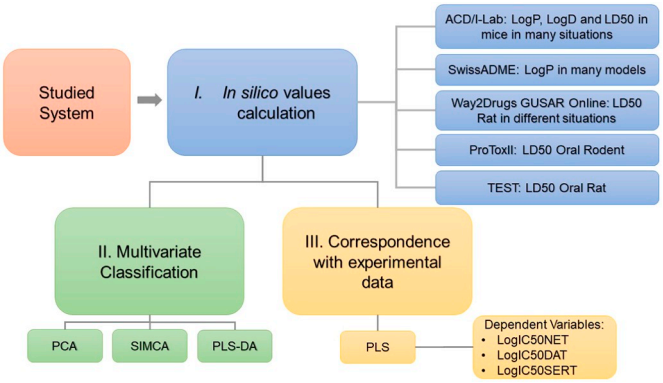


Fig. 1. Scheme of the computational procedure.

Table 2
In silico LogLD50 values in Mice (ACD/I-Lab 2.0 [66,67]).

Pair#	LogLD50M-IP		LogLD50M - O		LogLD50M - IV		LogLD50M–SC	
	a	**c**	**a**	**c**	**a**	**c**	**a**	**c**
01	1.69	2.43	2.11	2.71	1.45	2.04	1.11	2.54
02	1.86	2.49	2.00	2.62	1.38	2.08	1.89	2.45
03	2.18	2.30	2.2	2.76	1.61	2.00	1.69	2.46
04	1.54	2.38	2.32	2.88	2.06	2.04	1.74	2.56
05	1.52	2.36	2.3	2.88	1.18	2.00	1.85	2.53
06	1.6	2.36	2.26	2.87	1.23	2.04	1.85	2.53
07	1.53	2.36	2.32	2.88	1.18	2.00	2.08	2.52
08	1.91	2.38	2.26	2.80	1.23	2.04	1.85	2.53
09	2.08	2.41	2.53	2.79	1.66	2.00	2.46	2.48
10	2.00	2.40	2.56	2.82	1.63	1.96	2.45	2.45
11	1.83	2.43	2.46	2.74	1.60	2.04	2.83	2.53
12	1.54	2.36	2.34	2.89	1.18	2.04	1.72	2.54
13	1.53	2.36	2.32	2.88	1.18	2.00	2.08	2.52
14	2.00	2.40	2.56	2.82	1.63	1.96	2.45	2.45
15	2.04	2.40	2.63	2.84	1.60	1.93	2.43	2.43
16	1.96	2.26	2.57	2.95	1.46	1.68	2.11	2.38
17	1.92	2.26	2.58	2.89	1.45	1.67	2.11	2.34
18	2.18	2.48	2.48	2.92	1.73	1.76	2.45	2.58
19	2.11	2.46	2.65	2.94	1.71	1.73	2.43	2.57
20	2.04	2.46	2.45	2.95	1.62	1.70	2.40	2.54
21	2.20	2.20	2.64	2.86	1.56	1.49	2.49	2.53

4.2. Multivariate classification

In all classification methods, amphetamines and cathinones are shown in black and red, respectively. Fig. 2 corresponds to PCA analysis and shows the three-dimensional view. Three principal components described around 99.62% of the whole information. Two well-defined classes existed.

Fig. 3(a) and 3(b) illustrate the two-dimensional projection for the combination of factors and depict the diagrams for Scores and Loadings, respectively. Separation occurred in the third factor. Table 6 lists Scores numeric values for each Factor. Loadings numerical values are shown in Table 7. All LogP, LogDD, and LogDS values had major negative contributions to Factor 3, whereas LogDJ, LogDB, LogDC, all LogLD50 for Mice (ACDI-labs), and LogLD50-Pro-Tox had positive contributions. LogLD50R-IV, LogLD50R-IP, and LogLD50R-SC had slightly negative contributions, while LogLD50R-O had slightly positive contribution. (See Table 7.)

Fig. 4 displays projections for SIMCA evaluation in (a) three dimensions with the hypersurface represented as dots and (b) two dimensions. Three principal components modeled the classes, which were well separated.

Table 8 contains results obtained by SIMCA. There was no misclassification between classes. Besides, interclass residuals and

Table 3
In silico LogLD50 values obtained with different software (ProToxII [70], Way2Drug GUSAR Online [69], and TEST [71])

Pair#	LogLD50-Protox		LogLD50R-IP		LogLD50R-IV		LogLD50R-SC		LogLD50R-O		LogLD50R-OTest	
	a	**c**	**a**	**c**	**a**	**c**	**a**	**c**	**a**	**c**	**a**	**c**
1	1.00	2.60	2.57	2.48	1.78	1.66	2.19	2.84	2.38	2.62	2.72	3.25
2	1.40	2.45	2.59	2.46	1.64	1.67	2.44	2.66	2.32	2.47	2.50	2.93
3	1.32	2.70	2.50	2.56	1.79	1.75	2.28	3.00	2.39	2.71	2.84	3.01
4	1.18	2.20	2.06	2.15	1.39	1.34	2.41	2.73	2.23	2.48	2.63	3.11
5	2.21	2.20	2.14	2.31	1.57	1.50	2.69	2.91	2.44	2.73	2.80	3.30
6	1.88	2.60	2.11	2.12	1.43	1.38	2.58	2.78	2.37	2.54	2.70	3.44
7	1.88	2.60	2.09	2.08	1.47	1.40	2.52	2.76	2.45	2.51	2.45	2.74
8	2.45	2.45	2.20	2.41	1.28	1.53	2.65	2.95	2.52	2.66	2.91	3.06
9	2.65	2.65	2.03	2.51	1.34	1.33	2.62	2.46	2.23	2.48	2.73	2.71
10	2.27	3.33	2.19	2.55	1.48	1.44	2.88	2.89	2.42	2.75	2.86	2.44
11	2.48	2.20	2.00	1.76	1.53	1.47	2.16	2.24	2.10	2.38	2.77	2.38
12	1.76	2.20	2.10	2.20	1.48	1.41	2.90	2.94	2.26	2.49	2.56	3.01
13	1.76	2.35	2.25	2.34	1.50	1.45	2.91	2.90	2.36	2.60	2.66	3.19
14	2.65	2.65	2.23	2.44	1.42	1.33	2.83	3.02	2.24	2.54	2.83	2.77
15	2.27	3.33	2.32	2.49	1.43	1.36	2.85	3.10	2.46	2.61	2.96	2.76
16	2.20	2.54	2.02	2.15	1.44	1.33	2.54	2.72	2.50	2.58	2.53	2.80
17	2.20	2.54	2.06	2.04	1.48	1.41	2.54	2.57	2.80	2.59	2.59	2.99
18	2.65	2.65	2.11	2.24	1.18	1.17	2.67	2.76	2.41	2.51	2.59	3.25
19	2.07	3.33	2.18	2.20	1.17	1.13	2.80	2.85	2.41	2.55	2.85	2.93
20	2.27	2.65	2.15	2.24	1.17	1.16	2.74	2.89	2.48	2.54	2.67	2.98
21	2.20	2.54	2.26	2.36	1.49	1.43	2.50	2.49	2.66	2.69	2.60	2.97

Table 4
In silico LogD values in different situations (ACD/I-Lab 2.0 [66,67])

