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SUMMARY

01
AVALIAÇÃO DE RISCO

Amphetamines/ecstasy use by university students of health and related areas: the profile and associated risks

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Introduction: In 2021, is estimated 36 million people in the world, aged 15-64, had used amphetamines and 20 million had used *"ecstasy"*. In Brazil, the last study was realized in 2015, which demonstrated that around 1 million people, aged 12-65, had used amphetamines. Furthermore, university students tend to be more vulnerable, as they experience meeting new people and being separated from their family, as well as the pressure to maintain their grades and carry out other academic activities, which can cause a change in thinking about the use of drugs and increase their propensity to use. There are few studies on the use of drugs of abuse in health courses, but most research only reach one or two undergraduate courses, and not the entire health area. **Objective:** Characterize the profile of amphetamine/ecstasy users and evaluate the associated risk factors in university students in the health and related areas of Maringá-PR. **Methods:** A study carried out with a sample of 355 students from the Biological Sciences, Physical Education, Medicine, Physiotherapy, Nutrition, Nursing, Pharmacy, Biomedicine, Psychology, Dentistry, Speech Therapy, Biochemistry and Biotechnology courses, in the year 2023. The instruments "Alcohol, Smoking and Substance Involvement Screening Test" and "Self-reporting questionnaire-20" combined with sociodemographic questions were used for data collection. The results were analyzed using descriptive statistics and the risk analysis was performed using the chi-square test and Poisson regression for bivariate analysis and demonstrated an estimated value for the relative risk (RR). For all statistical tests, a 95% confidence interval and significance were considered for a value of p≤0.05. This study was approved by the Ethics Committee for Research Involving Human Beings of the State University of Maringá under number 3,430,374. **Results:** Of 355 participants, 56 had used amphetamines/ecstasy at some time in their lives and 37.5% using them in the last three months before answer the questionnaire. Users of this substance are, in their majority, women (70%), white (82%), aged 18-22 years (66%), who do not follow a specific religion (59%), with a monthly income of up to 4 minimum wage (68%), who live with other people (70%) and do not present a significant score for mental suffering (55%). Furthermore, they are from a public higher education institution (61%), from the first years of the course (63%), from courses related to the health area (56%), and from full-time studies (61%). A statistical association was observed between having used amphetamine/ecstasy at least once and being white (RR: 2.12; CI: 1.11 – 4.05: $p<0.05$), not following a specific religion (RR: 2.77; CI: 1.70 – 4.50; $p<0.05$), living with other people (RR: 0.40; CI: 0.24 – 0. 64; $p<0.05$), studying in a public educational institution (RR: 1.66; CI: 1.01 – 2.72; $p<0.05$) and being in the first years of graduation (RR: 0. 57; CI: 0.35 – 0.93; $p<0.05$). As for use in the last three months, an association was noted with not following a specific religion (RR: 3.87; CI: 1.6 – 9.34; p<0.05) and with living with other people (RR: 0.22; CI: 0.10 – 0.52; $p<0.05$). **Discussion/Conclusion:** According to the World Drug Report 2023, most amphetamine/ecstasy users are men, which differs from our study. Probably because of the characteristic of health courses having more women than men. Moreover, the results highlight the possible risk factors associated with the use of amphetamines/ecstasy, as well as the need for more studies regionally and for other drugs, to better understand the subject and assist in the development of preventive strategies. **Acknowledgments:** I would like to thank the State University of Maringá, my postgraduate program and the CAPES funding agency.

Assessment of the Brazilian Raw Commodity Food Intake Using IBGE POF 2017-2018 Data – Update of the ILSI Brasil Consumption Database

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Background: Dietary risk assessment encompasses all chemical compounds that may be present or added to food, including contaminants (e.g., mycotoxins, metals), pesticide and veterinary drug residues, and food additives. ANVISA's normative RDC 295 (July 29, 2019) outlines criteria for assessing the risk associate with residues resulting from pesticide application to agricultural commodities. This evaluation must be carried out in the registration, post-registration, re-evaluation of pesticides and to assess the results of food monitoring analysis, thus contributing to adequate risk communication for consumers. To assess population exposure to contaminants, food consumption data must be available. However, the National Household Survey (Portuguese acronym POF) by IBGE isn't directly applicable since consumption is reported as prepared dishes. To address this, food preparations must be broken down into their ingredients, which, in turn, can be linked to the agricultural commodities that originated them. Between 2013 and 2017, ILSI Brasil developed an unprecedented project to convert the foods reported in POF 2008-2009 into raw commodities and released two free databases containing average and 97.5 percentile consumption, taking into account foods in all forms of consumption. They also included the mean body weight and a compilation of unit weight of commodities (whole and edible part), crucial for calculating acute exposure. **Objective:** Update ILSI Brasil per capita consumption databases (average and 97.5p and Brazilian body weight) from population aged 10+ years using POF 2017-2018 data. **Methods Consumption data:** this project has used the raw data from the IBGE's POF 2017-2018 dated March, 2021. For the average consumption calculation, it was used SAS – Statistical Analysis System and for the 97.5p consumption, data was imported into Microsoft Excel®. Both were then uploaded into Microsoft Access® to obtain the raw commodity consumption. Recipes: a recipe is the combination of the ingredients of a food and its preparation mode (raw, cooked with or without oil, fried, baked, etc.). Using literature and internet search, new recipes were settled and included to the ILSI Brasil database from 2008-2009. Body weight: reported body weight of the 46164 residents that responded to the survey was used. Data expansion with factors provided by IBGE was performed to estimate the Brazilian population's weight. **Results:** About 1230 new recipes were added to the databases, expanding it to more than 3200 recipes. Per capita consumption data (average and 97.5p) to each region (Midwest, North, Northeast, South, Southeast) of 440 food commodities (fruits, vegetables, meat, cereals, dairy, etc.) and water were obtained, by relating reported food to their recipes. This was achieved using Microsoft Access®. Some similar commodities were grouped and their consumption was calculated. A Microsoft Excel® file with consumption data, as well as the estimated body weight and commodity unit weight (whole and edible part) was published in December, 2023 at https://ilsibrasil.org/projetogtpof/. **Discussion/Conclusion:** A valuable and updated source of information was achieved as a result of this project. It can be used not only for pesticide registration and monitoring programs, but also for robust dietary risk assessment of other types of contaminants. However, as the survey only gets information for residents aged 10+ years, no inference about infants and children's consumption can be made. **Acknowledgments:** The author would like to thank ILSI Brasil and Pesticide Task Force members for supporting this important project and for giving me the privilege to lead it, and to Carlos Ledo (Embrapa Mandioca e Fruticultura, Universidade Federal do Reconcavo da Bahia – UFRB) and Paulo T. Yatsuzuka (Well Done Consultoria Ltda.) for their valuable participation during data handling.

Assessment of *in vivo* dermal exposure of rural workers to Tebuconazole and Pyraclostrobin residues

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Background/Introduction: Pesticides are chemical products widely used in crops to control pests, prevent diseases and improve crop yields, however, rural workers are classified as one of the main risk groups for exposure to these products, due to their handling at different times, from transportation, mixing, loading and application. Such occupational exposure can occur through different routes, but the inhalation and dermal routes stand out. **Objective:** Carry out an *in vivo* study to evaluate the dermal exposure of rural workers to residues of Tebuconazole (TBZ) and Pyraclostrobin (PIRA) in onion crops. **Methods:** The evaluation was previously approved by the Ethics and Research Committee of the Federal University of Pernambuco, under CAAE number: 56545822.2.0000.520. The assessments were carried out on five rural workers in the municipality of Belém do São Francisco - Pernambuco. Each individual prepared a dilution of the commercial products, Rival200 EC® (Tebuconazole 200g/L) and Comet250® (Pyraclostrobin 250g/L), according to the leaflet, to be applied to onion cultivation, using a backpack spray pump, for 4 hours. Each worker was wearing their usual work clothes, and the assessment took place by attaching cotton sections to their clothes, in different regions (back of the neck, right forearm, left forearm, right thigh, left thigh, right foot and left foot), before starting spraying. In addition to collecting the stratum corneum, by tape stipping, after finishing spraying. All samples were kept at -6 ºC until extraction and quantified by liquid chromatography coupled to mass spectrometry. **Results:** The dermal exposure values, expressed in mg/body region, for Tebuconazole were from 0.73 to 138.28 while for Pyraclostrobin the values found were from 0.89 to 145.43. Regarding penetration factors, PIRA presented values that varied between 9.99-93.79 and 23.60-71.74% for the neck and arms, respectively, and TBZ values between 0.85-99.72 and 14.88-68.83%, for the same locations. **Discussion/Conclusion:** Data on dermal exposure during the application of pesticides by spraying are scarce in the literature. It is not clear whether dermal exposures are related only to the spraying process or to the handling of the pesticide product as a whole. In the case of family farming, the worker is generally responsible for the acquisition and correct use of personal protective equipment (PPE) and in this study, none of them wore appropriate clothing, as indicated in the product leaflets. Decisions on the use of PPE represent a challenge, as some workers are unaware of the potential dangers of pesticides and/or are often not informed about the type of PPE that should be used, and/or do not have the resources to purchase such equipment. The above demonstrates the need for actions aimed at educating and training rural workers regarding good practices in the use of pesticides, as actions of this nature are associated with high levels of safety behavior. **Acknowledgments:** Instituto Nacional de Ciência e Tecnologia – Rede Norte Nordeste de Fitoprodutos (INCT–RENNOFITO). Projeto: 465536/2014-0.

Brazilian aluminum exposure from green tea consumption

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Introduction: Aluminum (Al) is one of the most abundant chemical elements in nature and its numerous uses result in various sources of human exposure. High concentrations of this element in food are mainly due to the use of additives that contain aluminum in their composition and the migration of utensils, packaging and/or pans made with this metal. There is no evidence that aluminum, even in trace amounts, is essential for living organisms. In recent years, its toxicity has been the subject of debate and it is still not fully understood. Some studies indicate that high concentrations of aluminum in living organisms can trigger neurodegenerative diseases and other cognitive impairments, as well as breast cancer, but there is no confirmation of these toxic effects. Its neurotoxicity and cytotoxicity, especially to bone, are well-known. **Objective:** The purpose of this study was to estimate the intake of aluminum by the Brazilian population from the consumption of green tea. **Methods:** A deterministic model was used to calculate dietary exposure to aluminum, applying the following equation: Dietary exposure = Σ (aluminum concentration in green tea × tea consumption/body weight - b.w.). Aluminum concentrations in green tea were obtained from the World Health Organization's GEMS/Food (Global Environment Monitoring System - Food Contamination Monitoring and Assessment Programme) database for the years 2000 to 2019. Green tea consumption was obtained from the average consumption in the 24-hour food record of the 2017/2018 Family Budget Survey (POF). This data was extracted using the statistical program SAS® (Statistical Analysis System) OnDemand for Academics and transcribed into Microsoft® Excel Office 365 version. **Results:** The average concentration of aluminum in dried green tea was 696 mg/kg. The average aluminum intake for the population was established at 3.136 mg/kg b.w., reaching 6.888 mg/kg b.w. at the 95th percentile (p95) of occurrence, based on an average daily consumption of 0.6 mL of green tea and considering 100% extraction of the analyte from the matrix in the preparation of the beverage. The average exposure to aluminum was 0.042 mg/kg b.w., reaching 0.098 mg/kg b.w. on p95 . The risk assessment indicated that green tea consumption contributed, on average, 4.6% and on p95 10.5% of the Tolerable Weekly Intake (TWI) of aluminum (1 mg/kg b.w.). In the urban population, exposure was slightly higher, with average of 4.7% and p95, 10.3% of TWI. In the Southern Region of Brazil, the percentages were even higher (11.5% and 25.4% of TWI, respectively). **Discussion/Conclusion:** The study revealed that green tea consumption contributed significantly to aluminum intake, especially in the Southern Region of Brazil and in urban areas. However, the method used to prepare green tea influences the extraction of this element from the beverage and its bioaccessibility, with factors such as pH, compounds with an affinity for aluminum, temperature and preparation time, as well as the use of aluminum utensils, affecting its solubility and transfer to the beverage. Although the levels were lower than TWI, the influence of tea preparation on the extraction and bioaccessibility of aluminum suggests the need for a more detailed analysis of preparation, consumption and its impact on health. **Acknowledgments:** CNPq; CAPES; FAPEMIG.

Copper levels in fruits and processed fruit-based products and dietary exposure

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Introduction: Copper (Cu) is an essential micronutrient for human metabolism, but excessive exposure to this element can be harmful and result in damage to human health. Dietary exposure is the main source of copper. The concentration of metals in food, regardless of its origin, depends on the environmental conditions where food was produced (use of agricultural pesticides, soil composition), as well the techniques used in its processing (food additives, utensils and equipment used in processing) and storage conditions. **Objective:** The study aimed to compare the concentrations of copper in different fruits (apple, tomato, orange, grapes, apricot, citrus fruits, and pear) in their raw state and processed (sauce, compote, juice, canned, dehydrated) and estimate dietary exposure from these products. **Methods:** Data of copper levels in fruits and processed fruit-based foods were obtained from the GEMS/Food database (Global Environment Monitoring System - Food Contamination Monitoring and Assessment Programme) of the World Health Organization without limitation of sampling period. Data were analyzed by descriptive statistics using 95th percentile (p95) and average. Copper intake was estimated by a deterministic model [Exposure = Σ(Cu content (mg/kg) x fruit consumption (kg)/ body weight (kg))]. Food consumption data for the Brazilian population was obtained from a 24-hour dietary recall described at the 2017/2018 Household Budget Survey report. **Results:** It was observed significant variation in copper levels depending on the fruit type and processing method. Red or green grapes had the highest copper values among raw fruits (p95=1.96 mg/kg, average=1.13 mg/kg). Reconstituted concentrated grape juice showed lower levels of copper (p95=0.36 mg/kg, average=0.09 mg/kg) than grapes, demonstrating a dilution factor of 8%. White raisins had lower copper contents (p95=1.88 mg/kg, average=1.00 mg/kg) than grapes, but the results were in the same magnitude and were from different origins and varieties. Copper levels in red apple with skin (p95=0.54 mg/kg, average=0.34 mg/kg) and orange (p95=0.66 mg/kg, average=0.46 mg/kg) were lower than in their respective juices (apple juice: p95=7.47 mg/kg, average=1.36 mg/kg; orange juice p95=17.22 mg/kg, average=0.72 mg/kg). Lower copper levels were observed in compotes for all categories, such as pear (p95=1.02 mg/kg, average=0.74 mg/kg) to canned pear in light syrup (p95=0.49 mg/kg, average=0.39 mg/kg); red apple with skin to apple compote (p95=0.43 mg/kg, average=0.43 mg/kg) and light apple fruit compote (p95=0.46 mg/kg, average=0.41 mg/kg). This can indicate that water and sugar do not contribute to copper levels in fruit products. The exposure of the Brazilian population to copper from fruits and fruit-based products was low (0.17% of the acceptable daily intake - ADI of 0.05 mg/kg b.w.). Fruit compotes were the products with higher contribution to the exposure (0.0007 mg/kg b.w). Oranges, apples and fruit juices contributed with less than 0.1 0g/kg b.w. each. **Discussion/Conclusion:** Notable variations were observed among different types of fruits and processed fruits, showing that copper levels can increase or decrease depending on the fruit and processing method used. Fruits and fruit-based products did not represent a potential source of copper exposure for the Brazilian population. Future research should explore the mechanisms of the variations found and expand the analysis to other food categories. The monitoring of copper in food should be constant due to the increase of using copper-based pesticides. **Acknowledgments:** CAPES (Funding Code 001); CNPq; FAPEMIG.

Development of a Pesticides Occupational Exposure Study following Technical, Regulatory, Ethical and GLP Requirements in Brazil

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Background/Introduction: The assessment of occupational risk arising from pesticide use is a regulatory tool implemented in several countries and regions, such as the USA and Europe. It's conducted to determine whether the use presents acceptable risks to worker health. To achieve this, the exposure must be estimated usually from predictive models based on generic data from occupational exposure studies. This type of study shall follow harmonized international guidance to compose databases that estimate exposure in similar scenarios (combining activities, application equipment, crop characteristics, etc.), aiming to reduce the number of studies conducted in human subjects. Considering the implementation of a Brazilian regulation by Agencia Nacional de Vigilancia Sanitaria (ANVISA, Public Consultation 987 of December 15, 2020) and the need to have representative Brazilian agriculture scenarios, a pilot study conducted. **Objective:** Describe the steps to design, to obtain consent and conduct an occupational exposure study with human subjects in Brazil following technical, regulatory, ethical and GLP requirements. **Methods:** 1. Technical requirements: study design under guideline OECD/GD(97)148 – Series on Testing and Assessment No. 9, defining the agricultural scenario to be monitored, including routine activities that would be monitored (mixing, loading and/or application), type of application, sprayer, formulation, crop and test substance. Mapping possible locations to conduct the study once the scenario is defined. 2. Regulatory requirements: the study was conducted using regular working clothing: pants, long-sleeved shirt, sturdy shoes, gloves and safety glasses. This allows measuring exposure under realistic conditions. To be conducted without PPE required by label, an Experimental Use Permit (EUP) was issued by MAPA in agreement with ANVISA and IBAMA and supported by a preliminary risk assessment conducted using available tools (USEPA and EFSA). 3. Ethical requirements: pesticides exposure varies depending on many conditions during the work routine. As this study requires the participation of experienced and trained workers, the study project was submitted to Comissao Nacional de Etica em Pesquisa – CONEP, with supporting information to guarantee study participants' physical integrity, health and protection of personal data. All risks involved in such study were listed and mitigation actions were presented. 4. Compliance to Good Laboratory Practices - GLP: this type of study must be conducted under GLP to be accepted by ANVISA or by other regulatory agencies. Due to the study characteristics, a new area of expertise was created by INMETRO. **Results:** Once the study design and the preliminary risk assessment were carried out, consents from regulatory bodies and the Ethics Committee were granted. The first and second phases of the study were successfully carried out in 2022 and 2023. Several professionals were trained, mentored by international experts. Members of ANVISA staff were present during field phase. **Discussion/Conclusion:** The main benefits of the conduction of such studies in Brazil are: a. to provide representative data of the local agricultural scenario to compose a Brazilian database; b. to conduct high quality local exposure studies following clear parameters and procedures for local and global data use; c. to improve industry, academia, and governmental entities technical expertise; d. to contribute with scientific data for the appropriate recommendation of PPE on pesticide labels. As a final result, by considering not only the hazard but also exposure, the health and safety of the agricultural workers are prioritized which includes their comfort, which will result in increased adherence to the use of PPEs. **Acknowledgments:** ProHuma would like to thank all company members that supported this project, and to the authors – scientific specialists of the ProHuma technical committee. ProHuma also would like to thank to the Toxicology General Office – GGTOX from ANVISA for the valuable support throughout the development of this project.

Dietary exposure assessment to sodium cyclamate for Brazilian adolescents with probabilistic modelling

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Introduction: Sodium cyclamate, an artificial, non-caloric sweetener, has a low level of toxicity. However, when it is metabolized by intestinal bacteria, cyclohexylamine is formed, increasing significantly the level of toxicity and it can be a health concern. The United States and Japan do not allow the use of cyclamic acid and its salts (cyclamate) in foods and drinks. However it is allowed by Codex Alimentarius and in Brazil the National Health Surveillance Agency (ANVISA) determines in which foods it is allowed and its respective Maximum Permitted Levels (MPL). The Acceptable Daily Intake (ADI) of cyclamate, established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), is 11 mg/kg of body weight (bw). Previous studies suggest that some population groups, such as adolescents, may exceed the ADI. **Objective:** This study comprises a risk assessment of sodium cyclamate in food for the Brazilian adolescent population using a probabilistic approach. **Methods:** Dietary exposure, based on Brazilian consumption data and food occurrence of this additive, was processed using Monte Carlo simulation method with the @Risk® statistical program. 10,000 simulations were calculated for each population subgroup (sex, household situation, and Brazil's regions), considering general adolescents and consumers only (a population that reported consumption). The consumption of foods and beverages with sodium cyclamate was obtained from the average consumption, of two non-consecutive days, of the 24-hour food recall of the 2017/2018 Family Budget Survey (POF). These data were collected by two researchers using the statistical program SAS® (Statistical Analyses System) OnDemand for Academics and transcribed to Microsoft® Excel version Office 365. The occurrence of sodium cyclamate was obtained from MPLs established in the Brazilian legislation. The presence of cyclamate in food categories was identified by analyzing the list of ingredients on food labels. Data collection of 3,335 foods/beverages with the presence of the sweetener was carried out in a Belo Horizonte supermarket, in 2021. **Results:** Estimated dietary exposure for adolescents was, on average, 5.047 mg/kg bw. For consumers only, it reached 57.440 mg/kg bw at the 95th percentile. The food categories that contributed the most to the intake of this additive were soft drinks, powder drink mix, and dairy beverages for the general adolescents, and soft drinks, dulce de leche and powder drink mix for consumers only. The probability of male adolescents exceeding the ADI was 0.2%, considering the mathematical model. No probability of female adolescents exceeding the ADI was observed. For the urban and rural areas, the probability of exceeding the ADI was 0.2% and 0.0%, respectively. Comparing the Brazilian regions, populations from Southeast and South regions showed higher risk of exceeding the ADI (3.4% probability each). For consumers only, 80.0% of adolescents, in the subgroup of high consumers, were at risk of exceeding the ADI. **Conclusion:** Based on the data obtained, Brazilian adolescents, in general, have a low probability of exceeding the ADI of sodium cyclamate. In contrast, consumers only have a higher probability that can cause serious side effects on the intestinal microbiota. Considering that a conservative approach has been adopted on the sweetener levels used in the estimation, additional refinements can be made if cyclamate levels are available. **Acknowledgments:** Coordination for the Improvement of Higher Education Personnel (CAPES). Funding Code 001. Joint Institute for Food Safety and Applied Nutrition, University of Maryland, United States of America.

Dietary intake of high-intensity sweeteners by a population of children aged 1 to 3 years in the city of Campinas-SP, Brazil

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Sweeteners are food additives with the ability to give foods a sweet taste. Its intake is considered safe when it is below the Acceptable Daily Intake (ADI); however, there are few studies on exposure to sweeteners in Brazil, especially in relation to children. Therefore, this study aims to evaluate the intake of high-intensity sweeteners (acesulfame potassium, aspartame, sodium cyclamate, neotame, saccharin, sucralose and steviol glycosides) by children aged 1 to 3 years in the city of Campinas-SP, Brazil. For this, food consumption data from 24-hour dietary recalls of 92 children were used combined with analytical concentrations of sweeteners in commercial products obtained through high-performance liquid chromatography coupled to mass spectrometry. Using this information, it was possible to analyze different exposure scenarios in order to characterize the risk of sweeteners intake by comparing them with the ADI. It was observed that for the general population, which includes all study participants, the average intake values of the sweeteners analyzed were below the ADI, suggesting that there is no toxicological risk to health. For the high consumer of the general population, exposure to sodium cyclamate and saccharin represented 132.5% and 149.5% of the respective ADIs, while the intake of other sweeteners was below the safety value. In relation to the intake of the consumers only subgroup, that is, which only includes participants who consumed at least one food that may contain sweeteners, mean intake values for acesulfame potassium and sodium cyclamate were above the ADI (101.5 % and 165.2% of the ADI, respectively) while for the high consumer of this subgroup, most sweeteners, except neotame and sucralose, presented intake values above the safety value. Thus, the results suggest that exposure to sweeteners can vary considerably depending on the food consumption profile, highlighting the importance of evaluating different scenarios to characterize the toxicological risks associated with these additives. The study has conservative considerations and can be refined through the use of probabilistic exposure assessment techniques.

Estimative exposure to inorganic arsenic by rice consumption for Brazilian adolescents, adults and elderly population

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Introduction: Inorganic arsenic (iAs) is a genotoxic carcinogen with additional epigenetic effects. The main source of non-occupation exposure to arsenic is by ingestion of contaminated water and food. In 2010 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) revoked the provisional tolerable weekly intake (PTWI) of 15 µg/kg bw set for iAs based on the Benchmark Dose (Lower Limit) Level (BMDL) (3 µg/kg bw day) for a 0.5% increased incidence of lung cancer. However in 2023 the European Food Safety Authority (EFSA) published a new report considering the BMDL 0.5 of 0.06 µg iAs/kg bw day as a reference (end)point for skin cancer based on a case–control study. Rice is the most important food source to iAs exposure worldwide. Rice is an important staple-food for Brazilian population with a consumption prevalence of over 70%. **Objective:** This study comprises a risk assessment of Inorganic arsenic (iAs) intake for the Brazilian adolescents, adult and elderly population. **Methods:** The mean was estimated from 294 Brazilian rice samples reported in GEMS/Food. The middle bound approach was used for left censored data treatment. The tAs level was converted in iAs (0.7x tAs level) based on a conservative approach. The consumption of rice was obtained from the average consumption, of two non-consecutive days, of the 24-hour food recall of the 2017/2018 Family Budget Survey (POF). These data were collected by two researchers using the statistical program SAS® (Statistical Analyses System) OnDemand for Academics and transcribed to Microsoft® Excel version Office 365. The risk was estimated based on Margin of Exposure (MOE). An MOE of 1 would correspond to an exposure level that is associated with a 5% increase relative to the background incidence for skin cancer, based on the available data. **Results:** The estimative of mean of iAs levels was 0.07 mg/kg. For tAs, the mean was 0.10 mg/kg and 0.23 mg/kg (percentile 95). By EFSA the mean values of iAs varied of 233 µg/kg in red rice (Lower Bound = Upper Bound) and 128–131 µg/kg (Lower Bound–Upper Bound) in brown rice among the rice samples. The mean of white rice consumption observed in your study varied between 1.63 g/kg bw day and 2.54 g/kg bw day for elderly and adolescent respectively. The estimative of iAs intake from white rice varied for 0.11 µg/kg bw day to 0.17 µg/kg bw day. The iAs Brazilian exposure only from white rice consumption was similar than observed for EFSA of the dietary chronic mean to iAs across European dietary surveys (0.10 – 0.16 µg/kg bw day). The dietary iAs exposure from rice consumption is higher in all age groups and therefore the respective MOEs were smaller than 1. Based on this preliminary study the MOE raises a health concern associated with a 5% increase relative to the background incidence for skin cancer. **Conclusion:** The risk of an increase in 0.5% of the incidence of skin cancer presented concern in all scenarios for white rice consumption, especially for adolescents. However, a conservative approach to establish the iAs levels was used. Therefore, is important to improve an analytical method to quantify iAs level to conduct a more realistic risk exposure. Also to consider the impact of rice-derived foods consumption, such as (bakery products, pasta, cookies, biscuits). **Acknowledgments:** The authors are grateful to the Ezequiel Dias Foundation (FUNED) and the Farmacy Faculty of Federal University of Minas Gerais State. (FAFAR/UFMG). Coordination for the Improvement of Higher Education Personnel (CAPES). Funding Code 001. **Disclosure statement:** No potential conflict of interest was reported by the authors.

Exposure to cadmium in infusions: a study in the Brazilian context

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Introduction: Cadmium (Cd), a heavy metal, is released into the environment both by natural processes, including rock weathering and volcanic activity, and by anthropogenic activities, including mining, the use of phosphate fertilizers and the combustion of fossil fuels. Human exposure to this metal occurs primarily through the ingestion of plant-based foods, the inhalation of tobacco smoke, and industrial emissions. Characterized by its environmental persistence and bioaccumulation, especially in organs such as the kidneys, liver and bones, cadmium can remain in the human body for decades. Nephrotoxicity is a significant adverse effect, with a latent period of up to ten years. Monitoring cadmium levels in food is essential to mitigate their impact on human health and ensure food safety. **Objective:** The purpose of this study was to estimate the cadmium intake by the Brazilian population, derived from the consumption of teas and infusions. **Methods:** This study assessed the average cadmium concentrations in four categories of infusions recognized by Brazilian legislation (Collegiate Board Resolution (RDC) No. 716 of July 1, 2022). Tea was defined as the product derived from Camellia sinensis, including the categories green tea and black tea, with the other species classified as 'other infusions'. Cadmium exposure was calculated using a deterministic model, according to the equation: Dietary exposure = Σ(Cd concentration in the infusion x infusion consumption / body weight (b.w.)). Cadmium concentrations were obtained from the World Health Organization's GEMS/Food database (1998-2021). An extraction efficiency of 100% of the analyte from the plant matrix was considered for the infusions ready for consumption. The consumption of infusions was based on average data from the 2017/2018 Household Budget Survey (POF), extracted using SAS® (Statistical Analysis System) OnDemand for Academics and recorded in Microsoft® Excel Office 365 version. **Results:** Infusions of yerba mate and chamomile showed the highest concentrations of cadmium (0.502 mg/kg and 0.374 mg/kg, respectively), while 'other infusions', black tea and green tea, showed lower levels (0.089 mg/kg, 0.022 mg/kg and 0.047 mg/kg, respectively). The estimated weekly intake of cadmium, based on an average daily consumption of 36 mL of yerba mate and 7 mL of chamomile, indicated exposure of 0.126 mg/kg b.w. and 0.21 mg/kg b.w., respectively. It was found that the southern region of Brazil has the highest exposure to cadmium, via consumption of infusions, with an average of 0.007 mg/kg b.w. and 0.021 mg/kg b.w. in the p95 of occurrence with percentage of 40.5% on average and 69.9% on the 95th percentile (p95), relative to the Tolerable Weekly Intake (TWI) of 2.5 µg/kg b.w. Yerba mate was the main contributor to cadmium exposure, with 96.7%, followed by chamomile with 3.1%. **Discussion/Conclusion:** The study determined that infusions, including teas, were a significant source of dietary exposure to cadmium. Data from POF 2017/2018 indicated that the population of the southern region of Brazil, due to its high consumption of yerba mate, was the most exposed to cadmium from this food category. These findings reinforce the need for continuous monitoring and regulation of cadmium concentrations in food products to protect public health.

Integrated Pre-Clinical and Clinical Evaluation Strategy to Measure Ocular Irritation of Children's Products with Potential for Eye Contact (Rinsable)

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Introduction: Ocular irritation is an important safety concern for children's rinse-off products, such as shampoos and soaps. The determination of non-irritation and ocular comfort of products with a risk of exposure to the eyes is carried out through an integrated approach, involving preclinical and clinical evaluation stages. Initially, a risk analysis is performed, elucidating through literature data and in silico analysis the ocular irritation potential of each ingredient in the finished product. Then, using standardized in vitro approaches, such as protocols referenced by the OECD, we ensure the absence of ocular irritation potential in the developing formulation, concluding the pre-clinical evaluation stage with high criticality. With this, clinical tests are carried out, as a confirmatory stage of all evaluation and to prove the attributes of the formulation, such as "ophthalmologically tested", "no sting", "no tears", among others. **Objective:** To elucidate pre-clinical and clinical evaluation strategies to prove the absence of ocular irritation in children's products with potential for eye contact. **Methodology:** The risk analysis of each ingredient in the formulation was carried out using highly credible toxicological bases (ECHA, CIR, SCCS) and in silico tools (QSAR, StopTox). In the in vitro analysis stage, the OECD 492 protocol was applied, using a commercially available corneal epithelium model (SkinEthic - Episkin). The formulation was tested at a concentration of 10%, established as a concentration that simulates the product's rinsing condition. For clinical trials, the ocular instillation protocol was performed on adult volunteers (18 - 60 years old) who met the inclusion criteria. In the protocol, 0.1 mL of the test substance was applied to the periocular region of one eye, while 0.1 mL of the negative control (sterile water) was applied to the other eye, following a random distribution. The parameters of irritation, discomfort, redness and tearing were determined through self-assessment and technical analysis carried out by an ophthalmologist before and after instillation (immediately after 30 seconds), 5 minutes, 15 minutes and 60 minutes after application. To complete the clinical panel, dermal compatibility and acceptability tests with ophthalmological monitoring were carried out according to the methodology described in the Anvisa Guide (2012). **Results and Discussion:** A large number of tests were carried out based on this evaluation strategy, with this, toxicological dossiers for the risk assessment of the ingredients were prepared. The proficiency of the test substances established in the OECD was completed and the tested formulations did not show potential for ocular irritation. The ocular instillation method is an important analysis to establish with high criticality the absence of irritation and discomfort from the test formulation. Through comparative analyzes with the negative control, the absence of clinical symptoms and signs in the studied population was demonstrated. Combined with the range of clinical studies standardized by Anvisa, the integrated strategy is robust to ensure that the tested formulations can be used safely in children and allows establishing attributes such as: no sting, no tears and tear free. **Conclusion:** The integrated preclinical and clinical evaluation strategy offers a comprehensive approach to evaluate the ocular irritation potential of rinsable children's products with a high probability of contact with the eyes, improving the acceptability of this class of products in highly vulnerable target populations. **Acknowledgments:** Grupo Boticário and Medcin.

Key Considerations for Surrogate Selection in Nitrosamine Risk Assessments

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Since the discovery of the potent carcinogen nitrosodimethylamine (NDMA) in batches of valsartan in 2018, increasing numbers of drugs [Schlingemann et al 2022] have been affected by a structurally-diverse range of nitrosamine impurities. Global regulatory guidance documents for nitrosamine risk assessment [e.g. ANVISA, EMA, FDA and others] permit the use of read-across to determine the acceptable intake of a novel nitrosamine drug substance-related impurity (NDSRI) in cases where insufficient carcinogenicity data for the compound itself exists. In 2023 the carcinogenicity potency categorization approach (CPCA) was included in these guidance documents from many agencies, which enables pragmatic and conservative method for determining acceptable intake limits (AIs) for NDSRIs. This has provided more achievable limits for some NDSRIs than the prior default of 18 ng/day; however, the approach still applies this limit or other low limits to many NDSRIs, and therefore read-across may be needed to set a limit that is both achievable via control measures – and indeed detectable analytically. Read-across, or surrogate selection, for the determination of safety limits is established as a method across a number of fields beyond nitrosamines, including agrochemicals, European REACH assessments, extractables and leachables, and various methodologies have been proposed to systematise what is currently a manual and expert process. The potential for, and problems associated with, this subjectivity have been evident in application of read-across for NDSRIs, where discrepancies exist between health authorities and indeed the acceptability of read-across for a given compound has changed over time. For example, nitrosovarenicline was proposed by the sponsor to be read across to nitrosohexamethyleneimine at 313 ng/day [Ponting et al 2022]; health authorities requested read-across to nitrosotetrahydropyridine at 37 ng/day, and at the time of writing this has been superseded in some regions by the CPCA-derived limit of 400 ng/day. Critical considerations for nitrosamine read across include the following: 1) Robustness of the carcinogenicity data of the proposed surrogate. While robust data exists for relatively few nitrosamines, methods have been proposed in the past year for the utilisation of less robust data [Felter et al 2023], including the use of the lower confidence interval [Thomas et al 2023] - as health authorities have done when nitrosodiphenylamine is the surrogate. 2) Structural features in the NDSRI and surrogate. Some features, as presented in the CPCA and recent literature [Thomas et al 2022, Ponting et al 2022, Cross and Ponting 2021], have statistically-significant effects on potency, and these should be evaluated alongside other reactivity-affecting features. 3) DMPK-relevant properties such as molecular weight and logP. While these are expected to differ between the small molecule analogues and the NDSRI, consideration should be made, ensuring that distribution to the same organs is expected. Understanding the effects of these differences should also allow the use of molecular weight scaling when determining the AI [Fine et al 2023]. 4) Overall similarity and localised similarity. Calculated as cheminformatic descriptors, these can capture additional effects not directly listed in the expert assessment above, with localised similarity being useful for reactivity, and global similarity being useful for assessing the DMPK-relevant properties. The use of the considerations above allows for more robust selection of read-across surrogates for nitrosamines, or a category thereof [Dobo et al 2022], for many NDSRIs for which the CPCA-derived limit is unachievable. This should allow for the continued marketing of the affected drugs with confidence that there is no exposure of patients to unacceptable increases in carcinogenic risk.

Next-generation risk assessment: Integrating *in vitro* data and physiologically-based kinetic (PBK) modeling for chemical kidney toxicity evaluation

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Vancomycin is an antibiotic employed in the hospital for the treatment of infections caused by Gram-positive bacteria. Nevertheless, its use is limited due to the narrow therapeutic window and its high probability of causing nephrotoxicity. Despite efforts to evaluate vancomycin nephrotoxicity, few studies have demonstrated the relationship between vancomycin concentration in renal cells and toxicity in humans. Then, strategies for risk assessment of vancomycin-based nephrotoxicity in humans are urgently needed. Nowadays, the next-generation risk assessment is moving toward a new approach method of integrating physiologically-based kinetic (PBK) modeling and relevant human cell-based assays to inform a priori the point of departure (PoD) for human health risk.[1,2] Considering this, we aimed to develop a population-based human PBK model for vancomycin to correlate the internal vancomycin concentrations from *in vivo* exposure associated with nephrotoxicity to the PoD concentrations derived from *in vitro* studies and demonstrate this model as a promising alternative to animal toxicity testing. The PBK model was developed and validated employing PK-Sim Software version 11.1. Due to the scarcity of clinical data for vancomycin-induced nephropathy, a renal cell model for vancomycin exposure was developed using information obtained from PBPK models for rats and mice and extrapolated to humans through sensitivity analysis and parameter estimation tools. A critical parameter for model building was the kidney permeability (interstitial-intracellular), which was approximately 10-fold lower in humans compared to the average values for rats and mice. After the rigorous testing and model validation, the kidney intracellular value estimated in healthy volunteers was about 4-fold higher than in plasma, corroborating with published *in vivo* studies from mice and rats. Further, the Benchmark Dose Software (BMDS) from the Environmental Protection Agency (EPA) was utilized to generate the Benchmark Dose Lower Limit (BMDL). This was calculated using input parameters obtained from *in vitro* nephrotoxicity data. The BMDL values obtained ranged from 0.3 to 1.0 µM, representing the concentration range that leads to 10% of kidney cells becoming nonviable. The BMDL value of 0.3 µM was employed to calculate the PoD, considering exposure to vancomycin for 14 days of intravenous administration, the dose predicted by the PBK model was 0.02 mg/kg. Subsequently, the PoD was applied to establish the Health-Based Exposure Limit (HBEL) by multiplying the PoD achieved by 50 kg, resulting in a value of 1 mg daily dose of vancomycin permitted for humans with normal renal function. In our sense, the uncertainty factors to calculate Permitted Daily Exposure (PDE) were not considered in our dose metrics since the factors were already considered in the PBK model developed. In conclusion, this work presents that it is possible to combine PBK modeling of human exposure with *in vitro*- derived toxicity information to predict the potential risk of different exposure levels in humans, as an alternative to risk assessments based on the results of animal testing.

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Occupational Exposure Limit (OEL) derivation for the psychedelic drugs psilocybin and lysergic acid diethylamide (LSD) under investigation for the treatment of psychiatric diseases

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Background/Introduction: The therapeutic potential of serotonergic psychedelics, including lysergic acid diethylamide (LSD) and psilocybin, in treating psychiatric disorders like depression and anxiety is currently being investigated in clinical trials. Given that these drugs are manufactured and administered in clinical trials, careful attention must be given to potential occupational exposures. It is imperative that the risk of occupational exposure connected with these drugs be assessed, particularly in light of the possibility of psychedelic effects at lower dosages. **Objective:** To determine the occupational exposure limits (OEL) for psilocybin and LSD by considering comprehensive information on their mechanisms of action, reported illicit uses, clinical studies, adverse effects, pharmacokinetics, and nonclinical toxicity. **Methods:** An extensive literature search was conducted using databases such as PubMed, Google Scholar, PubChem, and ClinicalTrials.gov to gather relevant information for the assessment of LSD and psilocybin. Various keywords related to psychedelic substances and their effects were used. The OEL was established by applying conservative uncertainty factors (UF) to the point of departure (PoD), enabling extrapolation to a safe threshold level within the intended worker population to ensure an ample margin of safety. **Results:** Serotonergic psychedelics, including LSD and psilocybin, primarily interact with the 5-hydroxytryptamine (5HT)2A receptor and other serotonin receptors, resulting in profound effects on perception, cognition, emotion, and neuroplasticity. These effects have sparked interest in their potential for treating psychiatric disorders. Phase 2 studies have demonstrated that low doses of LSD and psilocybin can improve depression and anxiety scores without serious adverse effects. Although psychedelics can cause temporary distortions in perception, mood alterations, hallucinations, depersonalization, ataxia, hyperreflexia, agitation, psychosis, amnesia, anxiety, hypertension, tachycardia, hyperthermia, abdominal pain, and gastrointestinal symptoms, these effects are generally mild and self-resolving. In unsafe environments, however, psychological effects can escalate to dangerous behaviors, including aggression towards oneself or others. Hallucinogen persisting perception disorder (HPPD) may also occur. Regarding pharmacokinetic aspects, psilocybin is rapidly metabolized to its active form, psilocin, after oral administration. Both orally administered LSD and psilocybin have a fast absorption, distribution, and elimination from the body with a relatively long duration of action. Nonclinical safety data of these drugs are limited. However, available experimental data indicate that LSD is associated with possible reproductive and developmental toxicity, as well as equivocal genotoxicity. Considering the manufacturing and administration of these drugs in clinical trials, it is crucial to consider potential occupational exposures. The primary concern in occupational settings is the acute mind-altering effects observed at low doses, emphasizing the need for protective occupational exposure limits. Considering the above-mentioned factors, an OEL was derived by integrating historical data, clinical studies, and available animal studies to establish a safe exposure limit for workers over a typical working lifetime (8 hours per day, 5 days per week, for 40 years). The derived OELs for both substances are found to be below 0.05 µg/m^3. **Discussion/ Conclusion:** The present study extensively discusses the pharmacological, toxicological, and health risks associated with LSD and psilocybin, with the goal of establishing inhalation exposure limits to ensure worker safety. The low occupational inhalation doses underscore the significance of implementing robust occupational health and safety measures when handling serotonergic psychedelics in occupational settings. These findings highlight the need for strict adherence to safety protocols to minimize potential risks in the workplace.

Recommendation of a safety framework for undergraduate forensic chemistry/ toxicology teaching laboratories based on the RAMP risk-based management system

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Background/Introduction: With the advancement of the forensic sciences, several universities across the globe have developed and implemented undergraduate forensic programs, covering several areas including drug chemistry and forensic toxicology. A key component of any undergraduate program with a forensic science concentration is laboratory-based courses. In any forensic chemistry/ toxicology teaching laboratory, implementing a safety program is of paramount importance. Undergraduate forensic chemistry/teaching, experiments can include numerous types of hazards (chemical, physical, and biological) present in a range of potentially hazardous procedures – including but not limited to – sampling, non-biological and biological sample homogenization, preparation of quality control samples, extractions using organic solvents and acids or bases, acid digestion and execution of color tests for drug identification. **Objective:** In this work, we apply the RAMP (Recognize Hazards, Assess Risk, Minimize Risk, Prepare for Emergencies) risk management system as a set of recommendations specifically relevant to teaching chemical safety in forensic laboratory courses. This can be used by instructors interested in developing a forensic chemistry/toxicology course or implementing specific forensic experiments in an existing analytical course. **Methods:** Current scientific literature and standards relevant to chemical safety and traditional procedures normally performed in forensic chemistry/toxicology teaching laboratories for undergraduate students were reviewed with how applying the RAMP risk-management system could improve safety. **Results:** The proposed framework recommendations, based on RAMP, are structured in five main points as follows: (I) *Recognize*: Recognize that hazards may come in many forms in forensic teaching laboratories (chemical toxicants, biological pathogens and toxins, physical hazards, and process hazards) and use systematic identification procedures; (II) *Assess*: Evaluate the likelihood of occurrence and the consequences that may result from uncontrolled hazards and prioritize and set risk acceptance levels; (III) *Minimize*: Reduce all risks to a determined acceptable level, minimize priority risks (those that are highly likely to occur and/or result in severe consequences by first using the high-level controls (elimination, substitution, engineering)) and layer controls for redundancy; (IV) *Prepare*: Do not assume that events cannot occur and prepare for unexpected incidents should controls fail and (V) *Student Learning Outcomes*: Develop student's safety competencies in forensic experiments through education and training using a risk-based management system. **Discussion/Conclusion:** In undergraduate programs with a forensic chemistry concentration, forensic chemistry and toxicology teaching laboratories are important components of the curriculum that provide students with practical experience in performing procedures that can be routinely performed in real forensic laboratories. In these courses, it is important to ensure that students develop competencies in risk-based safety applicable to forensic chemistry/toxicology. Adopting the well-established RAMP risk-management system to recognize hazards, assess and minimize any risks, and prepare for any incidents in a forensic teaching laboratory establishes a framework for practicing forensic analysis safely.