Pair#	LogDS		LogDD		LogDJ		LogDB		LogDC	
	a	**c**	**a**	**c**	**a**	**c**	**a**	**c**	**a**	**c**
01	-1.28	-2.63	-1.28	-2.48	-1.10	-1.13	-0.58	-0.31	-0.06	0.12
02	-1.46	-2.66	-1.45	-2.51	-1.04	-1.14	-0.31	-0.32	0.25	0.10
03	-0.95	-2.04	-0.94	-1.87	-0.49	-0.45	0.26	0.36	0.82	0.75
04	-0.85	-1.86	-0.85	-1.80	-0.77	-0.73	-0.44	0.11	0.01	1.79
05	0.03	-1.16	0.03	-1.10	0.11	-0.03	0.44	0.81	0.89	1.32
06	-0.65	-1.33	-0.64	-1.26	-0.57	-0.20	-0.23	0.64	0.22	1.15
07	-0.17	-1.00	-0.17	-0.94	-0.09	0.13	0.24	0.97	0.69	1.48
08	-0.79	-1.86	-0.79	-1.81	-0.62	-0.82	-0.10	0.02	0.43	0.55
09	-1.21	-2.06	-1.2	-2.01	-1.01	-0.99	-0.47	-0.14	0.06	0.38
10	-1.12	-1.51	-1.12	-1.45	-0.93	-0.43	-0.39	0.41	0.14	0.93
11	-0.91	-1.81	-0.9	-1.75	-0.68	-0.66	-0.11	0.18	0.43	0.69
12	-0.47	-1.41	-0.47	-1.35	-0.39	-0.28	-0.06	0.56	0.39	1.07
13	-0.17	-1	-0.17	-0.94	-0.09	0.13	0.24	0.97	0.69	1.48
14	-1.12	-1.51	-1.12	-1.45	-0.93	-0.43	-0.39	0.41	0.14	0.93
15	-0.95	-1.33	-0.95	-1.27	-0.76	-0.25	-0.22	0.59	0.31	1.11
16	1.01	0.12	1.01	0.33	1.26	1.81	1.87	2.6	2.42	2.97
17	1.67	0.51	1.68	0.72	1.93	2.2	2.53	2.99	3.08	3.36
18	-0.56	-1.26	-0.54	-1.07	-0.05	0.37	0.71	1.17	1.27	1.56
19	-0.05	-0.94	-0.04	-0.75	0.45	0.7	1.21	1.5	1.77	1.88
20	0.42	-0.59	0.43	-0.4	0.92	1.05	1.68	1.85	2.24	2.23
21	2.28	1.55	2.29	1.76	2.82	3.24	3.59	4.03	4.16	4.4

distances corroborated two well-defined classes: the residual of one class was lower than the residual of the other class, and the distance within the same class was null.

Fig. 5 shows PLS-DA results. Both classes were well separated. We used three principal components with 99.62% whole information to perform regression, and we evaluated the following characteristics:

- Q^2: internal correlation coefficient model cross validation = 0.88
- R^2: correlation coefficient for calibration = 0.90
- RMSEV: Root Mean Square Error of Validation = 0.35
- RMSEC: Root Mean Square Error of Calibration = 0.33

To ensure modeling quality, $R^2 > Q^2$ and RMSEC < RMSEV [85,86].

Table 9 lists the modeling power for SIMCA and the regression vector for PLS-DA. Values agreed for both supervised techniques: values were higher for most Partition Coefficients. The PLS-DA regression vector confirmed the behavior indicated by Loadings from unsupervised PCA classification: LogP, LogDD, and LogDS were related to the amphetamine group because these variables presented positive values. In all cases, they indicated the lipophilic character of these drugs. All LogLD50 values were around zero and had lower discrimination power. LogLD50-O was slightly negative in this case, around zero.

4.3. Correspondence with experimental data

Table 10 summarizes some literature values in which IC50NET, IC50DAT, and IC50SERT responses were evaluated from human

Table 5
In silico LogP values (ACD/I-Lab 2.0 [66,67], SwissADME [68])

Pair#	LogP (ACD/I-Lab)		LogP (iLOGP)		LogP (XLOGP3)		Log (WLOGP)		LogP (MLOGP)		LogP (MLOGP)	
	a	**c**	**a**	**c**	**a**	**c**	**a**	**c**	**a**	**c**	**a**	**c**
01	1.81	1.16	2.08	1.33	1.76	1.10	1.58	1.22	2.19	1.17	1.94	1.47
02	1.86	1.22	2.20	1.55	1.90	1.20	2.14	1.78	2.61	1.59	2.35	1.89
03	2.58	1.93	2.36	1.83	2.49	1.79	2.34	1.98	2.91	1.89	2.60	2.15
04	1.94	1.40	2.49	2.07	2.07	1.61	1.84	1.48	2.49	1.47	2.35	1.89
05	3.01	2.46	2.75	2.54	3.30	2.49	2.62	2.26	3.06	2.05	3.08	2.63
06	2.40	1.86	2.73	2.41	2.43	1.97	2.15	1.79	2.78	1.76	2.84	2.39
07	2.94	2.39	2.84	2.27	2.87	2.41	2.40	2.04	3.06	2.05	3.21	2.77
08	1.86	1.47	2.62	2.24	2.28	1.58	1.85	1.49	2.12	1.15	2.38	1.94
09	1.81	0.81	2.59	2.36	2.15	0.64	1.57	1.21	1.53	0.58	2.38	1.92
10	2.34	1.34	2.75	2.62	2.68	1.95	1.96	1.60	1.81	0.86	2.74	2.28
11	2.00	1.45	2.46	1.95	2.17	1.71	2.40	2.04	2.91	1.89	2.77	2.32
12	2.48	1.93	2.55	2.02	2.48	1.98	2.23	1.87	2.78	1.76	2.71	2.26
13	2.94	2.39	2.92	2.41	2.92	2.34	2.54	2.18	3.06	2.05	3.21	2.77
14	2.34	1.34	2.93	2.67	2.52	1.79	1.96	1.60	1.81	0.86	2.74	2.28
15	2.87	1.87	3.06	2.86	3.04	2.32	2.35	1.99	2.08	1.14	3.11	2.65
16	4.12	3.65	3.20	3.12	4.02	3.45	3.11	2.75	3.46	2.45	3.90	3.43
17	4.58	4.11	3.48	3.35	4.39	3.81	3.42	3.06	3.71	2.69	4.40	3.94
18	2.92	2.00	3.17	2.91	3.11	2.38	2.06	1.70	2.34	1.40	3.10	2.62
19	3.45	2.53	3.31	2.79	3.63	2.90	2.45	2.09	2.60	1.66	3.45	2.98
20	3.98	3.06	3.52	3.38	3.99	3.26	2.84	2.48	2.85	1.91	3.82	3.35
21	5.35	4.88	3.59	3.53	5.27	4.70	4.27	3.91	4.21	3.20	4.99	4.52

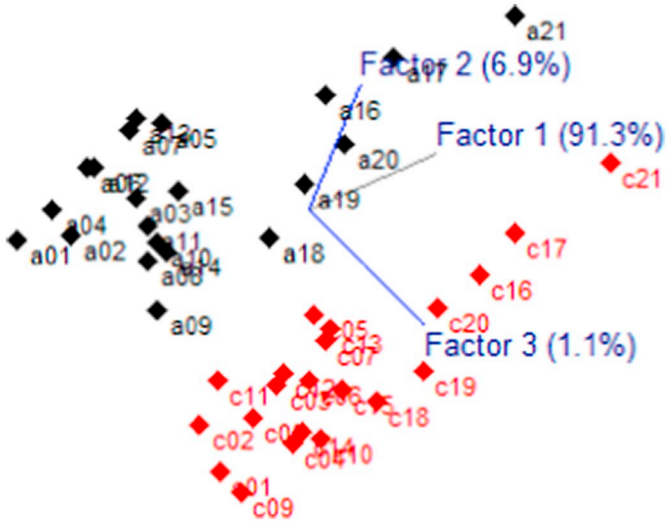


Fig. 2. Three-dimensional view for PCA.

embryonic 293 cells (HEK 293). The drugs had high purity (> 98.5%), and a racemic mixture was used, except for D-methamphetamine [30–33]. This technique expresses values obtained in human NET, SERT, and DAT with similar tendency to the tendency verified for brain transporters [30,34].

As in Tables 2–5, letters **a** and **c** indicate amphetamines and cathinones, respectively. The first column corresponds to pairs listed in Table 1. In this case, IC50 was related to the minimum compound concentration required to reduce the organism population growth by 50% in *in vitro* conditions [21].

All values presented in Table 10 were obtained through the same experimental method [30–33]. Some compounds were reported in more than one reference, and values shown herein are within the experimental error range. Therefore, even if values were not equal in some of the references, they were within the range of values of the method. Values in blue are related to lower values.

To evaluate how calculated values corresponded with experimental vales, we performed a PLS. Table 11 shows results for each model considering LogIC50 for NET, DAT, and SERT as dependent variables. The cathinone **c21** presented outlier behavior, so we removed it from the modeling, which was performed with 26 molecules (nine amphetamines and 17 cathinones;). In all models, we selected four principal components with > 99% cumulative information. We obeyed requirements for modeling in all cases since $R^2 > Q^2$ and RMSEC < RMSEV [85,86].

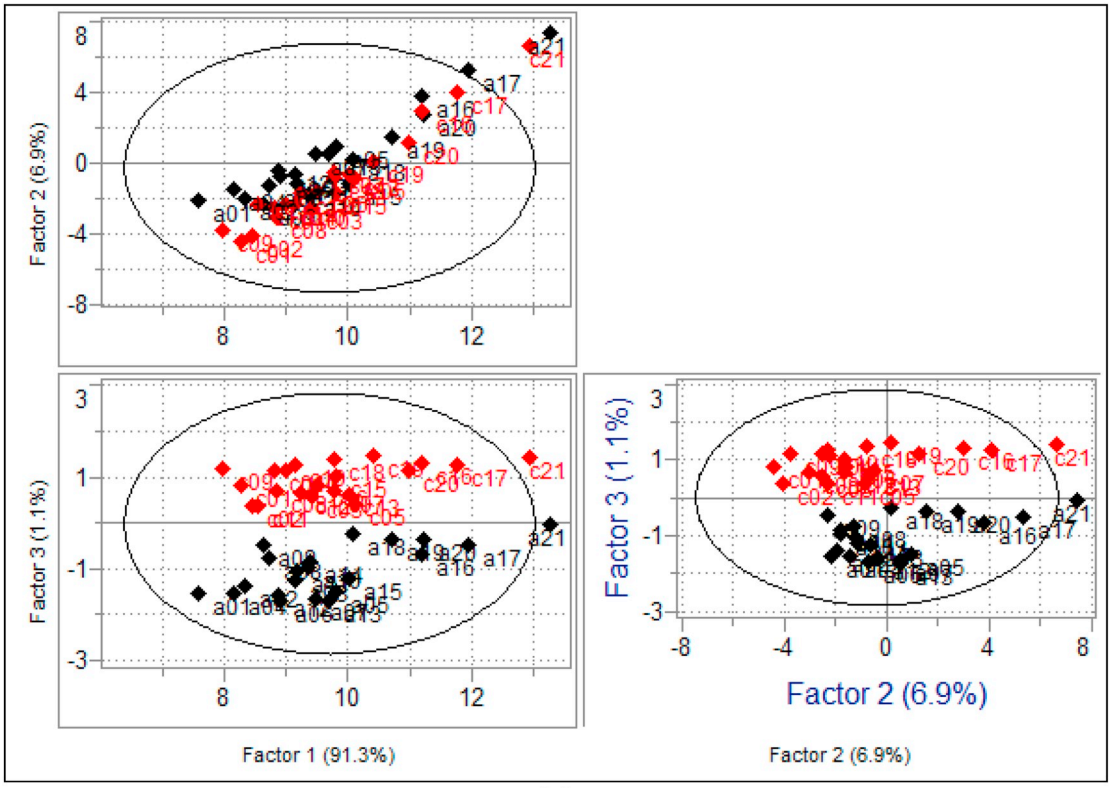
Table 12 presents the regression vector for all models, and it shows the importance of each *in silico* variable in describing each LogIC50 (dependent variables). Variables with major contribution are in bold. These contributions are different for each dependent variable, indicating that they exert distinct influence depending on a specific transporter.

5. Discussion

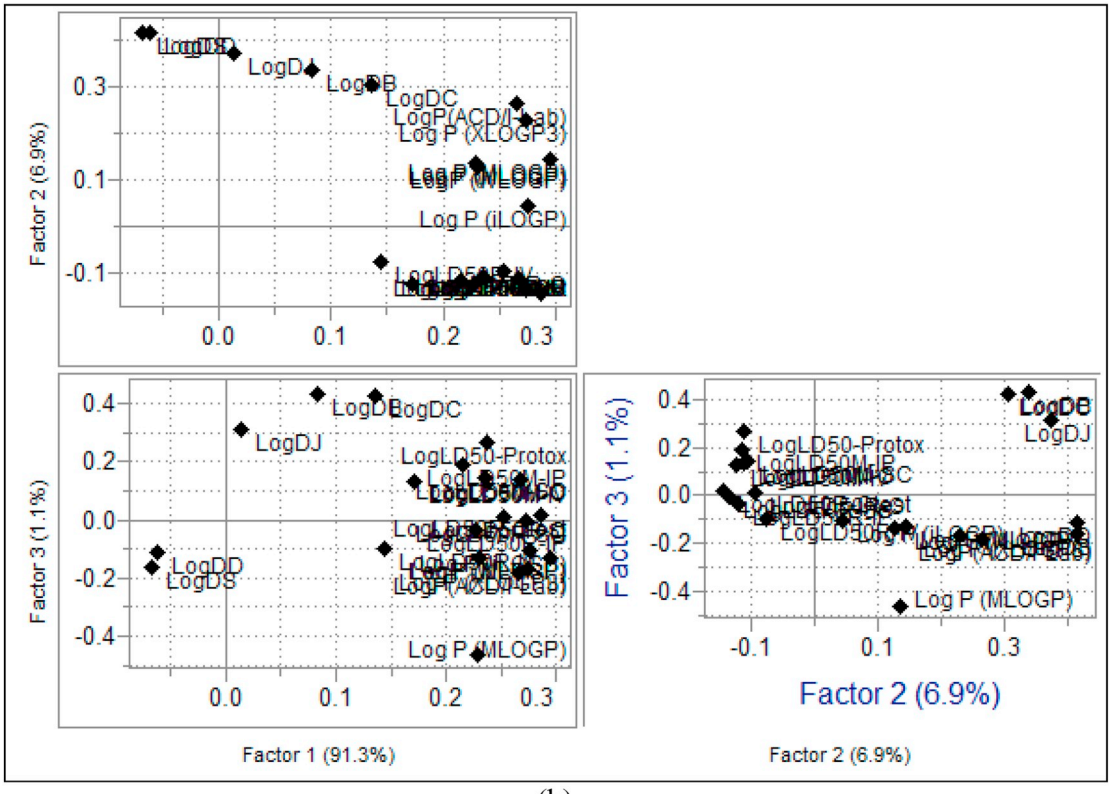
The therapeutic or recreational action of drugs results from their several interactions in the biological system. Depending on their structure and physicochemical properties, drugs produce different effects on each individual. These chemical factors influence drug interaction in the body. Two structurally similar drugs bearing only one atom occupying a different position in the molecules might present different physicochemical properties and, consequently, distinct biological activities from both quantitative and qualitative viewpoints [20].

As for *in silico* values calculations, amphetamines and cathinones behave differently in terms of toxicity. Amphetamines have higher LogP values: these molecules do not display the carbonyl group, so they are less ionizable than cathinones and therefore have greater lipophilic character. LogDS and LogDD values corroborate this observation. Because pH is acid in the stomach and duoden, cathinones can be protonated at the carbonyl group, which increases their hydrophilicity and decreases LogD values. As a consequence, these values are larger for amphetamines, confirming their higher lipophilicity.

On average, LogLD50 values agree with experimental values. Although these values do not influence group separation, they follow a similar trend: in general, amphetamines are more toxic than cathinones. LD50 intravenous data for rats and mice are the only exception for *in silico* data: cathinones have lower values than amphetamines in rats; the



(a)



(b)

Fig. 3. Two-dimensional view for the combination of factors for (a) Scores and (b) Loadings.

opposite trend is observed in mice. Rats and mice differ widely, and factors such as animal mass and body surface, among others, may require different dosages. Additionally, receptors and behavior vary depending on the species. Therefore, extrapolating drug behavior between rats and mice is impossible [87–89].