Risk assessment associated with lack of safety information on labels of hazardous chemical products

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The labeling of hazard materials is mandatory safety information in Brazil. However, in industrial and educational environments, this practice is sometimes overlooked or carried out incorrectly. Substances known to be harmful to human health and the environment are therefore handled without awareness of the intrinsic dangers, circulating without proper identification during road transport across the country and being stored without necessary precautions. The absence of chemical product identification not only violates strict laws and regulations requiring appropriate labels, but also carries serious legal consequences for the involved organizations, including significant penalties and damage to the companies' reputation. The lack of identification also poses a serious risk to occupational health, exposing workers to unidentified chemicals, which can result in immediate health problems and contribute to the development of long-term occupational diseases. Additionally, the inadequate identification of these substances has a significant environmental impact. Soil, water, and air contamination occurs more prominently, affecting local ecosystems and contributing to broader environmental issues such as pollution. Given these concerns, this article proposes an assessment of risks associated with the lack of safety information on labels of chemical products, based on responses obtained through a questionnaire distributed to students in the Chemistry program at Oswaldo Cruz Colleges. The central objective is to conduct a comprehensive survey on the topic and analyze the risks resulting from deficiencies in hazard communication. For this analysis, twenty responses were collected from Chemistry students in an online and anonymous format. The questionnaire addressed the following questions: (1) Have you received safety training related to the handling of these chemical products?; (2) Do you find the hazard information on the labels of chemical products clear and understandable?; (3) What type of hazard communication do you encounter most frequently on labels of hazardous chemical products in the environment(s) you frequent?; (4) Have you experienced situations where the lack of safety information resulted in unsafe practices? The data were analyzed quantitatively. Of the participants, 40% stated they had not received safety training related to the handling of chemical products in their companies. Surprisingly, 95% reported understanding hazard information on labels, with 85% of these labels adopting the hazard communication of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). It is noteworthy that 55% of respondents experienced situations where the lack of safety information resulted in unsafe practices. The results reveal a disparity between understanding hazard information on labels and the occurrence of unsafe practices. The analysis of this data suggests that, although the majority demonstrates understanding of the information, the lack of safety training and the experience of unsafe situations highlight a significant gap in the effective implementation of safe handling. According to the CETESB Chemical Emergencies Report in 2010, of the 57 chemical emergencies attended to in UGRHI 5, 5 cases involved unidentified chemical products or residues, while in UGRHI 6, of the 184 emergencies, 37 cases presented the same lack of identification. These data support the concern present in real scenarios that persists to this day, emphasizing the need for effective chemical safety training and correct hazard communication for chemical products. This study provides valuable contributions to educational institutions, companies, and regulatory bodies, aiming to implement effective accident prevention measures and promote safer environments in the handling of chemical products.

Synergism: combined effect of fipronil, glyphosate and imidacloprid on HepG2 cells

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Introduction: The growing increase in the planting of transgenic soybean monocultures increased the average consumption of pesticides in relation to the planted area. Additionaly, the use of mixtures of pesticides is a common practice. The aim of this study was to evaluate the effects of mixtures of the three pesticides most used in soybean cultivation in Rio Grande do Sul, fipronil, glyphosate and imidacloprid on the human hepatoblastoma cell line HepG2. **Methods:** Mixtures were calculated using data obtained for each pesticide individually through the MTT test. Using the concentration addition (CA) and independent action (IA) models, additivity expectations were calculated for two mixtures, one equipotent based on the EC50 values of each pesticide (Mix A) and the other based on the acceptable daily intake (ADI) index of each pesticide (Mix B). The cells were exposed to Mix A and Mix B mixtures for 48 hours. In addition, the effect of the mixtures in mitochondrial membrane potential and release of liver enzymes was evaluated. **Results:** The pesticide mixtures caused synergistic effects ($p<0.05$) that were greater than those expected by both CA and IA predictions. It was possible to observe that the mixtures depolarized cell mitochondria in HepG2 and increased the enzymatic activity of transaminases in cell culture medium. **Conclusions:** In the study, the three pesticides showed a synergistic effect, regardless of the proportion of the mixture and the contribution of the single chemical to the overall effect. In this context, evaluating the cytotoxicity of isolated pesticides may underestimate the effect of mixtures. Interactions between pesticides occur simultaneously, even at single levels, and can result in significant harmful effects, justifying the need for more realistic assessments in order to assess safety to better predict the effects on human health.

Teratogenic effects, oxidative stress and neurotoxicity in zebrafish embryos exposed to a mixture of herbicides: dicamba and glyphosate

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Introduction: Exposure to pesticides during embryonic development can pose significant health risks, especially due to complex mixtures capable of triggering various responses not well known yet. **Objective:** Assess the acute toxicity, teratogenic effects, antioxidant system and neurotoxicity of the herbicides dicamba and glyphosate, isolated and in mixtures, in zebrafish embryos. **Methods**: The embryos were exposed to different concentrations of the herbicides: 18 (D1) and 72 (D2) mg/L of dicamba; 6 (G1) and 22 (G2) mg/L of glyphosate; and mixtures of the lowest concentrations (M1 - 18 mg/L of dicamba + 6 mg/L of glyphosate) and the higher concentrations (M2 - 72 mg/L of dicamba + 22 mg/L of glyphosate) for 96h. **Results:** The LC50 was 88.1 mg/L for glyphosate and 285.8 mg/L for dicamba. M2 showed greater lethality at 48 and 72 hours, underscoring the severity of the effects. The mixtures caused a significant increase in teratogenic effects. Generalized edema, predominant in the dicamba group, reached its highest incidence at 48 and 72 hours and decreased subsequently, except for M2, which did not return to normality. The yolk sac edema was significant only for M1. The M1 caused delayed eclosion at 72h, while M2 accelerated the process at 48h. The herbicides affect the antioxidant system in various ways: dicamba decreased SOD activity, while glyphosate increased it considerably. Dicamba increased the acetylcholinesterase activity, demonstrating its neurotoxicity, which was also observed in M2. **Conclusion:** This is the first study that highlights the effects of the mixture of these pesticides on zebrafish embryos. These results underscore the complexity and severity of the effects of these herbicides in combinations during embryonic development.

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The profile and associated risk of hypnotics and sedatives use by university students of health and related areas

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Introduction: Hypnotics and sedatives are a class of psychoactive substances that depress the nervous system, facilitating the onset of sleep and/or causing destruction, such as benzodiazepines and other non-neuroleptic components. In Brazil, there is still little studies of the abuse use of this specific class, being studied together with psychotropics and antidepressants in general. The university students go through major periods of change, such asmainly experience separation from their families and the pressure of maintaining grades and additional academic activities, which can change their thinking about psychoactive substances use and lead to initiation of use or an increase in its use. There are few studies on the use of psychoactive substances in health courses, but most research covers only one or two undergraduate courses and not the entire health area. **Objective:** Characterize the profile of hypnotics and sedatives users in university students in the health and related areas of Maringá-PR. **Methods:** A study carried out with a sample of 355 students from the Biological Sciences, Physical Education, Medicine, Physiotherapy, Nutrition, Nursing, Pharmacy, Biomedicine, Psychology, Dentistry, Speech Therapy, Biochemistry and Biotechnology courses, in the year 2023. The instruments "Alcohol, Smoking and Substance Involvement Screening Test" and "Self-reporting questionnaire-20" combined with sociodemographic questions were used for data collection. The results were analyzed using descriptive statistics and the risk analysis was performed using the chi- square test and Poisson regression for bivariate analysis and demonstrated an estimated value for the relative risk (RR). For all statistical tests, a 95% confidence interval and significance were considered for a value of p≤0.05. This study was approved by the Ethics Committee for Research Involving Human Beings of the State University of Maringá under number 3,430,374. **Results:** Out of 355 participants, 40 had used hypnotics at least once in their lives, and 23 had used them in the last three months before the questionnaire was administered. Most users are female (80%), white (65%), aged between 18-22 years old (71%), follow a specific religion (53%), with a minimum income of up to 4 salaries. minimum (85%), who live with other people (75%) and positive presence for suffering. Regarding the characteristics of the degree, 45% are from courses related to the health area, full-time (58%), and private educational institutions (55%). Regarding relative risk, a significant association was noted between use at least once in a lifetime and: income up to 4 minimum wages (RR: 2.77; CI: 1-5.53; p=0.05), living with other people (RR: 0.51; CI: 0.26-0.98; p<0.05), and a positive score for mental suffering (RR: 2.40; CI: 1.27-4.48; p<0.05). There was a statistical association between use in the last three months and: white race (RR: 0.42, CI: 0.19-0.93; p<0.05), monthly income of up to 4 minimum wages (RR: 9.0; CI: 1, 22-66.00; p<0.05) and a positive score for mental suffering (RR: 7.67; CI: 2.32-25.41; p<0.05). **Discussion/Conclusion:** According to the III National Survey on Drug Use carried out in 2015, the majority sex for the use of psychoactive is male, which differs from our study. Probably due to the characteristic of health courses having more women than men. Furthermore, the results highlight the possible risk factors and that more studies should be carried out for hypnotics and sedatives nationally to better understand the needs and, thus, develop preventive strategies. **Acknowledgments:** I would like to thank the State University of Maringá, postgraduate program and the CAPES.

02
BIOMARCADORES

Determination of cotinine in breast milk samples by liquid chromatography coupled to mass spectrometry

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Introduction: The habit of smoking is very common throughout the world. However, this practice can be extremely harmful to the exposed individual. There are different diseases that may be associated with exposure to nicotine, the active ingredient in cigarettes. Lung and heart diseases and their genotoxic and carcinogenic potential are some of the most reported effects. For lactating women, smoking deserves more attention, as it is known that nicotine can be transmitted through breast milk, creating risks for the newborn. **Objective:** Optimize, validate and apply methodology able to identify and quantify cotinine, the main metabolite of nicotine in breast milk samples, serving as a bioindicator of exposure for future assessments. **Methods:** 20 samples were collected from lactating volunteers living in the city of Alfenas MG, who agreed to participate in the study. The samples were collected with the help of the doctor responsible for monitoring the women during the pre and postpartum period. After collection, 1 mL of breast milk was placed in a falcon tube, followed by 2.5 mL of acetonitrile, 50 µL of sodium hydroxide and 500 µL of chloroform. The mixture was vortexed for 1 minute and centrifuged at 1550g for 10 minutes. After these processes, the supernatant was transferred to another falcon tube, adding 0.1g of sodium sulfate. The new supernatant was transferred to a vial and 10 µL injected by liquid chromatography coupled to mass spectrometry. For validation, parameters such as linearity, precision, accuracy, detection and quantification limits, robustness and stability were evaluated following recommendations from national and international validation guides. **Results:** Based on the recommendations of the validation guides used, the method presented precision and accuracy within the recommended limits, with a linear range of 30 µg L-1 to 1000 µg L-1. The detection limit was 5 µg L-1 while the quantification limit was adopted as the smallest calibrator in the range linear. The method showed satisfactory robustness and stability for 7 days at a temperature of -70ºC. Of the 20 samples analyzed, 7 showed cotinine in breast milk, when 5 of which were between the limit of detection and quantification and two samples above the limit of quantification with concentrations of 131.57 µg L-1 ± 3.12 and 283.66 µg L-1 ± 13.91 respectively. **Discussion/Conclusion:** The proposed and validated method was able to property identify and quantify cotinine in breast milk samples, proving the exposure of the newborn through breastfeeding. The use of this effect biomarker presented is important in assessing the risk of exposure to this substance, as it can be applied as a tool during this assessment. The lack of established cotinine limits for breast milk are difficulties currently faced. For this reason, it is understood that the presence of any concentration can be harmful to the baby. The possible relationship between dose and response is also a factor to be studied in future research. **Acknowledgments:** This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and FAPEMIG (APQ 00224-22; APQ 03243-22).

Determination of cotinine in urine samples by liquid chromatography coupled to mass spectrometry as a bioindicator of tobacco exposure

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Introduction: Direct or indirect exposure to nicotine can cause various effects on the health of the exposed individual. Respiratory problems are the main effects resulting from this exposure. Furthermore, the possible genotoxic effect related to nicotine exposure is also a reality to be faced. In research with farmers exposed to pesticide mixtures, smoking habit is an important variable that must always be evaluated, as it can cause effects similar to those resulting from exposure to pesticides. Therefore, to better assess the true agent causing changes in the effect biomarkers used in farmers' work, it is necessary to apply an exposure biomarker, which will reflect the concentration of cotinine in each participating volunteer. In this way, it will be possible to compare and investigate the existence or not of a relationship between changes in effects biomarker applied and the concentration of cotinine, the main metabolite of nicotine, in farmers' urine. **Objective:** Optimize and validate methodology able to identify and quantify cotinine in urine samples, serving as a bioindicator of exposure for future assessments in farmers exposed to pesticides. **Methods:** For the sample preparation 1 mL was placed in a falcon tube, followed by 100 µL of acetonitrile, 50 µL of sodium hydroxide and 1 mL of ethyl acetate. The mixture was vortexed for 1 minute and centrifuged at 1500g for 5 minutes. After centrifugation, the supernatant was transferred to a vial where 10 µL was injected by liquid chromatography coupled to mass spectrometry. For validation, parameters such as linearity, precision, accuracy, limit of detection, limit of quantification, residual effects and stability were evaluated, based on the regulations recommended by national (ANVISA) and international (FDA) validation guides. **Results:** A linear range from 50 µg L-1 to 1000 µg L-1 was found, with precision and accuracy within the limits recommended by the validation guides used. Furthermore, the method in question showed no residual effect and can be safely used in samples from volunteers as a tool capable of identifying and quantifying cotinine in urine samples. Regarding stability, a safe storage time was found following the guides' recommendations, in 7 days at a temperature of -70ºC. **Discussion/Conclusion:** The method proved to be an easy tool for be used in large numbers of samples, due to the simplicity of sample preparation. A pilot test with two samples collected from students at the Federal University of Alfenas - MG proved the efficiency of the method, where concentrations of 288 µg L-1 ± 12.90 and 320.96 µg L-1 ± 14.48 were found. Finally, the method was properly optimized and validated, being able to be applied to samples from volunteers, being used as a bioindicator of exposure for future research. **Acknowledgments:** This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and FAPEMIG (APQ 00224-22).

Development of a liquid chromatography-tandem mass spectrometry method for the determination of parabens and bisphenol A concentrations in human hair

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Introduction: Parabens and bisphenols are endocrine disruptors (ED) compounds widely present in consumer products. Reproductive disorders, breast cancer, obesity, hypertension, and asthma are known to be linked to the exposure to these compounds. Blood and urine are the usual matrices used in human biomonitoring studies. However, they are not adequate to evaluate long-term exposure to ED with a short elimination half-lifes, such as phenols or parabens. Therefore, hair has been proposed as an alternative testing matrix once it is less sensitive to short-term variations in exposure. **Objective:** The study aims to develop and validate an analytical method for the determination of methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), butylparaben (BuP) and bisphenol A (BPA), in hair samples using ultra-performance liquid chromatography-tandem mass spectrometry (LC- MS/MS). **Methods:** The method is based on incubation of 50 mg hair samples with acid acetic 1.5 % at 38 °C overnight, extraction with ethyl acetate. Deuterated analogs were used as internal standards. Analytes were derivatized with dansyl chloride to improve ionization efficiency at the electrospray ionization source. Chromatographic separation was performed using an Acquity UPLC BEH C18 (2.1 x 100 mm, 1.7 µm) column heald at 40 °C. The mobile phase was composed of ultra-purified water with 0.1 % formic acid (A) and methanol with 0.1 % formic acid (B), eluted in gradient mode. The total analytical run time was 5.5 min. **Results:** Intra-assay precision assays was lower than 9.09 % and inter-assay precision was lower than 8.75%. Accuracy was in the range of 100.71 to 108.58 %. Autosampler stability of derivatized extracts was demonstrated for 12 h, with maximum response differences after 12 h of 0.34, -6.23, -0.45, -1.45, and 10.37 % for MeP, EtP, PrP, BuP and BPA, respectively. The limits of quantitation were 25, 2.5, 5, 2.5, and 2 ng g-1 for MeP, EtP, PrP, BuP, and BPA, respectively. Extraction yields were higher than 48.38 %. The assay will be applied in an ongoing human biomonitoring study. **Discussion/Conclusion:** A sensitive assay for the simultaneous determination of parabens and BPA in human hair was developed and validated. Preliminary evaluations characterized the capability of the assay to measure concentrations of the target compounds in a Brazilian cohort. Hair levels of parabens and BPA will be related with lifestyle data of the participants of the cohort and with urinary biomarker concentrations. **Acknowledgments:** To the volunteers of the study and to the National Council of Scientific and Technological Development of Brazil (CNPq).

Hematological and biochemical biomarkers in exposure of Prochilodus lineatus to the fungicide carbendazim

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The use of pesticides has stood out as one of the main factors resulting in relevant environmental impacts, since these compounds, when used, can end up contaminating the soil, water and air. Among the different types of pesticides that are sold in Brazil, fungicides are widely used to prevent losses due to fungal contamination, and carbendazim (CBZ) stands out. However, this compound is considered highly persistent in the environment and very toxic to aquatic organisms and bees. Studies demonstrate its presence in different water bodies and even adverse effects on animals of different species. The objective of this work was to evaluate the effects of Derosal 500® (CBZ) using biomarkers on fish of the species Prochilodus lineatus, at concentrations of 5, 50 and 500 µg L-1, for 24 h. After the exposure period, the animals were anesthetized and euthanized and blood samples were taken for hematological analyzes and comet assay, as well as liver, gills, brain and muscle were taken for biochemical analyses. Aquarium water samples were collected for chromatographic analysis using LC-MS/MS. It was observed that CBZ induced a decrease in hematocrit, promoted hyperglycemia, hypernatremia, increased hepatic glutathione (GSH) and brain acetylcholinesterase (AChE) activity. Since fish exposed to contaminants generally present an increase in hematocrit as a response to a stressful situation, our results observed a decrease in hematocrit, since CBZ can result in hemolysis, which is consistent with results already described in the literature in Clarias gariepinus, in Oreochomis niloticus and rats. Hypernatremia may be related to dysfunctions in the cell membrane, with loss of water and interference with sodium uptake by red blood cells, which would result in an increase in plasma levels. Exposure to CBZ at the highest concentration tested resulted in hyperglycemia, which may also suggest a response to stress induced by the fungicide, similar to that described in work on African catfish exposed to CBZ. This work did not observe changes in the activity of carbonic anhydrase in gills during the period tested, which suggests that exposure to CBZ within 24 hours did not promote changes in respiration and acid-base balance. Regarding AChE, it is known that several pesticides act by promoting its inhibition, with hyperstimulation of cholinergic postsynaptic receptors. However, this work observed an increase in AChE activity in all concentrations for the brain, but without muscle enzymatic changes. Several studies suggest that AChE appears to be involved in the induction of apoptosis and cell proliferation, through effects on gene expression, and that ACh exerts anti-inflammatory effects, through the suppression of pro- inflammatory cytokines. In this way, we suggest that CBZ acts by stimulating inflammatory cytokines and consequently, apoptosis, in a similar way to what was observed in studies with Danio rerio. The increase in GSH in CBZ50 and CBZ500 suggests a response to combat oxidative stress caused by the contaminant, so that hepatic lipid peroxidation was not observed. Furthermore, no DNA damage was observed, which may be related to the experimental period, since studies with longer exposure times observed the induction of DNA damage in Daphnia magna and Eisenia foetida. Likewise, micronucleus formation was not observed within 24 hours, although this has already been described in other studies with longer periods of exposure to CBZ. Our results demonstrate that P. lineatus is sensitive to exposure to CBZ even in a short period of exposure and at low concentrations, which can result in biological damage to exposed animals, especially in longer periods of exposure.

03
DESREGULADORES ENDÓCRINOS

Assessing the performance of in silico platforms in predicting endocrine-disrupting chemical interactions with estrogen receptors

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Endocrine-disrupting chemicals (EDCs) are considered a serious health threat, contributing to major diseases. Classical targets of EDCs include nuclear receptors such as estrogen receptors (ER), androgen receptors (AR), thyroid receptors (TR), among others. The interaction with estrogen receptors (ERs) can affect the transcription of estrogen-controlled genes, leading to the induction or inhibition of cellular processes. This includes those processes necessary for cell proliferation, normal fetal development, and reproductive function. Perturbation of normal estrogenic systems may have the potential to trigger adverse effects on normal development (ontogenesis), reproductive health, and the integrity of the reproductive system. This study aimed to evaluate the performance of three in silico platforms against 38 chemical substances from the OECD proficiency lists 493 and 455 that assess the interaction with estrogen receptors. Three platforms with free access (Endocrine Disruptome [ED], Vega HUB [VH], and Danish QSAR [DS]) were utilized, and the results were assessed based on correct predictions, false positives, false negatives, out-of- domain/no predictions made, and total predictions. Sensitivity, defined as the ability to predict truly positive/active substances, and specificity, defined as the ability to predict truly negative/inactive substances, were also evaluated. OECD 455 has two proficiency lists that differentiate between agonist and antagonist substances. However, only ED provides analysis for both types of receptors. DS has models for alpha and other non-specific receptors, while VH only has non-specific models. Three sets of substances were evaluated: (i) list of proficiency substances for agonist assay (OECD 455), (ii) list of proficiency substances for antagonist assay (OECD 455) and (ii) list of proficiency substances for the competitive binding assays. Taking into account the overall number of predictions, both tools demonstrated strong performance, accurately assessing at least 90% of substances across the three analyzed datasets. The set (ii) had the lowest specificity (ED 42.8%, VH 28.6%, and DS 28.6%). ED was the software that performed the best in this set, likely because it is the only tool that includes the antagonist receptor. DS was regarded as the top- performing tool, achieving a sensitivity of at least 90% in the three evaluated sets and accurately predicting truly active substances. The results obtained demonstrated good performance for the three tools evaluated. However, it is necessary to carefully assess how each model was constructed and what it is specifically evaluating. Computational methods are widely used in toxicology and represent our future. Their applications are limitless, ranging from the initial screening of molecules in the absence of sufficient toxicity data to regulatory purposes in specific circumstances. Due to the animal testing ban for cosmetic purposes, some complex endpoints still lack in vitro methods capable of assessing the full complexity observed in in vivo models. Therefore, in silico approaches are extremely important to complement data from the literature or obtained through in vitro tests.