Multivariate classification can separate amphetamines and

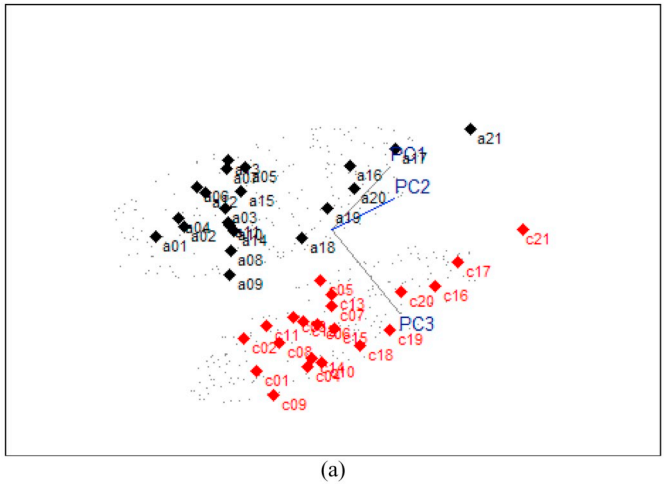
Table 6
Scores: sample coordinates for each principal component

	Factor 1 (91.3%)	Factor 2 (6.9%)	Factor 3 (1.1%)
a01	7.563217	−2.139141	−1.557328
a02	8.330724	−1.954409	−1.379920
a03	9.149360	−0.619033	−1.235453
a04	8.160300	−1.414766	−1.557923
a05	9.797864	0.943938	−1.508407
a06	8.915643	−0.715517	−1.687759
a07	9.468674	0.547626	−1.645497
a08	8.719831	−1.273201	−0.748531
a09	8.642337	−2.275245	−0.467382
a10	9.344657	−1.765589	−0.962186
a11	9.169171	−1.193822	−1.038495
a12	8.863600	−0.362363	−1.597110
a13	9.694367	0.497094	−1.718316
a14	9.373034	−1.808980	−0.851142
a15	10.024313	−1.113308	−1.209710
a16	11.202198	3.778037	−0.668758
a17	11.948937	5.324472	−0.480030
a18	10.088233	0.190297	−0.248979
a19	10.726814	1.509507	−0.364311
a20	11.230069	2.794828	−0.342600
a21	13.265943	7.415195	−0.051629
c01	8.253957	−4.389836	0.821793
c02	8.461538	−4.079805	0.381602
c03	9.410534	−2.549109	0.572152
c04	8.801560	−2.500181	1.155127
c05	10.107600	−0.879304	0.398052
c06	9.497741	−1.602768	0.811898
c07	9.764121	−0.525048	0.716087
c08	8.835814	−3.062866	0.700807
c09	7.970514	−3.774025	1.183059
c10	9.144510	−2.291923	1.264639
c11	8.542624	−2.293779	0.360219
c12	9.231749	−1.687373	0.656944
c13	10.014571	−0.617367	0.623162
c14	8.987629	−2.272696	1.123315
c15	9.816061	−1.628126	1.023039
c16	11.203341	2.989287	1.318569
c17	11.776567	4.079403	1.265263
c18	9.788733	−0.816540	1.390017
c19	10.410496	0.136336	1.466762
c20	10.971437	1.227926	1.156484
c21	12.937274	6.643055	1.440926

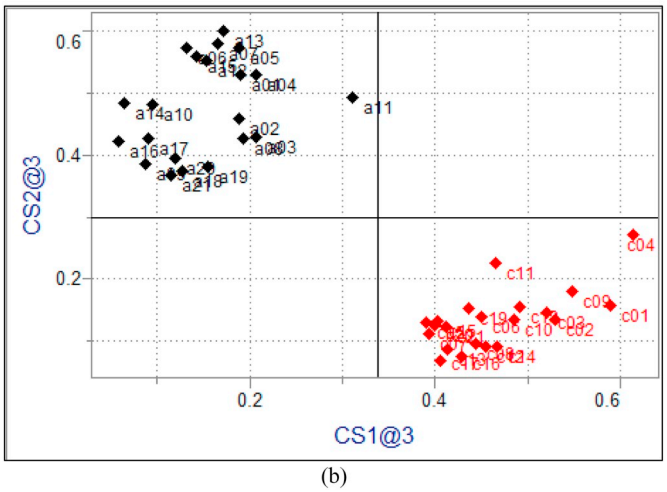
Table 7
Variables contribution to loadings

Variable	Factor 1 (91.3%)	Factor 2 (6.9%)	Factor 3 (1.1%)
LogLD50M-IP	0.215731	−0.11452	0.192327
LogLD50M-O	0.267141	−0.11129	0.137293
LogLD50M-IV	0.171827	−0.12326	0.13086
LogLD50M-SC	0.235167	−0.10569	0.141995
LogDS	−0.06797	0.414943	**−0.16033**
LogDD	−0.06169	0.415108	**−0.11132**
LogDJ	0.013565	0.372687	0.310766
LogDB	0.083498	0.337157	0.432679
LogDC	0.135547	0.303629	0.425273
LogLD50-Protox	0.236964	−0.11197	0.265828
LogLD50R-IP	0.22685	−0.12226	**−0.03698**
LogLD50R-IV	0.144251	−0.07686	**−0.09713**
LogLD50R-SC	0.2733	−0.13468	**−0.00306**
LogLD50R-O	0.253003	−0.09387	0.011816
LogLD50R-OTest	0.286685	−0.14338	0.01852
LogP (ACD/I-Lab)	0.265496	0.262975	**−0.18065**
LogP (iLOGP)	0.275379	0.043759	**−0.10252**
LogP (XLOGP3)	0.274344	0.22787	**−0.16774**
LogP (WLOGP)	0.230909	0.126867	**−0.13342**
LogP (MLOGP)	0.227982	0.134141	**−0.46441**
LogP (MLOGP)	0.29516	0.144563	**−0.13199**

cathinones according to their *in silico* toxicity references. Both supervised and unsupervised classification techniques provide information about how these molecules behave: results are similar, and



(a)



(b)

Fig. 4. SIMCA results for class projections in (a) three dimensions with hypersurface represented as dots and (b) two dimensions.

Table 8
SIMCA results

Misclassification			
	Class 1 (Predicted)	**Class 2 (Predicted)**	**No match**
Class 1 (original)	21	0	0
Class 2 (original)	0	21	0
Interclass residuals			
	Class 1	**Class 2**	
Class 1	0.16	0.48	
Class 2	0.47	0.14	
Interclass Distances			
	Class 1	**Class 2**	
Class 1	0	2.12	
Class 2	2.12	0	

techniques agree, indicating that these values are robust enough to be reproducible regardless of the statistical approach.

Finally, with respect to correspondence between *in silico* and experimental data, PLS regression affords different results – theoretical and experimental LogIC50DAT values correlate well (0.71), but LogIC50SERT (0.55) and LogIC50NET (0.45) values are lower. Table 13 displays literature values for the correlation (R^2) between IC50 and LD50 for different sets of compounds. Here, we used 26 molecules for PLS (obtained from Table 10); correlations could be improved when more experimental data are available. Even though this number is low, the values we found in this work are within the literature range. An

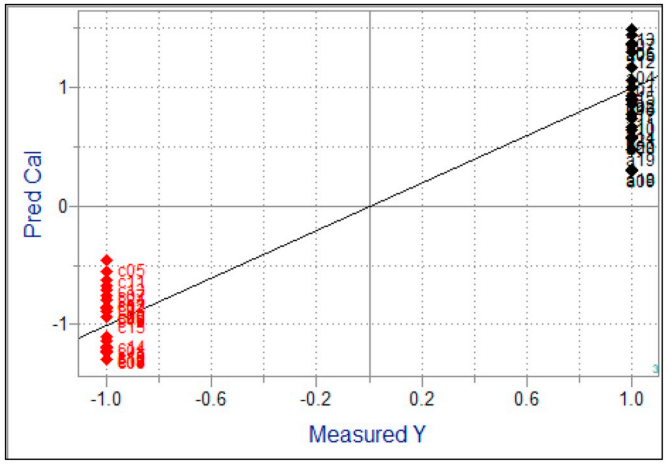


Fig. 5. PLS-DA classes for amphetamines and cathinones.

Table 9
SIMCA and PLS-DA results

Variable	Total modeling power for SIMCA	Regression vector for PLS-DA
LogLD50M-IP	0.374116	−0.165835
LogLD50M-O	0.480503	−0.138561
LogLD50M-IV	0.337094	−0.126914
LogLD50M-SC	0.562939	−0.113279
LogDS	**0.892326**	**0.194470**
LogDD	0.909880	0.146768
LogDJ	**0.941084**	**−0.258734**
LogDB	**0.908130**	**−0.361297**
LogDC	**0.842108**	**−0.342864**
LogLD50-Protox	0.608970	−0.191774
LogLD50R-IP	0.376904	0.016836
LogLD50R-IV	0.491034	0.049272
LogLD50R-SC	0.383068	−0.016189
LogLD50R-O	0.370953	−0.031900
LogLD50R-OTest	0.334192	−0.044400
LogP (ACD/I-Lab)	**0.896749**	**0.177386**
LogP (iLOGP)	**0.779644**	**0.139819**
LogP (XLOGP3)	**0.873983**	**0.191957**
LogP (WLOGP)	**0.811796**	0.095686
LogP (MLOGP)	**0.812273**	**0.364293**
LogP (MLOGP)	**0.875778**	**0.135244**

important observation is that, here, we conducted multivariate regression with all studied *in silico* variables; *i.e.*, we performed regression with 21 variables. Regression vectors in Table 12 indicate that this correlation could be improved if variables are selected to model LogIC50 values first. This means that each transporter can be described by different *in silico* values according to its characteristics. In this sense, different approaches can be adopted for calculated values to correlate them with the experimental ones. However, herein, our main objective was to verify how variables contribute to LogIC50 values.

Table 14 presents data collected from DRUGBANK [90–94]. We found only five of the molecules discussed in this study. An experimental value for toxicity is only available for amphethamine; all other LD50 values were calculated with AdmetSAR, a free tool to evaluate chemical ADMET [95]. This corroborates the lack of information about these drugs in the literature.

6. Conclusion

We have evaluated how *in silico* toxicity can provide information about new psychoactive substances. The idea is having an alternative way to increase our knowledge about these drugs given that traditional methods can be expensive and time-consuming [96]. Considering the speed at which these new drugs appear in the market, a faster methodology could provide useful information and reduce the lack of data on how these substances behave.

In silico methodologies may also be a strategy to meet the demand for generic bans on these drugs and to circumvent bureaucracies related to the scientific study of their effects because tests are conducted on the computer and not on living organisms.

In addition, planning harm reduction programs and adequate public policies for drug users can benefit from increased information. Early determination of these substances can be used to carry out harm

Table 10
Experimental values for inhibition compiled from the literature [30–33]

	IC50NET		LogIC50DAT		LogIC50SERT	
	a	c	a	c	a	c
01	-1.15	-0.7	0.11	1.14	1.65	2
02	-0.85	-0.92	0.041	0.38	1.26	1.6
03						
04	-0.7		0.57		1.28	
05		-0.21		0.4		2.13
06		-0.55		0.76		0.34
07		0.4		1.49		0.63
08	0.08	0.35	1.69	1.54	0.25	0.67
09	-0.44	-0.27	1.49	0.68	0.3	1.2
10	0.45	0.31	1.34	0.46	0.31	0.79
11	-0.66	-0.44	0.89	1.15	0.94	1.69
12	-0.7	-0.01	0.77	0.135	0.67	0.92
13		0.35		0.63		0.9
14	0.01	0.4	0.97	0.75	0.1	0.65
15						
16		-1.7		-1.4		2
17		-1.3		-1.15		1.36
18		-0.01		-0.28		1.88
19		-0.8		-0.96		1.18
20		-1.4		-1.3		0.98
21		-0.96		-0.66		-0.1

Table 11
PLS parameters for multivariate regression models for each IC50

	#PCs	Cumulative	SEV	Q^2	SEC	R^2
LogIC50NET	4	99.46	0.55	0.20	0.49	0.45
LogIC50DAT	**4**	**99.42**	**0.60**	**0.53**	**0.52**	**0.71**
LogIC50SERT	4	99.50	0.48	0.34	0.43	0.55

Table 12
Regression vector for each dependent variable

	Variable	LogIC50NET	LogIC50DAT	LogIC50SERT
1	LogLD50M-IP	−0.023319	−0.027099	**0.107325**
2	LogLD50M-O	0.045185	0.103662	0.061588
3	LogLD50M-IV	0.018625	0.069431	**0.140775**
4	LogLD50M-SC	**0.166522**	**0.312459**	−0.095349
5	LogDS	**0.219994**	**0.286916**	**−0.304039**
6	LogDD	**0.130658**	**0.162157**	**−0.232673**
7	LogDJ	**−0.167114**	**−0.361534**	**0.173099**
8	LogDB	**−0.168169**	**−0.410995**	**0.244504**
9	LogDC	−0.098365	**−0.294842**	**0.198998**
10	LogLD50-Protox	**0.200552**	**0.394050**	**−0.368371**
11	LogLD50R-IP	**−0.146554**	**−0.384602**	**0.169780**
12	LogLD50R-IV	**−0.144449**	−0.040315	**0.174624**
13	LogLD50R-SC	**0.224414**	**0.170348**	**−0.142853**
14	LogLD50R-O	−0.004070	0.031562	**0.134571**
15	LogLD50R-OTest	−0.042147	0.063160	0.098556
16	LogP (ACD/I-Lab)	**−0.152804**	−0.098730	0.054909
17	LogP (iLOGP)	**0.108595**	−0.051508	**−0.225728**
18	LogP (XLOGP3)	0.098352	0.037371	**−0.137835**
19	LogP (WLOGP)	**−0.164067**	**−0.103403**	**0.128970**
20	LogP (MLOGP)	**−0.342527**	−0.018991	**0.267175**
21	LogP (MLOGP)	0.002127	0.012653	−0.086261

Table 13
Literature data about the correlation between LD50 and IC50

YEAR	Number of compounds	R^2	Reference
1988	117	0.67	[36]
1986	22	0.72	[51]
1992	44	0.55	[40]
1998	50	0,61	[52]
1998	50	0.65	[52]
1998	50	0.64	[52]
1998	50	0.58	[52]
1998	33	0.73	[53]
1998	230	0.66	[54]
1999	50	0.65	[55]
1999	50	0.77	[55]
2000	50	0.65	[56]
2003	347	0.66	[57]
2008	97	0.56	[58]
2016	131	0.70	[41]
2017	162	0.77	[43]
2017	69	0.86	[43]
2017	285	0.51	[43]
2018	24	0.69	[48]

Table 14
DRUGBANK values for amphetamines and cathinones

	LD50	Obtained by	Accession number [90–94]
a01	180 mg kg^{-1} *	Experimental assay	DB00182
a01	3.2491 mol kg^{-1}	ADMESar	DB00182
a09	2.7501 mol kg^{-1}	ADMESar	DB01454
a14	2.6623 mol kg^{-1}	ADMESar	DB01566
c01	2.2186 mol kg^{-1}	ADMESar	DB01560
c06	Not available	Not available	DB13108

* LogLD50 = 2.26.

reduction practices in several countries.

This study has shown that computational methodologies may be useful to understand the toxicological behavior of amphetamines and cathinones. Multivariate statistics can provide information on these groups of drugs.

Prediction of LogLD50, LogP, and LogD values with the aid of different software reveals the same trends, and PLS regression indicates that they are correlated with *in vitro* cytotoxicity. An important observation is that many *in silico* values from different tools should be used to verify how the drugs behave. Experimental data are also commonly obtained by different methodologies, which prevent toxicological values from being reproducible. Although we have employed different methodologies for *in silico* calculations, values are reproducible and point out trends according to the chemical structure. Furthermore, these methodologies accelerate analysis and are an alternative approach to toxicity tests, dismissing the need for experiments on animals.

We believe that this study may be useful in drug harm reduction policies, meeting the multidisciplinary required by criminal science.