Investigating the endocrine-disrupting effects of paracetamol and dehp on reproductive development: a focus on the endocannabinoid system

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Endocrine-disrupting chemicals (EDCs) pose a significant threat to reproductive health, with substances like paracetamol and di-2-ethylhexyl phthalate (DEHP) being implicated in the alteration of sexual development. Both substances can adversely impact testosterone and insulin-like 3 synthesis and action, leading to disorders such as cryptorchidism, hypospadias, germ cell testicular cancer, and low sperm count in adulthood. Furthermore, females are not exempt, experiencing a reduction in the ovarian follicle pool, and an increased risk of polycystic ovarian syndrome and endometriosis due to exposure to EDCs. Despite these associations, the precise mechanisms underlying the endocrine-disrupting effects of paracetamol and DEHP remain unclear. In this study, we propose a novel hypothesis suggesting an interaction between these EDCs and the endocannabinoid system, given its relevance to physiological actions in the reproductive system. Previous evidence indicates that a metabolite of paracetamol, AM404, agonizes CB1 and CB2 receptors and binds to the active site of the FAAH enzyme. Docking studies further reveal DEHP's ability to bind to the CB1 receptor, implicating the endocannabinoid system in the potential mechanism of endocrine disruption. The primary objective of our investigation was to assess whether paracetamol and DEHP, administered during the critical male programming window (gestational days 15 to 18 in Wistar rats), could reduce testosterone production and alter the expression of key steroidogenic and endocannabinoid genes. Two experiments were conducted: the first involved an in vivo study exposing pregnant dams to 50 and 250 mg.kg.day-1 of paracetamol and 750 mg.kg.day-1 of DEHP. The second utilized an in vitro approach, incubating during 3 hours at 37ºC in M199 media naïve fetal testes with paracetamol 10 µM, MEHP 100 µM, rimonabant 10 µM (CB1 antagonist), AM404 10 µM, rimonabant 10 µM + paracetamol 10 µM, and rimonabant 10 µM + AM404 10 µM. Results from our experiments revealed that paracetamol did not significantly impact testosterone levels or alter the expression of cannabinoid and steroidogenic genes. Conversely, DEHP exhibited a noteworthy reduction in testosterone levels and steroidogenic gene expression in the in vivo experiment. Interestingly, DEHP also increased the expression of cannabinoid receptor type 2 (Cnr2) and anandamide-synthesizing gene Napepld in the developing testis, highlighting a potential mechanism for its endocrine-disrupting effects. Interestingly, MEHP increased the in vitro testosterone production that could be a reflex of a compensatory response to MEHP action. Contrary to studies conducted with mice, cell cultures, and epidemiological research that reported testosterone and insulin-like 3 reduction with paracetamol exposure, our findings with Wistar rats did not replicate these outcomes. This discrepancy suggests that the Wistar rat may not be the ideal animal model for investigating paracetamol toxicity in the developing testis. In conclusion, our study provides novel insights into the endocrine-disrupting effects of DEHP, demonstrating for the first time in Wistar rats that DEHP can induce an alteration in relevant endocannabinoid genes in the developing testis. This mechanism could potentially explain, at least in part, the observed reduction in androgen synthesis. Further research is warranted to unravel the intricate interactions between EDCs, the endocannabinoid system, and reproductive development, fostering a deeper understanding of their implications for human health. We thank CAPES for providing the doctorate scholarship to conduct this research.

Maternal exposure to plastificants alters prostate proteostasis of descendants and modulates proteins associated to prostate cancer: integrative analysis to the human secretome

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Global plastic production increased from 204 to 390.7 million tons between 2002 and 2021. Phthalates are compounds used as plasticizers to increase the flexibility of plastic products and can be found in many consumer products such as toys, blood bags, automotive products and personal care products. The non-covalent bond to the polymers allows the easy release of phthalates in drinking water (0.16-170 μg/dm^3), air samples (0.4-65 ng/m^3) and dust samples (2.38- 4.1 g/kg). The prostate is an essential gland for reproductive success and its development is regulated by androgens and growth factors during the fetal and perinatal period, making the gestational and lactational periods important windows of susceptibility to changes in prostate development. This study was performed to evaluate whether exposure to a mixture of phthalates during gestational and lactational periods could modulate the rat prostate proteome and to compare the results with the human secretome, in normal and tumor samples, aiming to obtain possible biomarkers for toxicity and oncogenesis. For this, pregnant rats (SD) were randomly divided into three experimental groups (n=10/group): C: control, T1: 20μg/kg/day and T2: 200mg/kg/day. The composition of the mixture was based on the proportion of phthalates found in pregnant women's urine. Treatment was carried out from gestational day 10 (GD10) to postnatal day 21 (PND21). On PND22 and PND120, the rats were euthanized and the ventral prostate (VP) was collected to obtain the proteomic profile. The differentially abundant proteins were compared with data available in "The Human Protein Atlas" for the human secretome. Common proteins were crossed with differential expression data obtained from the Xena Browser platform. Enrichment analyzes and construction of the protein-protein interaction network were performed on the KOBAS 3.0 and STRING platforms. The results showed that early exposure to phthalates slightly upregulated proteins present in the human secretome, however the treatment downregulated 31 proteins in common to the human secretome at PND22 in both treated groups. At PND120, 7 of these proteins remained downregulated in both treated groups and the others, depending on the mixture dose, were upregulated, downregulated or similar to the control. The main pathways enriched by these proteins are related to post-translational modifications, IGF regulation by IGFBPs, protein processing in the endoplasmic reticulum, stress response, prostate cancer, chemical carcinogenesis and IL-12 signaling. Differential expression analysis demonstrated that 17 targets modulated by the treatments (T1 and T2) presented altered gene expression in patients with prostate cancer. Additionally, the analysis showed changes in crucial proteins in the processing of proteins in the rough endoplasmic reticulum (chaperones HSP90AB1 and HSP90B1), which may be associated with the decrease in protein abundance as a result of treatment, observed in this study. Furthermore, many of the proteins that were downregulated by treatment are important for the early development of the prostate and their alteration in adulthood maintains an altered tissue microenvironment, can increase susceptibility to prostate diseases. **Support:** (FAPESP: 2022/11424-5; 2019/13823-1; 2022/12304-3) e (CNPq: 312028/2022-9).

Molecular docking as an in silico tool to assess estrogen receptor binding by OECD 493

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The estrogen receptor (ER) has also been considered a target protein for a variety of synthetic chemicals known as endocrine disruptors (ED). These substances are suspected to disrupt the endocrine system, induce undesirable effects on the reproductive system, and interfere with fetal development processes in both animals and humans. Due to the ban on animal testing for cosmetic purposes, in vitro and cell-based assays for detecting estrogenic EDs are valuable. These targeted tests can often be performed in a high-throughput manner. However, in some cases, the results may not be sufficient, requiring the use of alternative approaches such as in silico strategies to predict the endpoint. This study aimed to assess the sensitivity and specificity of molecular docking predictions for 14 proficiency chemical substances listed in the OECD 493. This test guideline describes the methodology for human recombinant in vitro assays designed to detect substances with estrogen receptor binding affinity (hrER binding assays). Molecular docking was performed using AutoDock 4.2 software, polar hydrogens and Kollman charges were added to the protein structure (PDB code: 1a52) and the number of torsions in the ligand was established. The simulations were performed with the Genetic Algorithm, to identify the best binding modes and calculate the affinity of the protein-ligand complex. Sensitivity, defined as the ability to predict truly positive/active substances, and specificity, defined as the ability to predict truly negative/inactive substances. Substances were considered positive, indicating a high probability of binding, when the binding affinity was ≥ -7.5. Substances that obtained values < -7.5 were classified as negative. The molecular docking achieved a sensitivity of 80%, meaning 8 correct results out of the 10 substances classified as positive in OECD 493. The specificity was 75%, with 3 correct results out of the 4 substances classified as negative. There is increasing scientific concern about the nature and safety of ingredients used by the cosmetics industry, particularly regarding their potential endocrine-disrupting effects. In silico approaches can be more effectively utilized in a hazard assessment context to pre- screen potential endocrine disruptors, the predictions can better guide in vitro studies, facilitating more in-depth data analysis. The obtained results were promising; however, new sets of substances need to be evaluated to more definitively establish the robustness of the model.

Trophic exposure to phthalates DBP and DiPeP causes endocrine disruption in males and females of the catfish *Rhamdia quelen*

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Introduction: Phthalates are utilized in the formulation of products for human use and can be part of the polymeric structure of some plastics, being non-covalently linked to act as softeners or plasticizers. For this reason, they can be dispersed in the environment at different moments in the production chain. Di-n-butyl phthalate (DBP) and diisopentyl phthalate (DiPeP) are phthalates with a high potential for endocrine disruption. **Objectives:** This study aimed to evaluate parameters of endocrine disruption to trophically exposed specimens of the neotropical fish Rhamdia quelen to DBP and DiPeP. Material and **Methods:** Males and females of Rhamdia quelen were exposed to DBP and DiPeP at the doses of 5, 25, and 125 ng phthalate/g fish, After 30 days of exposure, the animals were anesthetized with benzocaine diluted in water at a concentration of 10 mg/L. Blood was collected by puncture of the caudal vein. Then, fish were weighed and measured before being euthanized by spinal cord section. Plasma was used to evaluate estradiol and testosterone concentrations. Serotonin, dopamine, and their metabolites were evaluated in the brain. Vitellogenin expression was analyzed in the liver and the antioxidant system was analyzed in the gonads. The Ethics Committee approved this experiment on the Use of Animals at the Federal University of Paraná under certificate number 1180. **Results:** DBP caused alterations in the serotoninergic system of males and females of R. quelen and increased testosterone levels in females. DiPeP was able to alter the dopaminergic system in females, in addition to reducing plasma estradiol levels and hepatic vitellogenin expression and altering the antioxidant system in gonads. **Conclusion:** Taken together, our data suggest that, DBP and DiPeP may have different response patterns in females, the first being androgenic and the second anti-estrogenic. These findings provide additional evidence regarding the molecular events involving DBP and DiPeP endocrine disruption potential in juvenile specimens of Rhamdia quelen. **Acknowledgments:** The authors would like to thank the CNPq for financing the project (Edital Universal - CNPq number 421809 / 2018-3), for the studentship (CNPq number 141339 / 2016-0) to L. F. Oya-Silva, and for granting a junior postdoctoral (PDJ) to I.C. Guiloski (process 150098 / 2018-9). The authors would like to thank the Laboratory of Technology for the Reproduction of Cultivable Aquatic Animals (LATRAAC) at the State University of Western Paraná (UNIOESTE) and Prof. Dr. Robie Allan Bombardelli for providing the specimens of Rhamdia quelen used in this study.

04
ECOTOXICOLOGIA

Behavioral effects of antibiotic mixtures (sulfonamides and fluoroquinolones) on zebrafish larvae

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The development of low-cost pharmaceuticals with high efficacy for bacterial infections was an excellent advance for target species, on the other hand, antibiotics are now a threat to the aquatic ecosystem, being detected in surface freshwater from several countries. Antibiotics usually coexist in water matrices, and the toxic effects on aquatic organisms can be aggravated by this combination. Studies have shown that sulfonamides and fluoroquinolones can provide potential ecological stress for different invertebrates, testing classical endpoints at isolated antibiotic exposures. However, the effects of antibiotic mixtures on the behavior of vertebrate species remain limited. This study focused on analyzing the effects of mixtures containing five sulfonamides (sulfamethoxazole, sulfadiazine, sulfamethazine, sulfamerazine, sulfadimethoxine) and five fluoroquinolones (ciprofloxacin, norfloxacin, pefloxacin, ofloxacin, enrofloxacin) each one at environmentally relevant concentrations (0.1, 1, and 10 µg/L) on motor function of zebrafish larvae. Adult zebrafish were maintained in aquariums under standard conditions. Embryos were collected and kept in a POL-EKO climatic chamber (28.5 ºC, 12h:12h light:dark photoperiod) for 7 days. Then, 7 dpf larvae were randomly selected and placed in wells containing 1 mL of test solution (1 larva/mL) in 48-well plates, covered with aluminum foil, and kept in the dark for 24 h in a climatic chamber (28.5 ºC). Four typical behavioral types in zebrafish larvae were evaluated: 1) escape response provoked by a vibrating acoustic stimulus, known as startle response; 3) habituation to repetitive acoustic stimulation addressing non-associative learning; and 3) basal locomotor activity (BLA) in the dark; and 4) response to visual stimuli (visual-motor response - VMR). Behavior assays were performed using a high-performance system (DanioVision and Ethovision XT software version 9). After 24h of exposure, considering sulfonamide mixtures, 8 dpf zebrafish larvae presented a significant decrease in distance moved during the startle assay at the lowest (0.1 µg/L) and highest (10 µg/L) concentration group tested ($p < 0,01$ and $p < 0,001$ respectively, Dunn post hoc test). In addition, a significant increase in the distance moved during visual-motor assay for all concentration groups was pointed out for sulfonamides ($p < 0.001$, $p < 0.05$, $p < 0.05$, Dunn post hoc test, for 0.1, 1, and 10 µg/L treatments, respectively). Moreover, mixtures of low and intermediate doses of fluoroquinolone mixtures caused a significant decrease in the habituation response ($p < 0,01$, Dunn post hoc test, for 0.1 and 1 µg/L treatments). On the other hand, the basal locomotor activity was not significantly affected by either sulfonamide or fluoroquinolone mixtures. Basal locomotor activity, the startle response, habituation to repetitive acoustic stimulation, and the response to visual stimuli are important behavioral parameters that may indicate the risk of survival of aquatic species, since these responses may be associated with mechanisms of food absorption, predation, and energy costs. Nevertheless, it is evident that low concentrations of antibiotic mixtures from both classes (0.1 µg L-1) can cause behavioral stress in the animal model under study, considering the co-occurrence of these substances in the freshwater environment. We are grateful to São Paulo Research Foundation (FAPESP) [grant numbers 2020/11042-0; 2020/15087-8; 2022/12048-7]; EU and the State Research Agency (AEI) [grant number PCI2022-121990] in the framework of the collaborative international consortium PRESAGE financed under the ERA-NET AquaticPollutants Joint Transnational Call [grant number 869178].

Biochemical and Genotoxic Effects on *Oreochromis niloticus* (Linnaeus, 1758) exposed to concentrations of polyethylene (PE) and polystyrene (PS) microplastics

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Introduction: The escalating issue of plastic proliferation, driven by its durability and low cost, leads to environmental disasters such as marine pollution and soil contamination. Microplastics (MPs), plastic fragments under 5.0 mm, sourced from various origins, pose a serious threat to aquatic ecosystem health, inducing adverse effects in fish. This study focuses on the effects of polystyrene (PS) and polyethylene (PE) on Oreochromis niloticus fish, at concentrations (200 and 800 µg/L), analyzing oxidative stress and genotoxic damage biomarkers. The research supports the hypothesis that MPs trigger antioxidant responses and cause genotoxic damage, contributing to understanding the risks associated with plastic pollution in marine life. **Objective:** To investigate the impacts of microplastics (MPs), specifically polystyrene (PS) and polyethylene (PE), on O. niloticus fish. Using biomarkers and genotoxic tests, we aim to deepen the understanding of oxidative stress effects, supporting the hypothesis that MPs can trigger antioxidant responses and cause genotoxic damage at environmentally relevant concentrations, contributing to the comprehension of risks associated with plastic pollution in marine life. Materials and **Methods:** O. niloticus specimens were obtained from a fish farm in Espírito Santo, acclimatized for two months, and fed commercial feed. Fish were kept under a 12-hour light cycle, with water changed every two days. Water physicochemical parameters were regularly monitored. Polyethylene (PE) and polystyrene (PS) microspheres were acquired from Cospheric LLC, and biochemical assay reagents were from Sigma-Aldrich® or Merck-Millipore. The experiment, approved by CEUA-UVV, evaluated oxidative stress and genotoxic damage in O. niloticus fingerlings after exposure to PE and PS at concentrations of 200 and 800 µg/L. Fish were individually housed in aquariums, subjected to different treatments, including control and dispersing agents. After 48 hours of exposure, fish were anesthetized, underwent biometric evaluation, and blood was collected for the micronucleus test. Livers were analyzed for oxidative stress biomarkers (CAT and GST). Statistical analysis was performed using Sigma Plot 12.0, including ANOVA and Kruskal-Wallis test ($p<0.05$). **Results:** Biochemical analysis results show no significant differences in CAT enzyme activity between treatment groups ($p > 0.05$), as depicted in Figure 1. Regarding GST, statistically significant variations were observed, especially when comparing the control group with PE (200 and 800 µg/L) ($p < 0.05$), as illustrated in Figure 2. In the genotoxic analysis, micronucleus test results indicate no statistically significant differences in micronucleus frequency between treatment groups ($p > 0.05$), as shown in Figure 3. No differences were identified when comparing means of controls and means of groups treated with MPs ($p > 0.05$). **Discussion:** The antioxidant system (AOS) responds to oxidative stress caused by exposure to microplastics (MPs), influenced by factors such as particle , exposure time, and polymer composition. Although CAT enzyme activity showed no significant differences, GST exhibited a significant inhibition trend in PE treatments (200 and 800 µg/L), indicating sensitivity of this biomarker to MP exposure. Exposure time is a factor to be considered, as evidenced by previous studies on its influence on biomarker results. The micronucleus test (MN) revealed no significant differences in micronucleus frequency after fish exposure to different MP concentrations. However, scientific studies associate MP exposure with an increased micronucleus frequency, indicating potential genotoxic damage. MP transfer through the food chain negatively impacts fish DNA, suggesting adverse effects. The micronucleus test in fish is effective in evaluating toxicological risks, emphasizing the importance of a comprehensive approach in assessing MP impacts across different trophic levels and biological systems.

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Development and validation of a LC-MS/MS method for PFAS analysis in a marine tropical amphipod – a promising biomonitoring tool

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Background/Introduction: Per- and polyfluoroalkyl substances (PFAS) are synthetic compounds used as surfactants since the early 1950s in cookware, cosmetics, military equipment, and fabrics. Many of these compounds present long half-life, classified as bioaccumulators. Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are the most prevalent PFAS, known to be toxic for various organisms, including human beings. PFAS were already detected in distinct environments, even in polar regions. Despite the existing studies related to that compounds, environmental monitoring data are still few. Parhyale hawaiensis is an epibenthic marine amphipod that has circumtropical distribution, presents sensitivity to pollutants and has been highlighted as an experimental model in several areas. The hemolymph of these organisms was already used for the determination of other pollutants and has gained prominence for ecotoxicology as an alternative matrix, and it's advantageous because of the low volume necessary for analysis. **Objective:** To develop and validate an analytical method for quantification of PFOS, its isomer Br-PFOS and PFOA by LC-MS/MS in P. hawaiensis hemolymph. **Methods:** An analytical method for PFAS analysis was developed and validated on the LCMS8060 (Shimadzu®, Kyoto Japan) based on INMETRO and EPA guidelines with adaptations. Chromatographic separation was performed with an Acquity UPLC HSS T3 (1.8 µm, 2.1 mm x 50 mm, Waters, Milford, MA, EUA) column and the mass spectrometer was equipped with an electrospray ionization source, operating in negative mode. To prevent possible interferents presents on the system, a delay column (5 µm, 50 mm x 2,1 mm, Restek, Bellefonte, PA, EUA) was installed after the mixer and before the autosampler. The total run time of analysis was 10 minutes and the injection volume was 10 µL. A calibration curve of 0.1-100 ng/mL (LOD = 0.05 ng/mL) and quality controls (QC) at 0.3 (LQC), 50 ng/mL (MQC) and 80 ng/mL (HQC) were determined. An internal standard mix solution (IS) of MPFOS and MPFOA at 50 ng/mL was used. Calibrators and QCs were prepared with a dilute-and shoot protocol, which consisted on the dilution of 20 µL of PFAS' solution in 160 µL of reconstituted seawater (final salinity of 3,3 g/L) and the addition of 20 µL of IS. After the method validation and the preliminary exposure experiments, adult organisms (4, ≤ 8 months-old) were exposed to different PFASs concentrations (1, 10 and 100 ng/mL, in triplicate) in a 1:1 sex ratio for 96h. Then, the hemolymph of these organisms was collected with a thin glass needle and a pool with 4 hemolymphs was made in 20 µL of reconstituted seawater. After that, each pool was diluted with 160 µL of ultrapure water to reduce salt concentration, and 20 µL of IS was added for analysis. **Results:** For method validation, parameters as within and between-run and bias were evaluated for the QCs in a period of 5 days and they did not varied more than 4.8%, 19% and 15.1%, respectively, meeting the maximum variation established (up to 30%). For exposure experiments, following the concentrations of 1, 10 and 100 ng/mL, the average concentrations of PFOS in the hemolymph's pool were 6.3, 374.2 and 2576 ng/mL; for Br- PFOS were 7.1, 146.6 and 316.4 ng/mL; and for PFOA, 18.6, 484.5 and 1879.4 ng/mL. That results are an indicative of bioaccumulation potential of PFAS on P. hawaiensis. **Discussion/Conclusion:** Hemolymph analysis of PFAS could be successfully performed and the developed methodology seems promising as a biomonitoring tool. For the next steps, more experiments will be performed using different exposure periods to understand PFAS toxicokinetics in P. hawaiensis. **Acknowledgements:** This project is supported by CNPq (grant number: 140266/2022-4). Materials and reagents were also financed by CAPES and FAPESP.

Differences in pesticide sensitivity between female and male of solitary bees

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Bee diversity protection against pesticide effects is performed using ecotoxicological tests in female social bees from *Apis mellifera* species. However, a more protective approach would require the inclusion of more species and the sex difference sensitivity in pesticide risk assessment, especially in solitary bees that has generally a male-biased population. Nonetheless, the sex sensitivity difference is not known, and predominantly females are included in toxicological studies. Therefore, aiming to compare pesticide sex sensitivity across solitary bee species, we calculated the sex sensitivity ratio using toxicological data of pesticides available in the literature. A literature review was conducted using as keywords 'LD50' AND 'bee' to collect medium lethal doses of adult males and females exposed to pesticides under laboratory conditions. We used the databases Web of Science, Scopus, Scielo, and the Ecotox database from EPA (United States Environmental Protection Agency). Then, a sex sensitivity ratio (SSR) was calculated by dividing the LD50 value of females by the LD50 value of males. To isolate other variables that can influence the toxicity, in each SSR calculated we used only the LD50 values from females and males resulting from the toxicological test performed with the same bee population, and following the identical parameters: insect age, exposure procedures, exposure time, and observation time. The literature review resulted in fourteen toxicological tests, where 75% of the ecotoxicological studies on a bee dose basis (µg/bee) showed a higher sensitivity in males than females. However, when taking body weight into account (µg/g of bee), females tend to be more sensitive to pesticides than males in most cases. The use of body weight to normalize species differences is still uncertain in bee toxicology, and the current pesticide regulation is based on the medium lethal dose per bee. Therefore, our results showed that males can have a higher sensitivity to pesticides than females depending on the species, pesticide type, and exposure route. In these cases, the use of only female LD50 for solitary bees could underestimate pesticide risk. Thus, the estimative of pesticide effects on the bee population level needs to include the differences among the sexes to guarantee a conservative scenario. **Acknowledgments:** FAPESP 2019/27863-5 and FAPESP 2022/07920-7.

Ecotoxicological characterization of dairy wastewater from a small dairy industry in Minas Gerais, Brazil

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Dairy products are increasingly consumed worldwide as population grows. However, dairy products manufacture represents a high-risk to the environment. Among a series of concerns, one of the main environmental issues is the volume of wastewater discharged to environmental waters. This research proposes the physicochemical and ecotoxicological characterization of dairy wastewater generated through cheese and yogurt production processes, based on a case study of a small dairy industry from Esmeraldas/MG. The study sheds light on the broader implications of environmental impacts related to small dairy industries, particularly in Minas Gerais. Environmental concerns associated with dairy wastewater discharges are especially notable in smaller facilities which are submitted to less restricted regulations and occur in high numbers in Minas Gerais adding up high volumes of wastewater discharge. These concerns are addressed in this study by performing physicochemical and ecotoxicological characterization of the dairy wastewater compared to values stablished in scientific literature and evaluating its compliance with national and state levels regulations associated with wastewater discharge. Dairy wastewater sampling employed both composite and simple schemes. Physicochemical characterization included parameters such as Alkalinity, Chemical Oxygen Demand (COD), Hardness, Dissolved Oxygen, pH, total and dissolved solids. Following characterization, samples were preserved prior to ecotoxicological assays according to ABNT NBR 15469/2021. Ecotoxicological characterization encompassed acute and chronic tests using the following assays: Raphidocelis subcapitata (algae; ABNT 12648/2023), Daphnia similis (crustracean, ABNT 12713/2022), Artemia salina (crustracean, ABNT 16530/2021), Danio rerio larvae and adult (fish, ABNT 15088/2022 & ABNT 15499/2022), and Vibrio fischeri (bacteria, ABNT 15411:3/2021). Regarding acute effects, dairy wastewater was toxic to all evaluated organisms in the following order: Daphinia similis (FT = 32), Danio rerio (embryo) (FT = <16), Artemia salina (FT = 8), Danio rerio (adult) (FT = 2). Acute toxicity testes with V. fisheri were inconclusive probably due to high turbidity of wastewater samples. Meanwhile, for chronic toxicity results were as follows: Raphidocelis subcapitata (FT = 32) > Danio rerio (FT = 16). Results indicate the importance of preventing acute or chronic ecotoxicity in freshwater bodies and empha the need to develop knowledge about dairy wastewater toxicity and for a more specific approach on national and state regulations concerning ecotoxicity of this matrix. These assays offer insights related to test organism sensitivity to dairy wastewater providing more knowledge on potential ecological risks and impacts. Results revealed that Raphidocellis subcapitata is the best test organism to evaluate chronic ecotoxicity and Daphnia similis for acute ecotoxicity of dairy wastewater. **Acknowledgments:** The authors would like to thank FAPEMIG for funding this research (APQXX) and the dairy industry for providing effluent samples.

Ecotoxicological effect of diclofenac sodium on microalgae *Tetraselmis sp.*

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Diclofenac (DCF) is an anti-inflammatory drug widely used in human and veterinary medicine, and its presence in aquatic environments varies in concentrations from ng/L to µg/L, classifying it as an emerging contaminant, presenting risks to aquatic organisms. The microalgae of the genus *Tetraselmis sp*. It is used as a test organism in bioassays due to its sensitivity to xenobiotics, its biochemical composition, as well as its ease of cultivation. The objective of this work is to evaluate the toxic effect of Diclofenac sodium on microalgae of the genus *Tetraselmis sp.*, through cell density. The study was carried out at the Ecotoxicology Laboratory, at the Federal University of Maranhão (UFMA). To carry out the cultivation, maintenance and growth/inhibition tests of marine microalgae, the ABNT NBR 16181 standard of 2021 was followed. Initially, a microalgae growth curve was carried out (duration of up to 30 days), which was obtained after inoculating the cells in nutrient medium (Guillard f/2), under controlled conditions. Daily, a 1 mL aliquot was collected to count cell density using optical microscopy. To carry out the tests with the DCF compound, sensitivity tests were carried out using the reference substance Sodium Dodecyl Sulfate (DSS) under the same conditions as the growth curve. The tests with DCF had five concentrations (1, 2, 3, 4, 5 mg/L), a control with the culture medium and a control with solvent (culture medium with addition of 0.01% DMSO (dimethyl sulfoxide). The experiments were carried out in triplicate, with an initial microalgae concentration of 10^4 cells/mL, the same as that used in the sensitivity test. The toxicity of DSS and DCF were expressed as EC_{50} (72h) and EC_{50} (96h), respectively. These data were subjected to normality and homogeneity analysis. After 32 days, the results of the growth curve of the marine microalgae *Tetraselmis sp.* were obtained, acquired in four growth phases: adaptation phase; exponential phase (beginning between days 3 and 4); reduction phase and the stationary phase (starting on day 9 to 24), however, high peaks were observed on days 11 and 15 reaching maximum cell density with 1.27 x10^6 cells/mL, and there was no decline phase (death) . For the *Tetraselmis sp*. control chart, starting from sensitivity tests, a total of 9 tests were carried out, all of which remained within the upper and lower limits (± 2s) with an overall average of 6.22 mg/L , with no outliers. In all tests, we observed significant differences between the concentrations tested, in relation to the control, except for the concentration of 5.5 mg/L. The coefficient of variation (CV) was 19.96%. When analyzing the inhibition rates in the tests, it was observed that test 1 and test 2 at concentrations of 3, 4 and 5 mg/L showed similar inhibition rates, reaching an inhibition of up to 79%, with EC_{50} values (96h) of 0.716 mg/L in test 1 and 3.06 mg/L in test 2. However, test 3 obtained an inhibition of up to 30% at a concentration of 3 mg/L, which prevented the determination of the EC_{50} (96h) in this case. The results with DSS as a reference substance were significant and contributed to the reliability of bioassays with DCF, while in tests with DCF on *Tetraselmis sp.*, there was variation in sensitivity at different concentrations of the compound, inhibition rates between assays highlight the complexity of the interaction between the species and DCF, suggesting future research, with more in-depth studies to understand the mechanisms underlying the response of microalgae to diclofenac and evaluate the potential impacts on the health of these organisms.

Effects of the semiconductor $CuWO_4$ on the green microalgae *Raphidocelis subcapitata*

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Interest in semiconductor materials has been growing due to their applications in many areas, such as water decontamination, medicine, agriculture, cosmetics, and food industry. It is of great importance to assess the toxicity of these emerging contaminants on microalgae, since these organisms are at the base of the food webs, and disturbances to this trophic level may affect the aquatic ecosystems. This study aimed to evaluate the effects of the semiconductor $CuWO_4$ on the green microalgae *Raphidocelis subcapitata* since this semiconductor may, eventually, end up in aquatic environments. In the toxicity tests, we evaluated the cellular growth, physiological parameters of the photosystem II (PS II) and production of reactive oxygen species (ROS) of *R. subcapitata*, after a 96h-exposure. We analyzed them using flow cytometry and pulse-amplitude modulated (PAM) fluorometry. The initial of $CuWO_4$ was 161.5 ± 23.5 nm, measured by field emission scanning electron microscopy (FE-SEM); although it reaches up to ≈ 700 nm in the algal medium. Results show that, regarding cell growth, the preliminary nominal 96h-IC50 was 8.59 ± 0.58 mg/L $CuWO_4$. Around the 96h-IC50, the ROS levels significantly ($p < 0.05$) increased ≈ 2 times at 10 mg/L, compared to controls. According to physiological parameters, the maximum efficiency of the PSII (ΦM) significantly ($p < 0.05$) decreased ≈ 1.25 and 1.75 times at 50 and 70 mg/L, respectively, compared to control. Moreover, the efficiency of the oxygen evolving complex (OEC) was affected ($p < 0.05$) as the concentration increased, decreasing ≈ 3.3 times compared to controls. According to our findings, the ROS production probably affected the cell growth and, at higher concentrations, led to a disturbance of PSII. Our next steps will include biochemical analysis of the algae, and measurement of the dissolved $CuWO_4$ concentrations to better understand how this semiconductor affected *R. subcapitata*.

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Evaluation of metal levels in sediments in mangroves in the State of Pará, Amazon, Brazil

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Introduction: The coastal zone of Pará has an extensive area of mangroves, with a highlight being approximately 180 km2 of mangroves located in the city of Bragança, northeast of the state. These tropical mangroves are characterized by muddy and saline sediment, flooded daily by the tide, which is of utmost ecological and economic importance for the region. Despite its recognized importance, it is an ecosystem threatened by disorderly urban expansion and the asymmetric relationship between mangrove flows. Such factors can contribute to the contamination of the ecosystem by various contaminants, including heavy metals, which are deposited directly in the sediments. Heavy metals cause environmental concern as they are toxic, biodegradable, poorly soluble in water, and easily absorbed and bioaccumulated in mangrove sediments due to this area's physical and chemical properties. **Objective:** To evaluate the distribution of heavy metal levels in sediment samples from mangroves in Bragança, state of Pará, aiming to ensure ecosystem conservation and sustainable development. **Methods:** Twenty-seven samples of sediment from the mangroves were collected and analyzed in two different periods, under conditions of low tide and ebb, at pre-defined points, in which the materials were removed with the help of dredgers, identified, and stored for subsequent treatment. The sediment samples were dried, sieved, and then processed in microwave radiation using the acid digestion procedure. Metal concentrations were measured by Inductively Coupled Plasma Optical Emission Epectroscopy (ICP-OES) model VARIAN, Vista-MPX after the dissolution of 0.2 g aliquots. Each sample aliquot was digested using a mixture of HNO3, HCl, and HF at 200 °C until vaporization and diluted with deionized water. **Results:** The metals Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were analyzed with a frequency of 100% in the samples. Average metal levels generally did not show significant differences between the sediments during the two collection periods, except Ni, which showed greater availability and average concentrations in the rainy season. In general, the average concentrations in mg/Kg-1 were 24,476.18±9,332.58 (Al), 2.03±1.57 (Cd), 8.91±3.48 (Co), 43 .43±14.23 (Cr), 6.42±2.42 (Cu), 21,861.02±7,501.83 (Fe), 216.99±130.83 (Mn), 13.81±6.79 (Ni), 20.13±12.73 (Pb) and 41.87±18.70 (Zn). **Discussion/Conclusion:** No values were observed above the limit. CONAMA Resolution No. 454/2012 was established, showing guideline values for dredged sediments. However, higher concentrations of heavy metals, mainly the toxic metals Cd, Pb, and Ni were found in samples collected in rainy weather, consolidating the influence of seasonality in the quantification of the elements. This fact can be justified by the mobilization and transport of sediments containing metals from adjacent areas to the mangroves, which can increase metal concentrations. Natural sources of heavy metals are mainly derived from atmospheric deposition, soil erosion, and surface runoff. At the same time, anthropogenic inputs include several sources, such as agricultural runoff, domestic sewage, industrial effluents, mining, and river traffic. The distribution of metals in sediments may be linked to the hydrodynamic conditions of the estuary and seawater flow associated with flood tides. It is worth mentioning that although toxic metals such as Pb, Cr, Cd, and Ni are not found at high levels, they can have harmful effects even at deficient concentrations, requiring continuous monitoring in the region. **Acknowledgments:** FAPESPA and CNPq for logistical and financial support.

Evaluation of the effects of mercuric chloride (HgCl2) and selenomethionine (SeMet) in the diet of *Oreochromis niloticus* (Nile tilapia)

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Introduction: Fish contains selenium (Se) and is considered the greatest source of this essential nutrient, which is related to complex enzymatic and metabolic functions (Filho et al., 2010). However, fish is the largest source of mercury (Hg) intake in the human diet, representing a major problem, as a highly toxic metal, with high absorption levels and low excretion rates, being accumulated throughout the food chain. (Ferreira et al., 2012). However, toxicity can be reduced by the presence and interaction with selenium (Filho et al., 2010). **Objective:** It is to evaluate the interaction of mercury chloride (HgCl2) and selenomethionine (SeMet), through the diet of *Oreochromis niloticus* (Nile tilapia) for 60 days. **Methods:** The individuals were acquired from fish farms and acclimatized for 15 days. Afterwards, aquariums were set for 15 days and 45 days: Control (without contaminants); T1 (food with SeMet); T2 (feed with HgCl2); T3 (feed with SeMet and HgCl2); T4 (feed with SeMet for 15 days and HgCl2 for 45 days); T5 (feed with HgCl2 for 15 days and SeMet for 45 days). The commercial feed was enriched with 0.2 mg/kg of HgCl2 and 5.0 mg/kg of SeMet. During the experiment, on day 15 and 45, individuals from each treatment (n=6) were anesthetized to collect the liver and brain, after which the tissues were homogenized. The liver was analyzed using CAT (Aebi, 1984) and GST (Habig et al., 1974; Habig and Jakoby, 1981) analysis and for the brain, AChE analysis was used (Ellman et al., 1961). Total protein was determined according to Bradford (1976). The results obtained were evaluated with the ANOVA variance test ($p<0.05$). **Results:** Enzyme analyzes are important as they participate in the detoxification process (Camargo and Martinez, 2006). CAT at T5 45 days showed an increase in activity when compared to TC 45 days, possibly due to the interaction of HgCl2 and SeMet in the fish organism. In GST, only the T4 45-day treatment showed a statistical difference when compared to TC 45 days. The treatments that received the supplemented diet T2, T3, T4 and T5 for 15 days showed a difference when compared with the control group in the activity of the AchE enzyme. **Discussion:** Metals induce the production of reactive oxygen species (ROS) to exceed the neutralization capacity of the body's antioxidant defenses, leading to oxidative stress. CAT participates in reducing the risk of oxidative injuries arising from ROS, through the detoxification of H2O2 acting in the body's defense (Rodrigues, 2021). CAT activity tends to reduce in contact with organometallic compounds, with a reduction of around 85% in activity due to the addition of thimerosal to the system (Nascimento, 2021). GST is mainly responsible for the detoxification of xenobiotics, metabolizing hydrophobic and electrophilic substrates, resulting in water-soluble conjugates, reducing toxicity that facilitates excretion (Van Der Oost et al., 2003; Ezemonye and Tongo, 2010; Monteiro, 2011). Acetylcholinesterase is the enzyme responsible for hydrolyzing the neurotransmitter acetylcholine in cholinergic synapses, the metal inhibits the enzyme in fish and invertebrates (Costa, 2007). HgCl2 could break disulfide bonds, essential for maintaining the structure, in proteins, causing a disturbance in their tertiary structure, resulting in inactivation of the enzyme (Frasco et al., 2007). It is concluded that goups exposed to HgCl2 and SeMet showed changes in enzyme activity, indicating a possible protective effect of selenium in relation to mercury in O. niloticus, however further analyzes are needed to establish this interaction. **Acknowledgment:** Financial support was provided by the Fundação de Amparo à Pesquisa e Inovação do Espírito Santo – FAPES and Universidade Vila Velha – UVV.

Evaluation of the toxicological potential of pesticides used in irrigated rice cultivation detected in surface waterin *Boana faber* tadpoles

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Introduction: Brazil is one of the largest rice producers in the world, with the state of Santa Catarina standing out in national production. Despite bringing good income to the economy, the large amount of pesticides that are used in this agricultural activity can cause serious damage to human and environmental health. **Objective:** Evaluate the effects caused by the main pesticides used in the cultivation of irrigated rice in the mesoregion of Northern Santa Catarina, on tadpoles of the native anuran species Boana faber. **Methods:** River water samples were collected and analyzed, revealing the occurrence of 9 pesticides, the most abundant being the herbicide bentazone (BTZ), the insecticide chlorantraniliprole (CTP) and the fungicide tebuconazol (TBZ). Based on these data, an experiment was carried out for 16 days using tadpoles of B. faber, where the animals were exposed to the three most abundant pesticides and their mixture (Mix), and for both cases the lowest and highest environmental concentration found was used. **Results:** No significant differences were observed in the development of the animals. Tadpoles exposed to BTZ showed higher levels of malondialdehyde (MDA) in the liver. In animals exposed to CTP, MDA levels were lower. Animals exposed to the TBZ fungicide showed higher glutathione S-transferase (GST) and carboxylesterase (CbE) activity, as well as higher levels of protein carbonyls and MDA. Animals exposed to Mix showed higher activity in CbE and glucose-6-phosphate dehydrogenase (G6PDH) activity, as well as higher levels of MDA. In the brain and muscle of tadpoles exposed to Mix, acetylcholinesterase (AChE) activity was higher. Histological changes were observed in groups exposed to pesticides, such as increased occurrence of melanomacrophages, inflammatory infiltrates and congestion. **Conclusion:** Our data highlight the problem of contamination of aquatic environments and reinforce the need for more vigorous measures to prevent contamination of natural environments, which may be progressively contributing to the degradation of local biodiversity.

First report on metal and metalloid contet in Crab-eating foxes (Cerdocyon thous) ruin over at the BR-040 highway in Rio de Janeiro, southeastern Brasil

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The crab-eating fox (Cerdocyon thous) is endemic to South America and widely distributed across various ecosystems, from forests to urban areas. Its remarkable adaptability to disturbed environments, such as plantations and pastures, has been noted. The species exhibits generalist and opportunistic feeding habits, consuming a variety of prey, including insects, crustaceans, small mammals, birds, reptiles, amphibians, eggs, and fruits. Despite being classified as "Least Concern" by the International Union for Conservation of Nature (IUCN), it faces potential threats, such as pathogenic infections transmitted by domestic dogs. The fox's presence on roads, scavenging for roadkill, contributes significantly to the high incidence of road accidents, making it one of the most frequently road-killed species in Brazil (Fauna CPS, 2022). Awareness campaigns and traffic improvements are recommended to mitigate these impacts (Beisiegel, 2013). Wild canids, like the crab-eating fox, are globally recognized as sentinels for environmental contamination due to their opportunistic habits and adaptation to human disturbances. However, studies on this topic are scarce in South America, with only one assessment available for Brazil, focusing solely on the species fur (Curi et al., 2012). This study aimed to determine, for the first time, the levels of metals and metalloids in the state of Rio de Janeiro, southeast Brazil. Carcass samples (lymphonde, liver, fat and nails) of crab-eating foxes were collected along the BR-040 highway. The samples were acid digested with with bi-distilled nitric acid according to USEPA method 6020B (US EPA 2014) in closed vessels, avoiding loss of volatile elements (USP, 2013). Metal and metalloid contents were then analyzed employing a NexIon 300x Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Certified reference materials (ERMBB422 - fish tissue, European Commission), and sample blanks were used to confirm method accuracy. The limits of quantification and detection (LOQ and LOD) for each determined metal and metalloid were calculated according to Brazilian National Institute of Metrology, Quality and Technology guidelines (INMETRO, 2016). Data normality assessed by the Shapiro-Wilk normality test indicated a non-Gaussian distribution for several elements. Thus, an ANOVA test was applied to verify potential differences between inter- organ metal and metalloid crab-eating fox concentrations. Values under the LOQ were not considered in any ofthe statistical analyses. The findings revealed detectable Al, Ag, As, Cd, Cr, Cu, Fe, Hg, Mn, Mo,Ni, Pb, Se, Zn, as well as many Rare Earth Elements (Ce, Dy, Er, Eu, Gd, Ho, Lu, Nd, Pr, Sm, Tm,Yb) in all organs. No significant inter-organ differences were noted for any of the determined elements in different crab-eating fox tissues. Many of these elements which were reported for the first time for the species. This study contributes to the understanding of the ecology and health of these animals in altered environments, which may lead to conservation measures and awareness initiatives to reduce roadkill and its impacts on the population of this species in the studied region. **Agradecimentos:** FAPERJ, CNPq.

Fungicides from rice cultivation (tebuconazole and azoxystrobin) alters biochemical and histological markers of hammertoad tadpoles (*Boana faber*)

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Introduction: Tebuconazole (TBZ) and azoxystrobin (AZX) are fungicides frequently used in rice cultivation. Despite protecting crops against fungal diseases, these compounds can contaminate the natural environments close to the crops, exerting negative effects on non- target organisms. **Objective:** characterize the contamination by fungicides of a river that flows in an area dominated by rice cultivation in the north of the state of Santa Catarina, SC, Brazil. **Methods:** Concentrations of TBZ and AZX found in the field were used to evaluate their negative effects on development, biochemical biomarkers and histopatology of the liver of a native tadpole species, the hammerfrog (Boana faber). Tadpoles were exposed for 16 days to the lowest (1.20 µg/L) and highest (2.60 µg/L) concentration of TBZ, lowest (0.70 µg/L) and highest (1.60 µg/L) concentration of AZX, and the mix of both fungicides at lowest and highest concentration of each found in field analyses. **Results:** Exposure to the lower TBZ concentration and both concentrations of the Mix accelerated the development of tadpoles. AZX caused an increase in the activities of glutathione S- transferase (GST), carboxylesterase (CbE) and glucose-6-phosphate dehydrogenase (G6PDH) in the liver, an increase in the levels of protein carbonyls (PC) in the liver and an increase in the activity of acetylcholinesterase (AChE) in muscle of tadpoles. TBZ, on the other hand, generated an increase in GST, G6PDH, PC and histopathological severity scores in liver and in muscle AChE activity. The effects were more intense in the groups exposed to the Mix of contaminants. No treatment altered brain AChE. **Conclusion:** The data showed that the fungicides from in rice cultivation found in natural aquatic environments around the crops pose risks to the health of the animals, compromising their metabolism and development.

GC-MS metabolomic profiling of *Eugenia uniflora* exposed to drought and ozone stress

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Introduction: In an era where pollution and climate change are altering global ecosystems, understanding plant responses to environmental stressors is crucial. This study focuses on *Eugenia uniflora* L. (*Myrtaceae*), or Pitangueira, a key urban fruit tree native to Brazil's Mata Atlântica. Despite its ecological importance in sustaining diverse fauna and its use in folk medicine, the extent of Pitangueira's resilience to urban stressors remains unclear. Our research evaluates the response of *E. uniflora* seedlings under four scenarios: control (C), drought (D), ozone exposure (O), and combined drought and ozone stress (DO), reflecting some pressing environmental issues of our era. **Objective:** Investigate the metabolic changes in *E. uniflora* seedlings when exposed to different stress conditions: drought and ozone exposure – both singly and in combination. **Methods:** Twenty seedlings were cultivated in a greenhouse equipped with filtered air and controlled irrigation. The experiment was carried out within two distinct fumigation chambers. Seedlings, aged four months, were randomly assigned to one of four treatment groups: C, D, O and DO, with five biological replicates per treatment. The control group experienced exposure to filtered ambient air and a daily watering regimen of 50 mL. Drought stress was induced by ceasing irrigation three days before starting ozone fumigation, totaling 11 days without water. The ozone stress involved exposing plants to ozone concentrations ranging from 80-120 ppb for five hours each day (10 a.m. to 3 p.m.) over six consecutive days. In the DO group, seedlings initially experienced drought stress by withholding water for three days. Subsequently, they were exposed to the same ozone regimen as the O group while maintaining the drought condition. For metabolomic profiling, 20 mg of freeze-dried and ground leaves were extracted, separated into polar and nonpolar fractions, and then derivatized. Gas Chromatography coupled with Mass Spectrometry (GC-MS) was employed for analysis. Data processing was conducted using the Global Natural Product Social Molecular Networking (GNPS) platform, and statistical analyses were executed using MetaboAnalyst 6.0. **Results:** GC-MS analysis of the polar phase led to the annotation of 19 major compounds, falling into four categories: sugars, sugar alcohols, organic acids, and organic nitrogen compounds. The predominant components were sucrose, quinic acid, and myo-inositol. Heatmap and Principal Component Analysis (PCA) indicated overlapping distributions among treatments, suggesting a minimal impact from the stress factors, whether isolated or combined. A detailed examination revealed slight elevations in sugars and sugar alcohols under stress conditions, particularly with ozone and combined treatments. Similarly, organic acid levels exhibited a marginal increase under drought and ozone stress. Nonetheless, the ANOVA test indicated no statistically significant differences among the groups. In the non-polar phase, 22 compounds were annotated, including esters, fatty acids, terpenoids, and steroids. This composition remained consistent across all treatments, with a minor and non-significant reduction in ester levels noted in the combined stress condition. Both heatmap and PCA analyses corroborated these findings, showing no clear clustering or separation. **Discussion/Conclusion:** Overall, the metabolomic profiling of *E. uniflora* in both non-polar and polar phases indicates a relatively stable metabolic profile across the tested conditions. This stability suggests that the levels of ozone and drought stress applied in our experiments may not have been severe enough to elicit substantial metabolic alterations. However, this observation does not conclusively determine the plant's sensitivity to these stressors. To gain a more comprehensive understanding, further experiments are essential. These should include analyses of gas exchange to understand photosynthetic and respiratory adaptations, the activity of antioxidant enzymes to assess the plant's oxidative stress response, and relative water content in leaves to evaluate hydration status under stress. **Acknowledgment:** Research supported by the State of São Paulo Research (FAPESP) (Grant numbers: 20/07141-2; 22/13213-1; 2022/07326-8; and 2021/0622 7-3).