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References

[1] UCL Jill Dando Institute, What Is Crime Science? http://www.ucl.ac.uk/jill-dando-institute/about-us/jdi-background/what-is-crime-science, (2018) , Accessed date: 10 February 2018.
[2] M. Morelato, A. Beavis, M. Tahtouh, O. Ribaux, P. Kirkbride, C. Roux, The use of organic and inorganic impurities found in MDMA police seizures in a drug intelligence perspective, Sci. Justice 54 (2014), https://doi.org/10.1016/j.scijus.2013.08.006.
[3] M. Morelato, A. Beavis, M. Tahtouh, O. Ribaux, P. Kirkbride, C. Roux, The use of forensic case data in intelligence-led policing: the example of drug profiling, Forensic Sci. Int 226 (2013), https://doi.org/10.1016/j.forsciint.2013.01.003.
[4] O. Ribaux, A. Baylon, C. Roux, O. Delémont, E. Lock, C. Zingg, P. Margot, Intelligence-led crime scene processing. Part I: forensic intelligence, Forensic Sci. Int 195 (2010) 10–16, https://doi.org/10.1016/j.forsciint.2009.10.027.
[5] P. Reuter, B. Pardo, New psychoactive substances: are there any good options for regulating new psychoactive substances ? Int. J. Drug Policy. 40 (2017) 117–122, https://doi.org/10.1016/j.drugpo.2016.10.020.
[6] R.A. Glennon, M. Dukat, Synthetic Cathinones: A Brief Overview of Overviews with Applications to the Forensic Sciences, 4 (2017), pp. 1–7.
[7] G.G. Grecco, D.F. Kisor, J.S. Magura, J.E. Sprague, Impact of common clandestine structural modifications on synthetic cathinone “bath salt” pharmacokinetics, Toxicol. Appl. Pharmacol. 328 (2017) 18–24, https://doi.org/10.1016/j.taap.2017.05.010.
[8] J.B. Zawilska, D. Andrzejczak, Next generation of novel psychoactive substances on the horizon – a complex problem to face, Drug Alcohol Depend. 157 (2015) 1–17, https://doi.org/10.1016/j.drugalcdep.2015.09.030.
[9] A.M. Araújo, M.J. Valente, M. Carvalho, D. Dias Da Silva, H. Gaspar, F. Carvalho, M. de Lourdes Bastos, P. Guedes De Pinho, Raising awareness of new psychoactive substances: chemical analysis and in vitro toxicity screening of ‘legal high’ packages containing synthetic cathinones Ana, Arch. Toxicol. 89 (2015) 757–771, https://doi.org/10.1007/s00204-014-1278-7.
[10] United Nations Office on Drugs and Crime, MARKET ANALYSIS OF SYNTHETIC DRUGS. Amphetamine-type stimulants, new psychoactive substances, 1t ed., United Nations publication, Sales No. E.17.XI.6, Vienna, 2017. http://www.unodc.org/documents/scientific/Booklet_4_Market_Analysis_of_Synthetic_Drugs_ATS_NPS.pdf
[11] A. Matthews, R. Sutherland, A. Peacock, J. Van Buskirk, E. Whittaker, L. Burns, R. Bruno, I like the old stuff better than the new stuff? Subjective experiences of new psychoactive substances, Int. J. Drug Policy. 40 (2017) 44–49, https://doi.org/10.1016/j.drugpo.2016.11.004.
[12] R. Sutherland, R. Bruno, A. Peacock, S. Lenton, A. Matthews, C. Salom, P. Dietze, K. Butler, L. Burns, M.J. Barratt, Motivations for new psychoactive substances use among regular psychostimulant users in Australia, Int. J. Drug Policy. 43 (2017) 23–32.
[13] ANVISA: Agência Nacional de Vigilância Sanitária, Orientações sobre a classificação de substâncias proscritas por classes estruturais do grupo das catinonas sintéticas, (2017) 33.
[14] L. Harper, J. Powell, E.M. Pijl, An overview of forensic drug testing methods and their suitability for harm reduction point-of-care services, Harm Reduct. J 14 (2017), https://doi.org/10.1186/s12954-017-0179-5.
[15] A. Kloosterman, A. Mapes, Z. Geradts, E. van Eijk, C. Koper, J. van den Berg, S. Verheij, M. van der Steen, A. van Asten, The interface between forensic science and technology: how technology could cause a paradigm shift in the role of forensic institutes in the criminal justice system, Philos. Trans. R. Soc. B Biol. Sci. 370 (2015) 20140264, , https://doi.org/10.1098/rstb.2014.0264.
[16] W.C. Moreira, A. Beatriz, M. Rodrigues, T. Karen, M. Monte, J.M. Magalhães, C. Kilcia, C. Sena, Alcohol and other drugs: contributions of an academic league for nursing training, Rev. Enferm. Da UFPI. 6 (2017) 82–88.
[17] V.E. dos Santos, C.B. Soares, C.M.S. Campos, Redução De danos: Análise das concepções que orientam as práticas no Brasil, Physis 20 (2010) 995–1015, https://doi.org/10.1590/S0103-73312010000300016.
[18] J.E. Ruppenthal, Toxicologia, Rede e-Tec Brasil, Santa Maria – RS (2013).
[19] B.V. em Saúde, Descritores em Ciências da Saúde: DeCS [Internet], BIREME / OPAS / OMS (2017).
[20] L.C. Tavares, QSAR: A abordagem de Hansch, Quim Nova 27 (2004) 631–639, https://doi.org/10.1590/S0100-40422004000400018.
[21] A.B. Raies, V.B. Bajic, In silico toxicology: computational methods for the prediction of chemical toxicity, Wiley Interdiscip. Rev. Comput. Mol. Sci. 6 (2016) 147–172, https://doi.org/10.1002/wcms.1240.
[22] F.P. Mayer, L. Wimmer, O. Dillon-Carter, J.S. Partilla, N.V. Burchardt, M.D. Mihovilovic, M.H. Baumann, H.H. Sitte, Phase I metabolites of mephedrone display biological activity as substrates at monoamine transporters, Br. J. Pharmacol. 173 (2016) 2657–2668, https://doi.org/10.1111/bph.13547.
[23] G.E. Torres, R.R. Gainetdinov, M.G. Caron, Plasma membrane monoamine transporters: structure, regulation and function, Nat. Rev. Neurosci. 4 (2003) 13–25, https://doi.org/10.1038/nrn1008.
[24] A.R. Shalabi, D. Walther, M.H. Baumann, R.A. Glennon, Deconstructed analogues of bupropion reveal structural requirements for transporter inhibition versus substrate-induced neurotransmitter release, ACS Chem. Neurosci. 8 (2017) 1397–1403, https://doi.org/10.1021/acschemneuro.7b00055.
[25] D.G. Standaert, J.M. Galanter, Pharmacology of Dopaminergic Neurotransmission, in: D.E. Golan, A.H. Tashjian, E.J. Armstrong, A.W. Armstrong (Eds.), Princ. Pharmacol. Pathophysiol. Basis Drug Ther, 3, Lippincott Williams & Wilkins, Philadelphia, PA 19103 USA, 2013.
[26] A.E. Fleckenstein, T.J. Volz, E.L. Riddle, J.W. Gibb, G.R. Hanson, New insights into the mechanism of action of amphetamines, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 681–698, https://doi.org/10.1146/annurev.pharmtox.47.120505.105140.
[27] R.A. Vaughan, J.D. Foster, Mechanisms of dopamine transporter regulation in normal and disease states, Trends Pharmacol. Sci. 34 (2013) 489–496, https://doi.