Impacts of Barra Bonita Reservoir water in zebrafish spermatogenesis

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Introduction: Brazil has approximately 13.6% of the world's fresh water. The State of São Paulo is the most industrialized in Brazil, and problems associated with water quality degradation have been documented for some rivers. Barra Bonita Reservoir receives water from Tiete and Piracicaba rivers, both with intense agricultural and industrial activities leading to a permanent contamination input. Therefore, the increase in pollutants and the lack of inspection of the emission of chemical compounds in natural environments, has increased the risks and mortality of natural species and, consequently, human contamination. In this work, we will assess the toxicity of water, and its effects in fish gonads exposed in vivo. We will attempt to identify possible altered biological pathways, as well as possible biomarkers for environmental contamination. Additionally, we hope to contribute to the elucidation of new mechanisms of action of environmental contaminants and in the awareness and inspection of the dumping of industrial and domestic waste in natural environments. **Objectives:** Our aims are to evaluate several contaminants the water from Barra Bonita reservoir, and to assess the toxicity of water from Barra Bonita reservoir in the gonads of adult male zebrafish (Danio rerio) exposed in vivo. **Methods:** Water from the Barra Bonita reservoir will be collected during the wet period of 2024. Water quality will be evaluated as well as the presence of several contaminants such as glyphosate, atrazine, quinolone antibiotics and cyanotoxins. In addition, male fish will be exposed to this water during 48 h, 7 and 14 days (n = 15 for each period evaluated). Testis histology and gene expression of genes related to spermatogenesis will be assessed. **Results:** We observed the presence of several contaminants such as pesticides, metals, antibiotics and cyanotoxins in the water. In addition, our results showed water from Barra Bonita reservoir can impair gene expression as well as morphological tissue changes in adult male zebrafish. **Discussion/Conclusion:** This approach allowed us to evaluate the effects of combine contaminants present in Barra Bonita water reservoir in male zebrafish. Furthermore, In the light of well-established contaminants and emerging ones, it its crucial to add new compounds to environmental laws as well as new regulations to water use such as recreational activities. **Acknowledgments:** This research is supported by São Paulo Research Foundation (FAPESP), Brazil – (2023/01319-2 granted to ACNM; 2021/06742-5 granted to RHN).

Metal toxicity in marine microalgae of the genus *Tetraselmis sp.*

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Coastal ecosystems play important roles in environmental, economic and social terms. However, they face increasing threats due to human interference, resulting in the loss of biodiversity and the decrease of water quality. Although regulations in Brazil limit the presence of chemical substances in aquatic environments, legally acceptable levels can harm organisms, depending on the particularities of each species and environment. Therefore, ecotoxicological tests are essential to assess such impacts. Microalgae, such as *Tetraselmis sp.*, stand out as ideal bioindicators due to their position in the marine food chain. In this study, we sought to evaluate the acute toxicity of Copper Sulfate ($CuSO_4$) for this organism. The study, carried out at the Ecotoxicology Laboratory of the Federal University of Maranhão, was based on the ABNT NBR 16181 standard of 2021 for cultivation, maintenance and growth/inhibition tests of the microalgae *Tetraselmis sp.* A 30- day algal growth curve was generated from cultivation in nutrient medium (Guillard f/2, salinity 35 g/kg, pH 8.0 - 8.5), under control conditions (light 8000 lux, temperature 24°C ± 2). Sensitivity tests with Sodium Dodecyl Sulfate (DSS) and toxicity tests with $CuSO_4$ (1.0 - 1.5 - 2.0 - 2.5 - 3.0 mg/L) were carried out in triplicate, with initial concentration of microalgae of 10^4 cells/mL, under the same conditions as the growth curve, and toxicity was expressed as effect concentration for 50% of the population (EC_{50} - 72h). After 32 days, the growth curve of the microalgae *Tetraselmis sp.* can be divided into four distinct phases: adaptation phase, exponential phase (days 3 to 4, persisting until the seventh day), reduction phase, and a stationary phase, with notable peaks on the 11th and 15th day reaching 1.27x 10^6 cells/mL, no decline (death). The control chart for sensitivity tests with the DSS was constructed with 9 tests, all within the limits of variation (± 2s), with an average value of 6.22 mg/L and CV of 19.96%. Therefore, the results with DSS were significant, giving credibility to the tests with $CuSO_4$, which revealed a significant effect on cell density in relation to the control from a concentration of 1.5 mg/L of $CuSO_4$. Regarding the EC_{50} (72h) of $CuSO_4$ for T*etraselmis sp.*, a value was found thirty times higher (1.41 mg/L) than that found in the literature for the genus (0.047 mg/L), showing low sensitivity of the species tested to environmental copper levels.

Multi-parameter Analysis of Diuron and its Metabolites (DCA and DCPMU) in different development stages of Zebrafish (*Danio rerio*)

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Background/Introduction: The use of pesticides in agriculture has been associated with environmental contamination and risks to human health. Diuron, the fourth most used pesticide in Brazil, has been a cause for concern due to its persistence in water and soil, as well as its degradation, which generates potentially toxic metabolites such as DCPMU and DCA. The use of the model organism Zebrafish (Danio rerio) for toxicological assessment is relevant in this scenario; mainly, because of the similarity and conservation of the metabolism of xenobiotics and genetic material, which is approximately 70% similar to that of humans. **Objective:** The objective was evaluate the toxicity of diuron and its metabolites, DCA and DCPMU using zebrafish (Danio rerio) in different stages of development. **Methods:** This study evaluates Diuron and its metabolites at environmentally relevant concentrations in Brazil ranging from 0.5 to 100.0 µM. For the embryo-larval stage, the OECD No. 236 protocol for the Fish Embryo Toxicity (FET) test was used, with an extension to 144 hours post-fertilization (hpf). Oxygen consumption was quantified in the 24 hpf embryos after immediate exposure. Embryos exposed for 96 hpf were assessed for acetylcholinesterase (AChE) enzyme activity and genotoxicity using the Comet Assay. Adult zebrafish were tested during 96 hours with acute exposure according to the OECD 203 Fish Acute Toxicity Test. The mutagenicity was assessed by the Micronucleus Test (MN). Additionally, cytotoxic and genotoxic effects were examined, including epigenetic considerations through Nuclear Anomalies (NA) in erythrocytes. These findings were substantiated by histopathological investigations. **Results:** The study showed that Diuron, DCA and DCPMU at concentrations of 5 and 10 µM have adverse effects in embryonic development of zebrafish. Specifically, Diuron at 10 µM caused significant changes in AChE enzyme activity, indicating neuromuscular toxicity. Exposure to Diuron also resulted in DNA damage, which became more prominent beyond 10 µM. DCA showed genotoxic potential in the 0.5-5.0 µM range, inducing nuclear pleomorphism at concentrations of 5.0-10.0 µM. While, DCPMU induces DNA damage at all tested concentrations, with nuclear abnormalities observed in the 1.0-10.0 µM range. No mutagenicity was observed in the analysis of erythrocytes. In adult zebrafish exposed to Diuron, no significant changes were observed in the organs, although there was slight cytoplasmic vacuolization in the hepatocytes. **Discussion/Conclusion:** The impact of Diuron, DCA, and DCPMU on zebrafish varies depending on their life stages, as indicated by ours results. The model used in the study was sensitive to the analyzed these parameters, showing changes after exposure to Diuron herbicide at concentrations compatible with already founded in the environment. Specifically, DCA and DCPMU show more impact on embryotoxicity than Diuron. Biochemically, DCA affected AChE activity, suggesting possible impairments in neurotransmission. However, the study found that diuron caused DNA damage and nuclear abnormalities, which is consistent with the effects observed for DCA and DCPMU. These results raise concerns about the potential adverse effects of diuron and its degradation products on zebrafish. The study highlights the importance of multiparametric assessment in understanding the impacts of herbicides and their metabolites on the environment and human health. These findings contribute to a comprehensive understanding of herbicide toxicity, supporting the formulation of regulations that promote the safe use of Diuron.

Occurrence and bioavailability of PAHs in surface waters in the Bragança Mangroves, State of Pará

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Introduction: Mangroves produce more than 95% of the food that humans capture from the sea. Its maintenance is vital for the subsistence of the fishing communities that live in its surroundings and is fundamental for the balance of the ecosystem. They are estimated to supply up to 10% of the dissolved organic carbon in the oceans from land. However, anthropogenic actions such as burning fossil fuels release contaminants into the water receptor, such as Polycyclic Aromatic Hydrocarbons (PAHs). PAHs are organic contaminants generated by natural and anthropogenic processes, formed mainly during the decomposition of organic matter. They are among the pollutants of most significant interest in the study of environmental contamination due to their recognized mutagenic, teratogenic, and carcinogenic potential, in addition to their relative persistence in the environment and the possibility of bioaccumulation. **Objective:** Evaluate environmental contamination by PAHs in the sediments of the mangroves of Bragança, state of Pará, investigating influencing factors, aiming to minimize socio- environmental impacts in the region. **Methods:** Twenty-eight surface water samples were collected at 14 collection points in the estuary during two sampling campaigns. The samples were collected in amber glass bottles with a capacity of 1L of sample for water analysis, according to the recommendations of the Standard Methods for the Examination of Water and Wastewater, in two distinct periods at pre-defined points. Samples were extracted by an automated solid phase extraction (SPE) system using C18 3 mg 6 mL columns, with ethyl acetate and dichloromethane, and analyzed by USEPA Method 8270 for Analysis of Semivolatile Compounds by Gas Chromatography/Mass Spectrometry. **Results:** HPAS fluoranthene, fluorene, naphthalene, phenanthrene, and pyrene were detected and quantified in 71.42%, 53.57%, 85.71%, 42.85%, and 67.85% of the samples. The total concentration of PAHs (∑PAH) ranged from 6.37 to 1638.25 ng/L-1. The average levels presented were 6.65±12 ng/L-1 (fluoranthene), 4.1±0.4 ng/L-1 (fluorene), 12.5±0.6 ng/L-1 (naphthalene), 520.1±500 ng/L-1 (phenanthrene) and 27.0±32.4 ng/L-1 (pyrene). Low molecular weight PAHs (2-3 rings) represented, on average, over 80% of PAHs at all sampling sites, while carcinogenic high molecular weight PAHs of 5-6 rings were not detected. **Discussion/Conclusion:** Low molecular weight (BPM) PAHs typically originate from moderate-temperature combustion processes such as coal combustion. In contrast, high molecular weight (APM) PAHs are primarily produced from high-temperature combustion processes such as exhaust vehicles. The abundant water concentrations of BPM PAHs can be justified by their high water solubility and relatively high vapor pressures. Pollutants found in mangrove surface waters are likely from combustion engines from boats and ships, oil contamination at study sites, and urban runoff. Pyrene and fluoranthene are formed during the combustion of fossil fuels and occur as a natural constituent of unaltered fossil fuels; they have been proven to be mutagenic. Anthracene is not genotoxic or carcinogenic, but it poses a threat to the environment due to its toxicity to aquatic life, mainly via photoinduced toxicity. Fluorene and phenanthrene are characterized by substances with potentially toxic and mutagenic properties to the environment and biota. Although the levels of PAHs found are relatively low, environmental monitoring is necessary to preserve aquatic and human life. **Acknowledqments:** National Research Council (CNPq) for logistical and financial support.

Toxicity of cobalt tungstate ($CoWO_4$) nanoparticles to freshwater microalga *Raphidocelis subcapitata* (Chlorophyceae)

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Innovative applications and the unique properties of nanomaterials (NMs) have attracted research attention in different areas. Given their applicability in products, such as personal care, pharmaceuticals, photocatalysis, sensors, there has been much discussion about the risks associated with NMs. In particular, cobalt tungstate NPs ($CoWO_4$) has excellent physical and chemical properties and has been used in environmental and technological areas. This can contribute to the presence of these NPs in the aquatic ecosystem and the contact of NMs with aquatic organisms can result in bioaccumulation and transfer of contaminants to higher trophic levels. Thus, the present study investigated the effects of $CoWO_4$ on freshwater microalga Raphidocelis subcapitata, evaluating cell growth, Chl a fluorescence and photosynthetic activity. The Chlorophycean was cultivated in L.C. Oligo culture medium at 25 ± 1°C, with light intensity (≅4000 lux LED light) and 12 h/12 h of light/dark photoperiod. The algal cells were exposed during 96 h to nominal concentrations of 10, 20, 40, 60, 80 and 100 mg L^{-1}. To evaluate the toxicity, we used flow cytometer and pulse amplitude modulated fluorometer (PHYTO-PAM). $CoWO_4$ had a final of 33.7± 8.7 nm aggregated in the culture medium, presenting a of 286± 3.4nm at 100 mg L^{-1}. Regarding biological data, our preliminary results showed that NP inhibits the growth of the algal cells from 40 mg L^{-1}. The chl a fluorescence (FL3-H) decreased ($p < 0.05$), suggesting that $CoWO_4$ affected pigment synthesis. Also, these results is corroborated by a significant decrease ($p<0.05$) of maximum quantum yield of photosystem II (PSII), mainly at 80 and 100 mg L-1 after 96h of exposure. Therefore, the photosynthesis processes may have been altered and cell growth inhibited. Considering that the freshwater ecosystem is an important route of NMs, it is essential to understand the toxic effects that $CoWO_4$ nanoparticles can have on microalgae, which are at the base of trophic chain, and any damage to this trophic level can endanger higher trophic levels. Financial support: this work was funded in part by the São Paulo Research Foundation - FAPESP (grant 2013/07296-2, 2014/14139-3, 2018/07988-5, 2021/13607-7, 2021/13583-0), Financiadora de Estudos e Projetos - FINEP, National Council for Scientific and Technological Development – CNPq (grant 316064/2021-1), and the Coordination for the Improvement of Higher Education Personnel - CAPES (finance code 001). M.A. was supported by the Margarita Salas Postdoctoral contract MGS/2021/21 (UP2021-021) financied by the European Union-Next Generation EU.

Toxicological evaluation of metylene blue degradation reaction products in aqueous systems in zebrafish larvae (*Danio rerio*)

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One of the major environmental problems faced in Brazil today is the contamination of water resources. Methylene blue (AM) can be used in industrial processes to analyze surfactants. Its disposal is an environmental problem, as it can cause the death of several aquatic species. The Department of Chemistry at UFMG synthed hybrid composites based on niobium and copper that act as catalysts for AM degradation in water. In this study, the objective was to evaluate the acute and chronic toxicity of the degradation products of MB solutions after oxidation by niobium and copper-based catalysts (CAT) in Zebrafish (ZB) larvae. The test was carried out according to the protocols described in NBR 15499:2015 and OECD236:2013, which are based on the assessment of acute and chronic toxicity during 96 hours of exposure, using recently hatched ZB larvae in samples of AM degradation reaction products in aqueous systems. ZB larvae were obtained from breeding pairs (2 males and 1 female), which were maintained according to the specifications in Annex B of NBR15499:2016. The water quality parameters of the aquariums were maintained in accordance with Annex B of NBR 15088:2016. Only newly hatched larvae that did not present abnormalities were used in the test. The larvae were randomly subdivided into groups and placed in a 24-well culture plate with a storage capacity of 2.0mLs/well: (1) Control (dilution solution N=20 larvae/plate – 1/well); (2) AM (1ppm - N=20 larvae/plate – 1/well) (3); CAT + AM (N=20 larvae/plate – 1/well); (4) Positive control (NaCl solution – 11.5 mg/L – N=20 larvae/plate – 1/well). The assays were performed in triplicate. At the end of the trial, mortality and morphological changes indicative of toxicity were evaluated. The results were plotted in tables and graphs with a 95% confidence limit in all experimental groups. The percentage of lethality in all groups was calculated. Chemical evaluations demonstrated that CAT produced a reduction in AM from 4ppm to 1ppm in the solution used in the tests. The NaCl group produced 100% mortality in ZB larvae after 24 hours of exposure. Both AM and CAT+ AM produced significantly different lethality compared to the control and NaCl groups (16.6±6.3% and 18.7±1.9% respectively, $p>0.05$ ANOVA), however, not statistically different each other ($p<0.05$ ANOVA). AM and CAT+AM induced tail and spine malformation in ZB larvae. Similarly to the test with adult ZB, diffuse accumulation of the dye was noticed in the skin of the larvae and of CAT+AM in the portions of the gastrointestinal tract (GIT) of the larvae. We concluded that the degradation products of AM solutions after oxidation by niobium and copper-based catalysts produced chronic toxicity in ZB larvae. An accumulation of the dye was observed in the skin and of CAT+AM in the GIT of the ZF. The authors thank to FAPEMIG for the foundation and contribution for this project development.

Toxicological, histopathological and bioaccumulative evaluation of products from the metylene blue degradation reaction after catalysis by niobium and copper based catalysts in zebrafish (*Danio rerio*)

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The textile industry plays an important role in the Brazilian economy and the state of Minas Gerais and is one of the most polluting sectors due to the release of recalcitrantly degrading textile dyes into bodies of water. These effluents are difficult to treat because they contain dyes that are difficult to physically, chemically and biologically degrade. Methylene blue (AM) has been used in industrial processes to analyze surfactants. Its disposal is an environmental problem, as it can cause the death of several aquatic species, mainly plants and fish. The Department of Chemistry at UFMG (DQUI/UFMG) synthed hybrid composites based on niobium and copper that act as catalysts for AM degradation in water. The objective was to evaluate the acute toxicity of the product synthed by DQUI/UFMG in adult Zebrafish (ZF). The test was carried out according to the protocols described in NBR 15088:2016 and OECD203:2019, which is based on the assessment of acute toxicity in adult ZF, after 48 h of exposure. ZF AB with an average length of 2.0 ±1.0 cm were randomly subdivided into groups: (1) control (dilution water – 1L N=10); (2) Sample (which corresponds to a series of serial dilutions of the composite synthed by DQUI/UFMG (five subgroups - CAT 100, 50, 25, 12.5 and 6.25% respectively - 1L N=10/ subgroup); (3) AM (1ppm, N=10); (4) NaCl (11.5g/L, N=10). Mortality and behavioral changes indicative of toxicity were recorded at 24 and 48 hours. At the end of the experiments, all fish were euthanized by an anesthetic overdose of eugenol (285 mg/L- CONCEA) to collect organs and tissues. Around 50% of the fish were subjected to macroscopic and histopathological analysis and the remainder were subjected to toxicity analysis through chemical characterization by HPLC of tissue samples from the head, muscles and viscera. The results were plotted in tables and graphs with 95% confidence limits in all experimental groups ($p<0.05$, ANOVA- Graph Pad Prism 8.0). At the end of the test the percentage of lethality in relation to the control was calculated in all groups. There was no mortality in the control groups and in any dilution of the samples. However, agitation and color changes were observed in the skin and viscera of the ZF, mainly at 100% sample concentration in all evaluated intervals. NaCl produced mortality in 100% of ZF 24 hours after exposure (FT=1). Histopathological evaluation demonstrated slight hypertrophy of the branchial epithelium in the AM group, and slight detachment of the surface of the intestinal epithelium at a concentration of 100% in the CAT group. Tissue chemical analysis demonstrated the presence of copper in all groups, although they were not statistically different from each other. High concentrations of niobium were found in the viscera and head, mainly in the 100% CAT solution. We conclude that AM degradation products after catalysis by niobium and copper-based catalysts do not produce acute lethality, despite the presence of histopathological alteration and high tissue concentrations of niobium in adult ZF. The authors thank to FAPEMIG, CAPES and CNPq for the foundation and contribution for this project development.

Triclopyr induced oxidative stress, cellular energy allocation, and neurotoxicity in zebrafish (*Danio rerio*) early life stage: a comprehensive analysis

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Background: Triclopyr is an auxin-like herbicide employed in agriculture and forestry to combat broadleaf weeds and invasive plants. In Brazil, sales of the active ingredient triclopyr TBEE, which degrades into triclopyr, reached 2,536.64 tons in 2020. Triclopyr has been detected at concentrations ranging from 0.000004 to 5.13 mg/L in various aquatic matrices, including surface water, groundwater, lakes, rivers, and estuaries. However, the mechanisms of toxicity of triclopyr for non-target organisms remain unknown. Therefore, triclopyr has been deemed of high environmental concern. **Objective:** This study proposes a comprehensive analysis of triclopyr toxicity mechanisms. Using the early life stage of zebrafish as a model organism, we intend to assess the triclopyr impact on the antioxidant defense system, cellular energy allocation, and neurotoxicity. **Methods:** Zebrafish embryos were exposed to 0.125, 0.470, 1.768, 6.648, and 25 mg/L of triclopyr up to 120 hours post- fertilization (hpf). Samples were collected for biochemical analyses, focusing on the enzymatic antioxidant system, including catalase, glutathione S-transferase, lactate dehydrogenase, and acetylcholinesterase. Additionally, the behavioral response of zebrafish larvae to light/dark transitions following exposure to triclopyr was investigated. The evaluation of cellular energy allocation on zebrafish larvae was conducted. The analysis of cardiac activity was performed at 48 hpf. **Results:** Catalase and glutathione S-transferase activities increased significantly at the 6.648 mg/L triclopyr concentration. LDH activity showed a significant increase at 0.125 mg/L and the 1.768 mg/L triclopyr treatment. AChE activity remained unaffected in zebrafish embryos exposed to triclopyr. Zebrafish larvae exhibited a significant decrease in available energy at 0.470, 6.648, and 25 mg/L triclopyr treatments with a simultaneous increase in total energy consumption at 0.152, 1.768, 6.648, and 25 mg/L. CEA significantly decreased at concentrations 0.125, 1,768, 6.648, and 25 mg/L of triclopyr. Zebrafish larvae exposed to 1.768 mg/L of triclopyr showed decreased movement in the dark compared to the control. Reductions in the average time moved in the dark were significant at 1.768 and 6.648 mg/L of triclopyr, with swimming velocity significantly decreased only at 1.768 mg/L. Increased inactivity was observed at 0.125, 0.470, 1.768, and 6.648 mg/L of triclopyr. There was a notable decrease in small distance moved in the dark across all triclopyr treatments. However, the duration of small movements significantly decreased only at 0.125, 1.768, and 6.648 mg/L of triclopyr. Large distance moved in the dark significantly decreased at 1.768 mg/L, while an increase was observed at 6.648 and 25 mg/L. The duration of large movements significantly decreased at 1.768 mg/L and increased at 6.648 mg/L of triclopyr. **Discussion/Conclusion:** The activities of the antioxidant enzymes CAT and GST exhibited a significant response at higher triclopyr concentrations indicating activation of the antioxidant defense system in response to triclopyr exposure. LDH, an enzyme associated with metabolic and stress processes, showed a significant increase even at the lowest tested concentrations, highlighting a metabolic response to sublethal triclopyr concentrations. AChE activity remained unaffected, suggesting potential enzyme resistance or adaptation in zebrafish embryos exposed to triclopyr. Energetic analysis suggesting a direct interference with energy allocation during early zebrafish development. Behavioral analysis suggests compromised motor activity in response to triclopyr exposure. **Acknowledgments:** We thank to CAPES for the scholarship funding (CAPES-PrInt process 88887.716831/2022-00).

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GENOTOXICIDADE, MUTAGÊNESE E CARCINOGÊNESE

Evaluation of the mutagenic potential of a residual aqueous fraction of the moss Sanionia uncinata.

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Introduction: Ultraviolet (UV) radiation is the primary exogenous inductor of skin damage, so photoprotection, becomes a meaningful way to control skin disorders. The Antarctic moss Sanionia uncinata has developed protective strategies against UV damage through biochemical pathways of secondary metabolites, such as phenolic compounds, and it is a substantial source of antioxidants. Recently, our research group showed that fractions from sequential partition, such as residual fraction aqueous (AF), of its defatted hydroalcoholic extract showed significant in vitro sun protection. It also absorbs the UV-vis spectrum, increases sun protection factor values of UV-filter benzophenone-3 and octylmethoxycinnamate, and does not induce embryotoxicity in zebrafish's early life stage. The phototoxicity was not detected. There is little data in the literature about toxicological research. **Objective:** The study aims to evaluate an aqueous residual sample's mutagenic and antimutagenic potential from hydroalcoholic extract moss Sanionia uncinata. **Material and Methods:** For the investigation of the mutagenic potential, the Salmonella/microssome assay was performed using the pre-incubation protocol. The mixture of 100 µL of the stationary culture of Salmonella enterica serovar Typhimurium strains (S. typhimurium) TA98 and TA100 and 500 µL of 0.2M sodium phosphate buffer (pH 7.4) or an exogenous metabolization (S9 mix, 4% w/v) was incubated for 20 min with 100 µL of different concentrations of the AF. Top agar was added and poured into a Petri dish containing minimal agar medium. After 72h the number of revertants was counted. Antimutagenicity assay was carried out by pre, co, and post-exposure protocols to investigate the potential of the AF on protection against an oxidant (4-nitroquinoline-N-oxide, 4NQO) and DNA adduct inductor (2-aminoanthracene, 2AA), using S. typhimurium (TA98 and TA100 strains) in the absence and presence of S9 mix fraction. **Results:** No mutagenic activity was detected for TA98 (frameshift mutation) and TA100 (base-pair substitution) strains either in the absence or presence of S9 mix fraction. AF significantly ($P < 0.05$) downregulated 4NQO in pre-treatment via the intracellular reaction route, reaching ~62% inhibition at 0.5µg/mL and ~58% inhibition at 0.005 µg/mL in the absence of S9 mix in strain TA100 and TA98, respectively. In the presence of S9 mix ~70% inhibition at 0.0005 µg/mL on pos-treatment (probably via DNA repair). The chemopreventive abilities were detected with more intensity in the presence of S9 mix (against 2-AA) in TA100 strain for the co-treatment, reaching 84% at 0.005 µg/mL). **Discussion/Conclusion:** The data obtained from AF supports its safety and effectiveness. The 4NQO, a quinoline derivative, is a potent mutagen and carcinogen that induces DNA lesions through Reactive Oxygen Species (ROS) production, generating superoxide radicals or hydrogen peroxide, which can induce DNA mutation at guanine residues (DNA target in TA100 and TA98). In the antimutagenicity assay, AF reduced the ROS damage formation, acting as a significantly reducing agent, and strongly inhibited the formation of DNA adducts. The AF should be of interest to further explore the possibility of using it as a potential photoprotective in dermatological applications. **Acknowledgments:** Rio de Janeiro State Foundation for Support Research (FAPERJ) and National Council of Technological and Scientific Development (CNPq).

Evaluation of the toxicity of an aqueous fraction from the Antarctica moss *Sanionia uncinata*

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Background/Introducton: Due to its geographic localization, the Antarctic continent suffers intense ultraviolet radiation (UV) effects. The earth's tilt relative to the sun exposes the continent to the sunlight for almost 24 hours during six months of summer. Besides global warming and the emission of harmful gases, the UV spectrum has been acting strongly over Antarctica. It is known that UV radiation has a large ability to penetrate the cells and act over the DNA, inducing mutations that can lead to genetic material injuries that can induce tumor formation. *Sanionia uncinata* moss, native to Antarctica, belongs to the bryophyte group and developed a mechanism that makes it resist to those damages, as the secondary metabolites production such as phenolic compounds. Previously, we reported that fractions from sequential partition, such as residual aqueous fraction (AF), of its defatted hydroalcoholic extract showed significant in vitro sun protection. It also absorbs the UV-vis spectrum, increases sun protection factor values of UV-filter benzophenone-3 and octylmethoxycinnamate, does not induce embryotoxicity in zebrafish's early life stage and phototoxicity was not detected. **Objectives:** Evaluate the cytotoxic potential of an aqueous residual fraction from the hydroalcoholic extract of *Sanionia uncinata* in absence and presence of UVA and UVB exposure. **Methods:** Cell death assays by WST-1 (water-soluble tetrazolium salt), LDH (lactate dehydrogenase), and TBE (tripan blue), with and without UVA and UVB exposure, were carried out with HaCaT cell lineage. The WST-1 assay is a colorimetric test that allows us to assess cell viability via mitochondrial dehydrogenase action. The LDH and Tripan Blue assays are also colorimetric assays that measure the death rate (LDH) and the viability rate (TBE) based on membrane injuries. The HaCaT cells were exposed to AF (0.0004-10 mg/mL) in the absence of UVA and UVB radiation by 24, 48 and 72 hours, except TBE test, which was made by exposing the cells for 24 hours. In the assays with UVA and UVB exposure, 24h exposure to AF (0.001-1 mg/mL) were performe, and the cells were washed and irradiated independently at 20 J/cm2 of UV-A and 50 mJ/cm2 of UV-B. **Results:** In the absence of UVA and UVB, the WST-1 and LDH assays (24, 48 and 72 hours) showed a significant mortality rate (above 30%) as from 1 mg/mL. The TBE assay without UV exposure showed a significant mortality rate (≥ 0.4 mg/mL, above 30%). The WST assay showed that the AF did not affect cell viability after UV-A and UV-B radiation. Using LDH and TBE assay, the AF fully protected HaCaT cells against UVA (up to 0.4 mg/mL) and UVB (up to 0.4 mg/mL) membrane integrity. **Discussion/ Conclusion:** Using LDH and TBE assay, the AF fully protected HaCaT cells membrane integrity against UVA and UVB radiation (up to 0.4mg/mL). Therefore, the AF does not induce decreased viability via WST-1 and protects the HaCaT keratinocytes against UV-induced damage by the cell membrane disruption pathway. **Acknowledgments:** Rio de Janeiro State University (UERJ), National Council for Scientific and Technological Development (CNPq) and Carlos Chagas Filho Foundation for Research Support in the State of Rio de Janeiro (FAPERJ).

Exploring cytotoxicity and in silico profiling of 2-(5'-amino-2'-hydroxyphenyl) benzoxazole sulfonamides

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The development of new bioactive compounds with potential as drugs is a complex process that requires multiple steps. The combinatorial chemistry approach appears as a promising strategy in medicinal chemistry; wherein the synthesis aims to conjugate two or more molecules to create new structures. Leveraging known compounds offers the advantage of expediting the creation of new medications. Within this scope, heterocyclic benzoxazoles and sulfonamides have emerged as important functional groups in the search for bioactive compounds, notably for their diverse pharmacological activities, such as antibacterial, antitumor, antiparasitic, anticonvulsant, analgesic and antimicrobial actions. The utilization of combinatorial chemistry underscores the imperative to assess the pharmacokinetic and toxicological properties during the early stages of new drug candidate development. In this context, in silico studies have gained prominence as a fundamental tool, complementing traditional in vitro and in vivo investigations. Their rapidity, cost-effectiveness, and potential to mitigate reliance on animal testing stand as significant advantages. Advancements in synthesizing derivatives through molecular combinations drive the exploration of effects and potential mechanisms of action, particularly in compounds like benzoxazole sulfonamides, representing the way for substantial prospects in new drug development. The assessment of the toxicity of chemical substances must be integrated into their synthesis process, especially in pharmaceuticals, after confirmation of positive effects through pharmacological screening. Cytotoxicity emerges as the first parameter to evaluate the potential toxicological risk in vivo, allowing toxicity to be estimated through cellular damage in vitro, replacing the use of animals in laboratory experiments. The MTT test, in this context, proves to be crucial in the initial phase of screening new molecules. In this study, the objective is to synthe, evaluate the cytotoxicity and predict physicochemical properties and in silico toxicological parameters of two new sulfonamides derived from 2-(5'-amino-2'- hydroxyphenyl) benzoxazole. The compounds 2-(p-toluenesulfonamide-2'-hydroxyphenyl) benzoxazole (**1**) and acid 2-(5'-p-cinnamicsulfonamide-2'-hydroxyphenyl) benzoxazole (**2**) were obtained in yields around 65% and their structures confirmed by spectroscopic techniques. Cytotoxicity was tested using the MTT colorimetric assay on the L929 mouse fibroblast cell line. In silico parameters were evaluated by theoretical simulations using online platforms such as Swiss ADME and Pro Tox-II to obtain ADMET parameters (adsorption, distribution, metabolism, excretion and toxicity). The cytotoxicity tests revealed that compounds 1 and 2 exhibited cytotoxic effects at concentrations of 53 and 46 µM, respectively, suggesting that lower concentrations are deemed safe for utilization. In silico studies indicated the potential for oral administration of these derivatives as pharmaceutical agents. Benzoxazole sulfonamides **1** and **2** exhibited no immunotoxicity, mutagenicity, or cytotoxicity, but manifested hepatotoxicity. However, both showed negative results in terms of carcinogenicity. Assessing the 2-(2'-hydroxyphenyl) benzoxazole derivatives via the BOILED-Egg diagram enabled interpretations regarding gastrointestinal absorption and passive cerebral penetration. The derivative 2-(5'-toluenesulfonamide-2'-hydroxyphenyl) benzoxazole displayed limited absorption, while the acid-2-(5'-p-cinnamic-sulfonamide-2'- hydroxyphenyl) benzoxazole revealed excellent intestinal absorption, permeated the blood- brain barrier effectively, and action as a substrate for P-glycoprotein. **Acknowledgments:** CAPES; CNPq; ULBRA and CEPPED.

Imidacloprid: evaluation of genotoxicity and oxidative stress in treated wistar rats

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Introduction: The neonicotinoids insecticides, which imidacloprid (IMI) was the first commercial pesticide launched (1991), became one of the most commonly used insecticides in the world due to the high toxicity of the organophosphates and carbamates, and also because your selectivity. IMI is an agonist of postsynaptic nicotinic acetylcholine receptors, widely used for crop protection against pests, home and garden protection, as well as veterinary applications to animal diseases control. **Objective:** To evaluate genotoxicity and oxidative parameters in rats after 45 days of treatment with an IMI-based commercial insecticide. **Materials and Methods:** Wistar adult male rats, obtained from Centro de Reprodução e Experimentação de Animais de Laboratório were used in this study. The experiments were approved by the University Ethics Committee (CEUA number 37572). The animals were distributed in four groups: the control group, which received distilled water, and the IMI treated groups, which received IMI suspension at 5, 10 and 15 mg/kg. The treatments were administrated daily by oral gavage for 45 consecutive days. Genotoxicity was evaluated by the alkaline comet assay in total blood, and the micronucleous (MN) frequency in the bone marrow. In addition, the number polycromatic (PCE), normocromatic (NCE) erythrocytes and the ratio between PCE/NCE was counted. The activity of the detoxifying enzymes catalase (CAT) glutathione peroxidase (GPx) and glutathione S-transferase (GST) was evaluated in blood. Lipoperoxidation (TBARS) and total non-protein thiols were measured in spleen, heart, liver and kidney. **Results:** In the micronucleus assay, the IMI 5 mg/kg treated group showed a significant lower ratio between PCEs and NCEs, and a significant increase in the frequency of the number of MN-PCEs ($p<0.05$, one-way ANOVA/ Bonferroni). A significant increase in CAT activity was observed in the group treated with 5 mg/kg of IMI when compared to the control ($p<0.05$, one-way ANOVA/Bonferroni). Spleen TBARS levels showed a significant increase at 5 mg/kg IMI when compared to the control group levels ($p<0.05$, one-way ANOVA/Bonferroni). The other parameters evaluated in the study did not show a significant statistical difference when compared to the control group. **Conclusion:** The findings indicate that IMI treatment might cause a possible genotoxicity and oxidative stress at 5 mg/kg dose. Further studies are necessary to evaluate the molecular mechanisms by how IMI acts on chromatin and oxidative parameters.
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In vitro cytotoxicity and genotoxicity evaluation of Cannabis sativa extract used by Brazilian family as a therapy in the control of seizures in children with refractory epilepsy

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Cannabinoid derivatives extracted from Cannabis sativa L., mainly cannabidiol (CBD), have shown efficacy in the treatment of severe epilepsy and in the reduction of seizures in epileptic patients refractory to conventional treatment. The medicinal use of CBD and D9 tetrahydrocannabinol (THC) is regulated in several countries and used as active ingredients in medicines. In Brazil, in 2016, through RDC 66/2016, the Brazilian National Health Surveillance Agency authorized the importation for personal medical use of C. sativa L, plant parts and extracts containing CBD and THC. Taking into account the patient safety and due to prolonged intended use, the aim of this study was to evaluate the in vitro cytotoxicity and genotoxicity of C. sativa extract on medium chain triglyceride (MCT). The extract, with 25 mg/mL of CBD and 1 mg/mL of THC, was prepared and standardized by the Toxicological Analysis Laboratory of UFRJ and used by a child of a Brazilian family to control seizures with refractory epilepsy. The in vitro Comet assay (CA) and the in vitro cytokinesis-block micronucleus (CBMN) assay in whole human blood cells (WHBC) were performed in the absence and presence of Arochlor 1254-induced rat liver S9 mixture. In the CA, treatment of WHBC with MCT vehicle (1.3 to 20%) and with C. sativa extract (CBD 0.3; 1.0; 2.0 and 5.0 mg/mL) in the absence of S9 did not induce significant DNA damage ($p > 0.05$) when compared to the untreated and corresponding MCT vehicle-controls, respectively. The extract of C. sativa (CBD 0.3; 1.0; 2.0 and 5.0 mg/mL) in the presence of S9 induced significant DNA damage ($p < 0.05$) in WHBC at the concentrations of CBD 1.0 and 2.0 mg/mL tested when compared to the corresponding TCM solvent control with S9. In the CBMN assay, treatment with C. sativa extract (CBD 0.3; 0.6; 1.3; 2.5 and 5.0 mg/mL) and MCT (2.5 to 20%), in the presence and absence of S9 mixture, did not induce chromosomal breaks and/or chromosome gain or loss in human lymphocytes. Therefore, it was concluded that the extract of C. sativa in TCM, in the 5 concentrations of CBD tested, was considered non-mutagenic in WHBC not inducing clastogenic and/or aneugenic effects in human lymphocytes, in the presence and absence of the S9 mixture.

Integrated biological assessment of air pollution in urban areas using passive biomonitoring of genotoxicity

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Background: Atmospheric pollution is generally monitored using physicochemical indicator systems. Variations of these indicators concerning environmental legislation parameters report on air quality, especially in large cities. However, the interpretation of environmental data on pollutants needs to be complemented, as the impacts of pollution on sensitive organisms serve as a warning to public health authorities. Using species adapted to the urban environment as sentinels enables continuous and integrated monitoring of environmental pollution effects on biological systems. **Objective:** In this study, we used the tropical plant species Tradescantia pallida, planted in ornamental beds at the main urban intersections in Uberlândia, Brazil, to monitor the genotoxic effects of atmospheric pollution under different vehicular traffic conditions. **Methods:** We collected and analyzed surface soil samples (volume = 100 cm3; depth = 5 cm) and T. pallida leaves at different intersections (in quintuplicate) to determine the concentration of heavy metals, including Lead (Pb), Chromium (Cr), Nickel (Ni), Cadmium (Cd), Copper (Cu), and Zinc (Zn). In four samples, we compared biological, physical-chemical, and traffic indicators at different intersections (in residential and commercial areas) during the restriction period of local commerce activities imposed by health agencies as a measure to face the COVID-19 pandemic. To monitor the effects of the variation in vehicle flow on particulate matter emission, we used daily data on the circulating fleet provided by the Municipal Traffic Department. The average daily levels of particulate material in the PM1, 2.5, 10 fractions at each evaluated site and period were obtained using a portable particulate matter sampler. We performed the Tradescantia micronucleus assay to evaluate the biological response of genotoxicity based on micronucleus frequency. **Results:** With the reduction in vehicle traffic due to the lockdown, our findings showed that T. pallida is sensitive to environmental changes in air and soil pollutants. The species bioaccumulated heavy metals under greater vehicular traffic exposure, with higher concentrations of these contaminants also detected in the soil. Furthermore, from the Micronucleus Test, we found that genotoxicity can be estimated using a multiple linear regression model, including (X1) chromium concentration in the soil and (X2) particulate matter in the atmosphere as the main independent variables. **Discussion:** The findings showed how vehicle traffic changes affect atmospheric particulate matter concentrations. We also demonstrate that the suspension of pollutant emission sources alters environmental metal concentrations but does not entirely remove them since chemicals, especially heavy metals, are transferred between different environmental compartments, such as by the air-soil-plant. Our results provide valuable insights for the (re) definition of parameters and models of Environmental Health Surveillance. **Acknowledgements:** Financial support is acknowledge to “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (Capes).

The impact of chronic exposure to acrylamide on the expression of microRNAs linked to renal carcinogenesis

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Acrylamide is a well-known food processing contaminant with toxic effects identified in in vitro and in vivo assays. Although less severe, the chronic exposure to acrylamide demonstrated damages to kidney, such as oxidative stress, degeneration of the glomeruli and renal tubules. All these effects can promote the process of renal tumorigenesis and induce cancer. Therefore, the aim of this study was to evaluate how acrylamide can induce the transcription of microRNAs associated with renal cell carcinogenesis and promote neoplastic pathways. Three cell lines (786-O, HEK-293, and HK-2) were exposure to low and high concentrations of the contaminant during 80 and 40 days, respectively. In addition to the expression of nine microRNAs (miR-21-5p, miR-34a-5p, miR-155, miR-193a-3p, miR-200c, miR-205-3p, miR-210-3p, miR-223-3p, and miR-429) using RT-qPCR, the impact of acrylamide exposure on cell proliferation was determined using the chronic exposure factor (Ĉ). The low concentration of acrylamide reduced cell proliferation in all cell lines while the same effect was observed for 786-O and HK-2 cells at high exposure concentration. Only HEK-293 cells demonstrated an increase in cell proliferation after 40 days of exposure. Moreover, acrylamide altered the expression of all investigated microRNAs in both tumor cells (786-O) and non-tumor cells (HEK-293 and HK-2). Based on the reported targets of each microRNA, the low concentration of the contaminant could induce the autophagy process as well as changes in cellular lipid metabolism. On the other hand, high concentration could cause cell death by necroptosis. In this way, the chronic exposure to acrylamide could promote cellular tumorigenesis through compensation of cell death, such as autophagy, and the induction of cell death by necroptosis. Furthermore, the changes in lipid metabolism are a well-known phenotype of neoplastic kidney cells. In conclusion, the chronic exposure to acrylamide, even at low concentration, can alter proliferation of tumorous and non-tumorous cells, as well as the induction of cancer cell phenotype.

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06
IMUNOTOXICOLOGIA

Evaluation of the quantitative of specific ige requests for food dyes in the primavera health network (2018-2022): specific ige for cochineal extract (f340) and specific ige for tartrazine (e102)

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Introduction: To intensify the color of foods and make them more attractive, different types of food colorings are used, whether of natural or artificial origin. However, the use of these additives can be questioned due to these substances being related to different types of allergic reactions and intoxication. Because it is potentially serious, allergies to food dyes are gaining notoriety and are increasingly being investigated. **Objective:** The present work aims to evaluate the quantity requestes of specific IgE proteins for cochineal extract (F340) and specific IgE for Tartrazine (E102) between the years 2018 to 2022 in Rede Primavera de Saúde. **Method:** The Primavera Health Network is made up of 5 clinics and 2 hospitals in the state of Sergipe, using the TASY System for data management. Annual reports were generated, through the TASY System, of specific IgE requests in the network for each year. To analyze the data, initially, a spreadsheet was created in the Microsoft Excel 2010® program. Using the program's resources, a bar graph was generated to represent the data. Considering that, in the present analysis, only information obtained from a database was used, in a grouped manner, without identifying subjects or consulting medical records, there was no need for assessment by the Research Ethics Committee. **Results and Discussions:** In Graph 1, the absolute number of exams requested in each year analyzed is presented. In 2018, 26 requests were made, 57.6% of requests were for specific IgE for cochineal extract (F340) and 42.3% specific IgE for Tartrazine (E102). In 2019, the total number of requests increased by 20 requests, with 71.7% of requests being specific IgE for cochineal extract (F340) and 28.2% specific IgE for Tartrazine (E102). In 2020, when the entire world was stopped by the COVID-19 pandemic, the total number of requests fell compared to the previous year. 42 requests were made, of which 50% for IgE specific to cochineal extract (F340) and 50% IgE specific to Tartrazine (E102). Still in the pandemic scenario, in 2021, even with some restrictions, the number of requests more than doubled compared to the previous year. 92 requests were registered, 61.9% for IgE specific to cochineal extract (F340) and 38.0% IgE specific to Tartrazine (E102). Finally, in 2022, the year in which Brazil declared the end of the health emergency and greater flexibility in restrictions, requests again doubled compared to the previous year. The total number of requests reached 202. 57.4% for IgE specific to cochineal extract (F340) and 42.5% IgE specific to Tartrazine (E102). The growing number of requests over the years is notable, however, it is not possible to state that this increase is only related to precautions, and may also be related to greater sensibility among the population due to exposure to these substances. **Acknowledgments:** I would like to thank Thatiane de Andrade Menezes, biomedical coordinator, and biomedical Bruno Cesar Fontes from the clinical analysis laboratory of Rede Primavera de Saúde for their assistance in obtaining the annual reports.