org/10.1016/j.tips.2013.07.005.
[28] E. Carboni, C. Spielewoy, C. Vacca, M. Nosten-Bertrand, B. Giros, G. Di Chiara, Cocaine and amphetamine increase extracellular dopamine in the nucleus accumbens of mice lacking the dopamine transporter gene, J. Neurosci. 21 (2001) RC141, https://doi.org/10.1523/JNEUROSCI.21-09-j0001.2001.
[29] C.L. German, A.E. Fleckenstein, G.R. Hanson, Bath salts and synthetic cathinones: an emerging designer drug phenomenon, Life Sci. 97 (2014) 2–8, https://doi.org/10.1016/j.lfs.2013.07.023.
[30] L.D. Simmler, A. Rickli, M.C. Hoener, M.E. Liechti, Neuropharmacology monoamine transporter and receptor interaction pro fi les of a new series of designer cathinones, Neuropharmacology 79 (2014) 152–160, https://doi.org/10.1016/j.neuropharm.2013.11.008.
[31] A. Rickli, M.C. Hoener, M.E. Liechti, Monoamine transporter and receptor interaction profiles of novel psychoactive substances: Para-halogenated amphetamines and pyrovalerone cathinones, Eur. Neuropsychopharmacol. 25 (2015) 365–376, https://doi.org/10.1016/j.euroneuro.2014.12.012.
[32] M.E. Liechti, Novel psychoactive substances (designer drugs): overview and pharmacology of modulators of monoamine signalling, Swiss Med. Wkly 145 (2015), https://doi.org/10.4414/smw.2015.14043.
[33] L.D. Simmler, T.A. Buser, M. Donzelli, Y. Schramm, L.-H.H. Dieu, J. Huwyler, S. Chaboz, M.C. Hoener, M.E. Liechti, Pharmacological characterization of designer cathinones in vitro, Br. J. Pharmacol. 168 (2013) 458–470, https://doi.org/10.1111/j.1476-5381.2012.02145.x.
[34] L.D. Simmler, A. Rickli, M.C. Hoener, M.E. Liechti, Monoamine transporter and receptor interaction profiles of a new series of designer cathinones, Neuropharmacology 79 (2014) 152–160, https://doi.org/10.1016/j.neuropharm.2013.11.008.
[35] M.L. Soria, Las nuevas drogas psicoactivas: populares y peligrosas, Rev. Española Med. Leg. 44 (2018) 64–72, https://doi.org/10.1016/j.reml.2017.11.004.
[36] W. Halle, E. Gores, E. Göres, Vorhersage von LD50-Werten mit der Zellkultur (Prediction of LD50 values by cell cultures), Pharmazie 42 (1988) 245–248.
[37] H. Spielmann, E. Genschow, M. Liebsch, W. Halle, Determination of the starting dose for acute oral toxicity (LD50) testing in the up and down procedure (UDP) from cytotoxicity data, Altern. Lab. Anim 27 (1999) 957–966 http://www.ncbi.nlm.nih.gov/pubmed/25490464 (accessed October 8, 2018).
[38] W. Halle, The registry of cytotoxicity: toxicity testing in cell cultures to predict acute toxicity (LD50) and to reduce testing in animals, Altern. Lab. Anim 31 (2018) 89–198 http://www.ncbi.nlm.nih.gov/pubmed/15612878 (accessed October 8, 2018).
[39] B. Ekwall, Correlation between cytotoxicity in vitro and LD50-values, Acta Pharmacol. Toxicol. (Copenh). 52 (1983) 80–99, https://doi.org/10.1111/j.1600-0773.1983.tb02685.x.
[40] W. Halle, H. Spielmann, Two procedures for the prediction of acute toxicity (LD50) from cytotoxicity data, Altern. to Lab. Anim. ATLA1 20 (1992).
[41] A. Svennebring, The connection between plasma protein binding and acute toxicity as determined by the LD50 value, Drug Dev. Res. 77 (2016) 3–11, https://doi.org/10.1002/ddr.21286.
[42] A.K. Tiwari, P. Narasimhamurthy, G. Nagendrappa, A. Thakur, QSAR modeling for relative toxicity prediction of (3-(2-chloroquinolin-3-yl)oxiran-2-yl)(phenyl) methanone derivatives, Curr. Res. Pharm. Sci 7 (2017) 16–24, https://doi.org/10.24092/CRPS.2017.070103.
[43] Y. Wang, S. Wang, X.N. Feng, L.C. Yan, S.S. Zheng, Y. Wang, Y.H. Zhao, The impact of exposure route for class-based compounds: a comparative approach of lethal toxicity data in rodent models, Drug Chem. Toxicol. 41 (2018) 95–104, https://doi.org/10.1080/01480545.2017.1320405.
[44] B. Semire, A.K. Oyebamiji, Theoretical studies on pyrazole derivatives as anti-breast cancer agents: DFT, QSAR and Docking methods, Bull. Pharm. Res 7 (2017), https://doi.org/10.21276/bpr.2017.7.3.3.
[45] A.K. Oyebamiji, B. Semire, Studies of 2-[5-(aryloxymethyl)-1, 3, 4-oxadiazol-2-yl-sulfanyl] acetic acid derivatives for anti-bacterial activities via DFT, QSAR and Docking approaches, Bull. Pharm. Res 7 (2017), https://doi.org/10.21276/bpr.2017.7.3.1.
[46] A. Omotayo, A.K. Oyebamiji, O.R. Oyewole, B. Semire, A DFT-Based QSAR and molecular Docking studies on potent anti-colon cancer activity of pyrazole derivatives, Glob. J. Med. Res. Pharma, Drug Discov. Toxicol. Med 18 (2018) 9–21.
[47] H. Feng, Y. Chen, W. Yue, C. Feng, The molecular topological research on acute toxicities of substituted arenes to aquatic organisms, IOP Conf. Ser. Earth Environ. Sci. 153 (2018) 022035, , https://doi.org/10.1088/1755-1315/153/2/022035.
[48] J. Ekowati, N.W. Diyah, K.A. Nofianti, I.S. Hamid, Molecular Docking of ferulic acid derivatives on P2Y12 receptor and their ADMET prediction, J. Math. Fund. Sci. 50 (2018) 1–17, https://doi.org/10.5614/j.math.fund.sci.2018.50.X.x.
[49] A.A. Lagunin, M.A. Romanova, A.D. Zadorozhny, N.S. Kurilenko, B.V. Shilov, P.V. Pogodin, S.M. Ivanov, D.A. Filimonov, V.V. Poroikov, Comparison of quantitative and qualitative (Q)SAR models created for the prediction of Ki and IC50 values of antitarget inhibitors, Front. Pharmacol 9 (2018), https://doi.org/10.3389/fphar.2018.01136.
[50] N.M. Esteves-Pedro, K. Sugibayashi, E.A. Ostrosky, M. Ferrari, B. da Silva Sufi, M.B. Mathor, P.R.H. Moreno, F.R. Lourenco, V.O. Consiglieri, A.R. Baby, T.M. Kaneko, Validation cytotoxicity assay for lipophilic substances, Curr. Top. Med. Chem. 18 (2018) 275–286, https://doi.org/10.2174/1568026618666180410142829.
[51] P. Knox, P.F. Uphill, J.R. Fry, J. Benford, M. Balls, The frame multicentre project on in vitro cytotoxicology, Food Chem. Toxicol. 24 (1986) 457–463, https://doi.org/10.1016/0278-6915(86)90092-X.
[52] B. Ekwall, F.A. Barile, A. Castano, C. Clemedson, R.H. Clothier, P. Dierickx, B. Ekwall, M. Ferro, G. Fiskesjö, L. Garza-Ocañas, M.J. Gómez-Lechón, M. Gülden, T. Hall, B. Isomaa, A. Kahru, G. Kerszman, U. Kristen, M. Kunimoto, S. Kärenlampi, L. Lewan, A. Loukianov, T. Ohno, G. Persoone, L. Romert, T.W. Sawyer, R. Shrivastava, H. Segner, A. Stammati, N. Tanaka, M. Valentino, E. Walum, F. Zucco, MEIC evaluation of acute systemic toxicity: part VI. The prediction of human toxicity by Rodent LD50 values and results from 61, In Vitro Methods., Altern. Lab. Anim. 26 (Suppl. 2) (1998) 617–658.
[53] H. Norlen, E. Berggren, M. Whelan, A. Worth, MEIC evaluation of acute systemic toxicity: part IV. In vitro results from 67 toxicity assays used to test reference chemicals 31–50 and a comparative cytotoxicity analysis, Altern. Lab. Anim. 26 (1998) 131–183.
[54] W. Halle, Toxizitätsprüfungen in Zellkulturen für eine Vorhersage der akuten Toxizität (LD50) zur Einsparung von Tierversuchen, (1998).
[55] B. Ekwall, Overview of the final MEIC results: II. The in vitro–in vivo evaluation, including the selection of a practical battery of cell tests for prediction of acute Lethal blood concentrations in humans, Toxicol. in Vitro 13 (1999) 665–673.
[56] B. Ekwall, M. Sjöström, MEIC evaluation of acute systemic toxicity: part VIII. Multivariate partial least squares evaluation, including the selection of a battery of cell line tests with a good prediction of human acute lethal peak blood concentrations for 50 chemicals, Altern, Lab. Anim. 28 (2000) 201–234.
[57] National Toxicology Program, U.S. Departament of Health and Human Services, Section 1: Introduction and Rationale for the Use of In Vitro Neutral Red Uptake Cytotoxicity Test Methods to Predict Starting Doses for In Vivo Acute Oral Toxicity Testing, in: Vitr. Cytotox. Test Methods Backgr. Rev. Doc, National Toxicology Program (2006), pp. 1–22.
[58] M. Sjöström, A. Kolman, C. Clemedson, R. Clothier, Estimation of human blood LC50 values for use in modeling of in vitro–in vivo data of the ACuteTox project, Toxicol. Vitr. 22 (2008) 1405–1411, https://doi.org/10.1016/j.tiv.2008.04.017.
[59] I. Kavakiotis, O. Tsave, A. Salifoglou, N. Maglaveras, I. Vlahavas, I. Chouvarda, Machine learning and data mining methods in diabetes research, Comput. Struct. Biotechnol. J. 15 (2017) 104–116, https://doi.org/10.1016/j.csbj.2016.12.005.
[60] D. Ravi, C. Wong, F. Deligianni, M. Berthelot, J. Andreu-Perez, B. Lo, G.Z. Yang, Deep learning for health informatics, IEEE J. Biomed. Heal. Informat 21 (2017) 4–21, https://doi.org/10.1109/JBHI.2016.2636665.
[61] United Nations Office on Drugs and Crime, World Drug Report 2013, 1st ed., United Nations publication, Sales No. E.13.XI.6, Vienna, 2013. http://www.unodc.org/unodc/secured/wdr/wdr2013/World_Drug_Report_2013.pdf
[62] E. Cockbain, G. Laycock, Crime science, OXFORD Res. Encycl. Criminol. Crim. JUSTICE. (2017) 1–35, https://doi.org/10.4324/9781843925842.
[63] M. Carvalho, H. Carmo, V.M. Costa, J.P. Capela, H. Pontes, F. Remião, F. Carvalho, M.D.L. Bastos, Toxicity of amphetamines: an update, Arch. Toxicol. 86 (2012) 1167–1231, https://doi.org/10.1007/s00204-012-0815-5.
[64] L. Karila, B. Megarbane, O. Cottencin, M. Lejoyeux, Synthetic cathinones: a new public health problem, Curr. Neuropharmacol. 13 (2015) 12–20, https://doi.org/10.2174/1570159X13666141210224137.
[65] A.T. Bruni, P.O.M. de Carvalho, C.H.P. Rodrigues, V.B.P. Leite, In silico methods in forensic science: Quantum chemistry and multivariate analysis applied to infrared spectra of new amphetamine- and cathinone-derived psychoactive substances, Forensic Chem. 9 (2018) 21–34, https://doi.org/10.1016/j.forc.2018.03.006.
[66] Advanced Chemistry Development Inc, ACD/ChemSketch Freeware, www.acdlabs.com, (2015).
[67] ACD/I-Lab, (n.d.).
[68] A. Daina, O. Michielin, V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep. 7 (2017) 42717, https://doi.org/10.1038/srep42717.
[69] A. Lagunin, A. Zakharov, D. Filimonov, V. Poroikov, QSAR modelling of rat acute toxicity on the basis of PASS prediction, Mol. Inform. 30 (2011) 241–250, https://doi.org/10.1002/minf.201000151.
[70] P. Banerjee, F.O. Dehnbostel, R. Preissner, Prediction is a balancing act: importance of sampling methods to balance sensitivity and specificity of predictive models based on imbalanced chemical data sets, Front. Chem 6 (2018), https://doi.org/10.3389/fchem.2018.00362.
[71] T. Martin, U.S. EPA, Toxicity Estimulation Software Tool (TEST), (2016).
[72] A. Masunov, ACD/I-Lab 4.5: an internet service review, J. Chem. Inf. Comput. Sci. 41 (2001) 1093–1095, https://doi.org/10.1021/ci010400l.
[73] A. Daina, O. Michielin, V. Zoete, iLOGP: a simple, robust, and efficient description of n -Octanol/water partition coefficient for drug design using the GB/SA approach, J. Chem. Inf. Model. 54 (2014) 3284–3301, https://doi.org/10.1021/ci500467k.
[74] T. Cheng, Y. Zhao, X. Li, F. Lin, Y. Xu, X. Zhang, Y. Li, R. Wang, L. Lai, Computation of Octanol – water partition coefficients by guiding an additive model with knowledge, J. Chem. Inf. Model. 47 (2007) 2140–2148, https://doi.org/10.1021/ci700257y.
[75] S.A. Wildman, G.M. Crippen, Prediction of physicochemical parameters by atomic contributions, J. Chem. Inf. Comput. Sci. 39 (1999) 868–873, https://doi.org/10.1021/ci9903071.
[76] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings 1PII of original article: S0169-409X(96)00423-1. The article was originally published in Advanced Drug Delivery Reviews 23, Adv. Drug Deliv. Rev. 46 (2001) (1997) 3–26, https://doi.org/10.1016/S0169-409X(00)00129-0.
[77] J. Taminau, G. Thijs, H. De Winter, Pharao: Pharmacophore alignment and optimization, J. Mol. Graph. Model. 27 (2008) 161–169, https://doi.org/10.1016/j.jmgm.2008.04.003.
[78] E.M. Leite, L.C. Amorim, Noções Básicas de Toxicologia, (2006), pp. 1–3.
[79] M.M. Hann, G.M. Keserü, Finding the sweet spot: the role of nature and nurture in medicinal chemistry, Nat. Rev. Drug Discov. 11 (2012) 355–365, https://doi.org/10.1038/nrd3701.

[80] L.S. Ramos, K.R. Beebe, W.P. Carey, E.S. M, B.C. Erickson, B.E. Wilson, L.E. Wangen, B.R. Kowalski, L. Scott Ramos, Kenneth R. Beebe, W. Patrick Carey, M. Eugenio Sfinchez, Brice C. Erickson, Bruce E. Wilson, Lawrence E. Wangen, Bruce R. Kowalski, Laboratory for Chemometrics, Department, Education, (1986), pp. 31–49.
[81] K.R. Beebe, R.J. Pell, M.B. Seasholtz, Chemometrics: A Practical Guide, 1, Wiley-Interscience, New York, 1998.
[82] Chemometrics Technical Note, Description of Pirouette Algorithms., Infometrix, Inc, http://www.infometrix.com/apps/19-0193_AlgorithmTN.pdf, (1993) (accessed April 8, 2014).
[83] I. Infometrix, Pirouette Multivariate Data Analysis Software V4.5, (2014).
[84] W. Infometrix Inc, Woodinville, Pirouette, Multivariate Data Analysis for IBM PC Systems, (2001).
[85] S. Wold, M. Sjöström, L. Eriksson, PLS-regression: a basic tool of chemometrics, Chemom. Intell. Lab. Syst. 58 (2001) 109–130, https://doi.org/10.1016/S0169-7439(01)00155-1.
[86] E. Szymańska, E. Saccenti, A.K. Smilde, J.A. Westerhuis, Double-check: validation of diagnostic statistics for PLS-DA models in metabolomics studies, Metabolomics 8 (2012) 3–16, https://doi.org/10.1007/s11306-011-0330-3.
[87] J.P. Balcombe, Laboratory environments and rodents' behavioural needs: a review, Lab. Anim. 40 (2006) 217–235, https://doi.org/10.1258/002367706777611488.
[88] V. Sharma, J.H. McNeill, To scale or not to scale: the principles of dose extrapolation, Br. J. Pharmacol. 157 (2009) 907–921, https://doi.org/10.1111/j.1476-5381.2009.00267.x.
[89] P.V. Hodson, A comparison of the acute toxicity of chemicals to fish, rats and mice, J. Appl. Toxicol. 5 (1985) 220–226, https://doi.org/10.1002/jat.2550050403.
[90] D.S. Wishart, Y.D. Feunang, A.C. Guo, E.J. Lo, A. Marcu, J.R. Grant, T. Sajed, D. Johnson, C. Li, Z. Sayeeda, N. Assempour, I. Iynkkaran, Y. Liu, A. Maciejewski, N. Gale, A. Wilson, L. Chin, R. Cummings, D. Le, A. Pon, C. Knox, M. Wilson, DrugBank 5.0: a major update to the drugBank database for, Nucleic Acids Res. 46 (2018) (2018) D1074–D1082, https://doi.org/10.1093/nar/gkx1037.
[91] V. Law, C. Knox, Y. Djoumbou, T. Jewison, A.C. Guo, Y. Liu, A. Maciejewski, D. Arndt, M. Wilson, V. Neveu, A. Tang, G. Gabriel, C. Ly, S. Adamjee, Z.T. Dame, B. Han, Y. Zhou, D.S. Wishart, DrugBank 4.0: shedding new light on drug metabolism, Nucleic Acids Res 42 (2014) D1091–D1097, https://doi.org/10.1093/nar/gkt1068.
[92] C. Knox, V. Law, T. Jewison, P. Liu, S. Ly, A. Frolkis, A. Pon, K. Banco, C. Mak, V. Neveu, Y. Djoumbou, R. Eisner, A.C. Guo, D.S. Wishart, DrugBank 3.0: a comprehensive resource for “Omics” research on drugs, Nucleic Acids Res 39 (2011) D1035–D1041, https://doi.org/10.1093/nar/gkq1126.
[93] D.S. Wishart, C. Knox, A.C. Guo, D. Cheng, S. Shrivastava, D. Tzur, B. Gautam, M. Hassanali, DrugBank: a knowledgebase for drugs, drug actions and drug targets, Nucleic Acids Res. 36 (2008) D901–D906, https://doi.org/10.1093/nar/gkm958.
[94] D.S. Wishart, DrugBank: a comprehensive resource for in silico drug discovery and exploration, Nucleic Acids Res. 34 (2006) D668–D672, https://doi.org/10.1093/nar/gkj067.
[95] F. Cheng, W. Li, Y. Zhou, J. Shen, Z. Wu, G. Liu, P.W. Lee, Y. Tang, admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties, J. Chem. Inf. Model 52 (2012) 3099–3105, https://doi.org/10.1021/ci300367a.
[96] J.C. Dearden, In silico prediction of drug toxicity, J. Comput. Mol. Des. 17 (2003) 119–127.