In vitro evaluation of the toxicity of flumetralin

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The use of pesticides has grown sharply due to agriculture having a consolidated base in the Brazilian economy. Tobacco farming is among the main agricultural activities developed, with Brazil being the leader in exportations. This is due to the fact that Brazilian tobacco has high quality and a high level of technology used in the crop. The immune system can be directly affected by exposure to pesticides and, therefore, autoimmune, neurodegenerative diseases, allergic diseases and even cancer can be developed. This work aimed to evaluate, through an in vitro model, the toxic effects of the use of flumetralin, a plant growth regulator widely used in tobacco cultivation. Mice macrophage cells RAW 264.7 and 3T3 fibroblasts were cultured in DMEM medium and incubated at 37°C in 5% CO2. The cytotoxicity of flumetralin was evaluated using MTT reduction and neutral red uptake assays in both strains after 24 hours incubations. Based on the cytotoxicity of flumetralin, EC20, EC40 and EC60 were obtained for each strain. For RAW 264.7 they were, respectively, 1.181, 1.552 and 1.945 mg/L and for the 3T3 lineage, respectively, 0.963, 1.037 and 1.103 mg/L. Additionally, the production of free radicals and mitochondrial membrane potential, were evaluated for both cell types. For the RAW 264.7 cells, nitric oxide and the cytokine profile, evaluating tumor necrosis factor (TNF) and interleukin-10 (IL-10) were also analyzed. There was an increase in free radicals production for both cell lines. In RAW 264.7 cells, flumetralin induced a mitochondrial hyperpolarization, however in 3T3 lineage, a depolarization was observed. In the cytokine profile, flumetralin decreased the expression of IL-10 and increased the expression of TNF at all concentrations tested. With this work, it was possible to observe the toxicity of flumetralin in both cell types tested and the effects caused on the immune system, affecting the anti-inflammatory response of macrophages.

Mitochondrial impairment related to the immunotoxicity of the herbicides clomazone, glyphosate and sulfentrazone in THP-1 cells

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Around 2 million tons of pesticides are used annually, of which herbicides account for 50%, followed by insecticides (30%), fungicides (18%), and other types such as rodenticides and nematicides. The risk is particularly significant for human health and occupational and environmental exposure, considering the trace amounts consistently found in food and water. Prolonged exposure to pesticides can result in neurological, reproductive, teratogenic, and immunological disorders. Dysregulation of the immune system by pesticides has been closely associated with predisposition to several diseases, especially those associated with immunosuppression or autoimmunity. Therefore, this study aimed to evaluate the toxicity of the herbicides clomazone (Gamit® 360 CS; 99.8% purity), glyphosate (Syngenta®, 620 g/L; 62% purity), and sulfentrazone (Boral® 500 SC; 92.73% purity) through the MTT assay on the human monocytic cell line THP-1 for 24h. Furthermore, pathways including mitochondrial impairment and interleukin profile were evaluated. Cytotoxicity was evaluated by the MTT reduction assay, mitochondrial membrane potential ($\Delta\psi m$) was accessed by measuring the inclusion of TMRE and the interleukin profile was performed using the Human Inflammatory Cytokine Kit BD™ CBA.The herbicides clomazone, glyphosate, and sulfentrazone produced concentration-dependent cytotoxic effects. Significant differences were found in the IC50 values of glyphosate (IC50 71.71 mg/L) compared to sulfentrazone (IC50 242.8 mg/L) and clomazone (IC50 576.0 mg/L). Glyphosate was the most potent herbicide tested. No significant differences were observed for clomazone and sulfentrazone IC50. A significant decrease in $\Delta\psi m$ was observed for all concentrations of clomazone and 100 and 150 mg/L glyphosate ($p<0.001$, ANOVA/Bonferroni). Interestingly, at all concentrations tested, sulfentrazone increased $\Delta\psi m$ ($p<0.01$ ANOVA/Bonferroni), indicating mitochondrial hyperpolarization. In the interleukin profile, glyphosate increased IL-8 levels. The results revealed a cytotoxic effect of the herbicides clomazone, glyphosate, and sulfentrazone on THP-1 immune cells after 24 hours of incubation. Among the mechanisms related to the cytotoxicity of herbicides on immune system cells, mitochondrial impairment is an essential target and should be investigated in depth.

07
MODELOS ANIMAIS ALTERNATIVOS E MODELOS IN VITRO

Adaptation of a reconstituted human ocular epithelium model (toxin ocular) to an animal-free condition

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Introduction: The vulnerability of the human corneal epithelium to damage from various substances and environmental factors highlights the significance of assessing eye corrosion and irritation for toxicological evaluations. Traditional approaches relied on animal models, but ethical concerns and scientific motivations spurred the development of alternative methods like reconstituted human corneal epithelium (RhCE) models. **Objectives:** This study aimed to create and validate an accessible and reproducible RhCE protocol based on the ToxIn Ocular model. The goal was to employ a chemically defined culture medium and animal-free conditions, addressing the limitations of previous models and methods. Materials and **Methods:** HaCaT keratinocytes were cultured on 24-well inserts coated with collagen I (bovine) at 5×105 cells/insert. Four media conditions (A, B, C, and D) were evaluated for tissue morphology and functionality. The selected medium (D) was used for further testing. The RhCE model's performance in ocular irritation assessment was measured by exposing tissues to 11 substances with established GHS classifications. Additionally, HaCaT cells were adapted to animal-free conditions using a straightforward process, involving the substitution of fetal bovine serum (FBS) with human serum (HS) in the culture medium, trypsin, and freezing medium. Morphology, gene expression (MKI67, CDH1 e CK14), and doubling time. **Results and Discussion:** No significant differences were observed in tissue morphology and functionality between the evaluated media conditions and the control. Substances categorized as non-classified achieved favorable cell viability (>60%), while others had viability below this threshold. This indicates the potential of the model for assessing irritant substances. Transitioning HaCaT cells to an animal-free environment, particularly using 5% HS supplementation, did not negatively impact cellular aspects. **Conclusion:** This study achieved successful adaptations to the Ocular ToxIn model, substantially reducing the reliance on animal-derived inputs. The protocol developed enabled the assessment of substance irritancy, showcasing the model's suitability for identifying non-irritating substances. The transition of HaCaT cells to an animal-free culture environment, particularly using 5% HS supplementation, displayed no adverse effects on vital cellular attributes. A bioinformatics analysis of networks was conducted to assess biological processes related to the evaluated genes. Thus, this research contributes to the advancement of more ethical, accessible, and reproducible methods for toxicological evaluations and cosmetics testing, reducing the need for animal experimentation, and addressing societal concerns.

Assessment of eye safety of rural workers exposed to the pesticides Tebuconazole and Pyraclostrobin

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Background/Introduction: Pesticides are products used in agricultural production with the aim of improving soil quality and production. However, the use of these products is associated with a wide range of risks to human health, especially when the protection used when handling them is insufficient. The most frequent routes of exposure to pesticides are inhalation and cutaneous, however, it should be noted that the ocular route must also be the object of attention because, after contact of chemical substances with the ocular surface (accidentally or not), the chemicals can permeate, causing many problems. **Objective:** To evaluate the in vivo eye pesticide exposition and ex vivo ophthalmic biodistribution of Tebuconazole (TBZ) and Pyraclostrobin (PIRA). **Methods:** The biodistribution assessment was carried out according to Cardoso et al., (2020). The commercial products Rival200 EC® (Tebuconazole 200g/L) and Comet250® (Pyraclostrobin 250g/L) were diluted in water to 0.2 and 0.4 mg/mL (TBZ), and 0.3 and 0.5 mg/mL (PIRA). 30 and 50 µL of the prepared dilutions were applied to the surfaces of porcine and bovine eyes respectively and kept in an incubator at controlled temperature and humidity. Aqueous humor was collected during test intervals and at intervals of 15 and 120 minutes the eyes were excised to collect internal structures, subsequently subjected to extraction and quantified by LC-MS/MS. In addition, tears were collected from 5 rural workers after 4 hours pesticide application in accordance with the ethics committee under CAAE number: 56545822.2.0000.520. The opacity and permeability test on bovine cornea (BCOP) was also carried out, in accordance with the OECD TG 437 guide, aiming to classify these products according to their irritating potential. **Results:** When a lower concentration dilution was applied for both pesticides, there was a decrease in pesticide concentration in aqueous humor after one hour, probably due to their biodistribution to other ocular structures faster than the pesticide elimination from the cornea. When higher concentrations were applied, the opposite was observed, with an increase in the total amount in the aqueous humor at end of the study (2 hours). Regarding biodistribution, for TBZ and PIRA, in bovine and porcine eyes, a greater amount of product was found in the iris, vitreous humor, choroid and retina. The application of higher concentrations of TBZ and PIRA also resulted in greater retention in all evaluated eyes internal structures. For in vivo study, the concentration found in the workers' tears was between 2.82-455.307ng/mL (PIRA) and 1.05-13.72ng/mL (TBZ). At BCOP, diluted products were classified as uncategorized, not being capable of causing serious damage to the cornea. Concentrated products, as undetermined, where no prediction can be made, been necessary more tests. **Discussion/Conclusion:** Hoon et al., (2023) states that evaluation using whole eyes has many advantages compared to the use of excised corneas in diffusion cells, as chemical compounds also penetrate the eye through the non-corneal route, that is, through the conjunctiva and sclera (TSAI et al., 2018). In relation to the presence of pesticides in tears, highlights the lack of use of personal protective equipment by rural workers, in addition to the lack of washing their hands after handling this equipment.

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Assessment of the toxicity of dimethyltryptamine DMT in an alternative model

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Introduction: Dimethyltryptamine (DMT) is an alkaloid responsible for the psychoactivity of Amazonian plant-based drinks such as ayahuasca and jurema wine, often associated with spiritual and transcendental experiences. Mimosa hostilis (synonymous M. tenuiflora), also known as "jurema-preta", is one of the plants that contain this alkaloid and given its psychoactive activity, the extract of the plant has generated interest among individuals looking for its recreational use, purchasing it online. On the other hand, the toxic effects of DMT have not yet been widely explored, particularly with toxicological preclinical trials. The nematode Caenorhabditis elegans is an alternative model for toxicity assessment studies due to its easy cultivation, short life cycle, and genetic similarities with more complex organisms, including humans. **Objective:** Evaluate DMT toxic effects in an alternative model. **Methods:** C. elegans (wild strain N2) were synchronized and, subsequently, 1500 L1 stage larvae were placed in plastic tubes and exposed for 30 min to concentrations (0.5; 1.0; 1.25; 1.5; 1.75; 2.0 mg/mL) of lyophilized Mimosa hostilis extract/powder dissolved in DMSO and diluted in M9 buffer (total volume of 1 mL), compared to the control groups (M9 buffer) and DMSO vehicle (50 uL). Three independent experiments of the assays of survival, pharyngeal beating, and development were executed in duplicate, the first two carried out in Petri dishes after 24 and 48h, respectively, and the development assay carried out on a slide under paralysis with Levamisole (1Mm), 48h after exposure. **Results:** A significant lethal effect was observed in all concentrations from 1.25 mg/mL; as well as a significant reduction in pharyngeal beats in concentrations of 1.75 and 2.0 mg/mL. There was a significant reduction of body area in all tested concentrations, however, in terms of , only concentrations of 1.0, 1.75, and 2.0 mg/mL were smaller compared to the control group. **Conclusion:** The toxic effects observed impact several parameters fundamental to the development and survival of the alternative C. elegans model. The mechanism under these results might be related to neuronal control, making it important to carry out more complex evaluations using strains with fluorescent labeling for neurotransmitters, enabling a greater understanding of the DMT neurotoxicity. **Acknowledgments:** The authors would like to thank the financial support by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Comparison of New Psychoactive Substances (NPS) acute toxicity using zebrafish (*Danio rerio*) embryo and larvae

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Background/Introduction: The emergence of New Psychoactive Substances (NPS) brings a challenge to analytical toxicology, since the demand for the identification of such molecules is constantly increasing, with new substances appearing on the streets and on web markets on a weekly basis. In toxicology, zebrafish (Danio rerio) assays achieved notability, due to several advantages over other in vivo models. **Objective:** Thus, the aim of this study was to evaluate the acute toxicity of ketamine derivatives [deschloroketamine (DCK) and 2-fluorodeschloroketamine (2F-DCK)] and the synthetic cannabinoid MDMB-4en-PINACA, using in vivo experiments based on embryonic and larval stages of zebrafish. **Methods:** All zebrafish experiments were approved by the Ethical Committee for Animal Research of the University of Campinas (protocol number 6096-1/2022 and 6096-1(A)/2023). To determine the toxicity of NPS in embryonic development stages, the Fish Embryo Toxicity (FET) test was performed following the OECD 236 recommendations, consisting of early exposure of the embryos to the substances. Exposures occurred in embryos between 0- and 4-days post-fertilization (dpf), at doses of 100, 250, 500, 1000, and 2000 µM for ketamine derivatives and 0.001, 0.01, 0.1, 1 and 10 µM for the synthetic cannabinoid. Hence, at least one endpoint was recorded as indicator of lethality and other changes as indicators of non-lethal effects. On the other hand, for larval experiments, the Maximum Tolerated Concentration (MTC) test was performed, with larvae between five and nine dpf, at the same doses tested in FET. For its evaluation, signs of acute locomotor impairment (hypoactivity, absence/decrease of response to touch, and loss of posture), deformations, lack of heartbeat, and death were registered. **Results/Discussion:** For FET, DCK and 2F-DCK produced low embryo mortality at the evaluated concentration range. The endpoints observed were embryos coagulation and lack of somite formation. The non-lethal effects were lordosis, kyphosis, scoliosis, lack of pigmentation, egg hatching delay, blood clotting and pericardial edema. Furthermore, MDMB-4en-PINACA caused high embryo mortality (30%) at 10 µM, in which the endpoints observed were coagulation, lack of heartbeat and lack of somite formation. The non-lethal effects observed in all doses were degrees of lack of tail detachment, lordosis, pericardial edema, yolk edema, and egg hatching delay. For the MTC of ketamine derivatives, 100% of the larvae died at 2000 µM after 96 hours post-exposure (hpe). In contrast, for MDMB-4en-PINACA, only 8% of the larvae died at the highest dose after 96 hpe. Hypoactivity (8%), deformations (8%), and absence of response to touch (17%) were also noted. With the obtained data, the lethal dose (LD50) for MDMB-4en-PINACA at FET was 1896 µM and for DCK and 2F-DCK at MTC, 1028 and 980.5 µM, respectively. **Conclusion:** Hence, it was possible to conclude that acute toxicity of MDMB-4en-PINACA was greater than that observed by ketamine derivatives through FET studies using zebrafish embryos, since this synthetic cannabinoid caused important changes and high mortality at all tested doses. Otherwise, for zebrafish larvae, DCK and 2F-DCK achieved higher morthality in MTC studies. **Acknowledgements:** Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (process number 2022/00037-0) and Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (process number 140157/2022-0).

Cytotoxic effects of the mixture of dicamba and glyphosate herbicides on the intestinal cell line CACO-2

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Introduction: Herbicides are the world's most widely used class of pesticides. Glyphosate (GLI) is the herbicide with the greatest agricultural impact, used in large monocultures. Dicamba (DIC) is commonly used for the same purpose, helping to combat selective resistance in some species, often in combination with glyphosate. The permanence of an isolated compound in the environment can generate an accidental mixture in the future due to the intense application of these substances. Exposure to pesticides can be considered a relevant factor in the development of diseases and poisonings (acute and chronic). The intestinal epithelium constitutes a barrier to these substances, having a complex system of permeability, absorption, and defense. Dysfunction in the homeostasis of this structure has been correlated with several endocrine, neurological, immunological diseases, and psychological disorders. **Objectives:** This work evaluated the cytotoxicity in the intestinal cells CACO-2 after exposure to the mixture of the herbicides Glyphosate and Dicamba **Methods:** The cells were exposed to concentrations of 0.1 to 10,000 mg/L of the isolated herbicides for 24, 48 and 72h to calculate the IC50. After the IC50 value, the cells were exposed to different concentrations of the mixture based on both the IC50 and the DWELL (Drinking Water Equivalent Level). The DWEL for glyphosate is 70mg/L and for Dicamba it is 18mg/L. The experimental groups were: control; M1 = Mixture 1 (5 mg/L glyphosate + 18 mg/L dicamba), M2 = Mixture 2 (25 mg/L glyphosate + 45 mg/L dicamba), M3 = Mixture 3 (70 mg/L glyphosate + 224 mg/L dicamba), M4 = Mixture 4 (124 mg/L glyphosate + 1123 mg/L dicamba), M5 = Mixture 5 (70 mg/L glyphosate + 18 mg/L dicamba). Groups with isolated herbicides were carried out in parallel. The cytotoxicity test with the mixtures was also carried out for 24, 48 and 72h.Cells were seeded in 96-well plates, at a concentration of 2.5x104 and analyzed by the PrestoBlue™ method. Statistics were performed using the GraphPad Prism program, Data were analyzed by the Shapiro Wilk normality test and one- way ANOVA, followed by Tukey's post-test. **Results:** The IC50 for glyphosate was 156.5 mg/L in 24h, 126.3 mg/L in 48h and 124.1 mg/L in 72h. For dicamba, the IC50 was 1560 mg/L in 24h, 1379 mg/L in 48h and 1123 mg/L in 72h. In the cytotoxicity assays with the mixtures and the control group, the M4 group showed cytotoxicity at 48h exposure with a 37% reduction in cell viability with the control and at 72h there was a 56% reduction in cell viability. For the other groups at 24 hours, there was no cytotoxicity in relation to the control group. **Conclusion:** Based on these results, it can be observed that an approximately 10 times higher concentration of dicamba is required to reduce the viability of 50% of exposed cells, compared to glyphosate, corroborating the hypothesis of greater cytotoxicity of glyphosate in relation to dicamba. Possibly, the changes observed in mixture 4 (M4) at 48 and 72 hours could be due to the effect of glyphosate. The results also demonstrate that toxicity increases with longer exposure times, as in the initial time of 24 hours there was no difference between the exposed groups and the control.

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Dermatotoxicology: innovative models of atopic dermatitis

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Background: The Reconstructed Human Epidermis (RHE) is a valuable tool in dermatological research, particularly in the study of atopic dermatitis (AD). AD, affect a significant global population portion (20% of children and 10% of adults), it represents a substantial public health challenge and financial burden on healthcare systems. It is characterized by symptoms like dry skin, intense itching, and eczematous lesions due to an impaired epidermal barrier. The complexity and variability of AD's pathophysiology make it a difficult condition to manage and treat. Therefore, the RHE for atopic dermatitis enable to examine the pathophysiology of AD and is an asset in developing treatments. **Aims:** This project aims to improve the RHE for Atopic Dermatitis (RHEAD) model, based on Hennies and Poumay (2021) research, incorporating innovative approaches like using atopic fibroblast-specific media. The focus is on exploring molecular markers linked to proliferation, epidermal differentiation, and markers altered in atopic dermatitis, to a better understanding and treatment of the condition. **Methods:** We updated the USP-RHE model with cells from healthy individuals (control group - RHE-C), generating three variants: 1. RHEAD-1, is the AD induction group (a cholesterol-lowering protocol and interleukins IL-4, IL-13, and IL-25); 2. RHEAD-2, is the AD induction plus supplementation with conditioned medium from atopic fibroblasts; and 3. RHE-FS, that receive only supplementation with conditioned medium from atopic fibroblasts. We evaluated these models using histology and immunofluorescence to identify markers of normal and atopic epidermal differentiation, such as FLG, LOR, IVL, CK10, CK14, CA2, TSLP, and Ki67. Molecular analyses included RT- qPCR and western blotting to assess mRNA and protein expressions, ensuring a thorough evaluation of the models' ability to mimic epidermal features accurately. **Results:** Morphological analysis revealed epidermal layer alterations typical of AD, with variations in the expression of structural proteins across groups. In RHEAD-1, the cell proliferation rate was consistent, whereas RHEAD-2 and RHE-FS showed statically trend ($p=0,0528$) to increases it, indicating the potential effect of atopic fibroblast-conditioned medium on cell growth. Immunofluorescence showed that RHE-FS displayed a decrease of terminal differentiation proteins (FLG, LOR e INV), while RHEAD-1 and RHEAD-2 were absence, except for FLG in RHEAD-1 and IVL in both groups, in accordance with AD's morphology. We observed a molecular decrease in FLG, LOR, and IVL expressions, alongside an increase in CA2, which may suggest an inflammatory response. **Conclusion:** The research, which developed variations of the RHE model, aims to replicate AD pathology more accurately. Our results revealed an inflammatory process and the decrease of important proteins for epidermal barrier dysfunction in the ADs groups indicating impairment of the epidermal barrier. An alteration in the proliferation rate was observed in the groups that received conditioned medium from atopic fibroblasts, suggesting that conditioned medium from atopic fibroblasts could influence the proliferation of skin cells. **Acknowledgments:** Fapesp, CNPq, CAPES, and Skin Biology Laboratory.

Ethical frontiers: integrating toxicology into development of animal-free skin models

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Introduction: The development of alternative methods to animal tests was a milestone in the life sciences field. It allowed us to fulfill ethical and scientific concerns, leading to advances at speed never seen before, and at a lower cost. However, most of such methods still heavily rely on animal-derived components. Besides ethical concerns, their use also raises concerns regarding relevance of results in studies focused on human health. The replacements of such components by human-derived or chemically defined alternatives are extremely relevant. **Objective:** Therefore, the aim of this project was to evaluate alternatives to animal- derived components used in the development of 3D reconstructed human epidermis (RHE) and full-thickness skin models. In this sense, we aimed to develop a more humanized, ethical, and relevant skin model. **Material and Methods:** Fibroblasts (HDFn) were adapted to an animal-free condition by gradually replacing fetal bovine serum by human platelet lysate and use of animal-free trypsin. Morphological analysis, proliferation rate and gene expression profile of key biomarkers (ki67, cdh2, col1, fn1 and vim) were used to compare the cells. A bioinformatic characterization was carried out for systemic evaluation of the role of these biomarkers in the physiology of the developed model. Animal-free fibroblasts, along with commercially available animal-free keratinocytes were used to develop the full-thickness and epidermal reconstructed skin models. Morphological analysis was performed to compare the control of an animal-free model. **Results:** Adapted fibroblasts maintained morphological characteristics and cell viability, but with reduced doubling time and differential gene expression of biomarkers. When these cells were combined with keratinocytes to develop full-thickness 3D skin models we achieved a tissue that presented better morphology and reduced contraction. In this sense, we have been able to successfully develop a RHE model 100% free of animal derived components. Furthermore, through systems biology evaluation, it was possible to conclude that the animal-free model had a significant improvement in biological processes and molecular functions important for the skin, demonstrating the benefits of using components that are not of animal origin. **Conclusion:** These models could be used in safety evaluation of substances and efficacy tests of cosmetic ingredients improving reproducibility and delivering a truly animal-free test. **Acknowledgment:** Grupo Boticário.

Galleria mellonella larvae as a model to evaluate the effect of psychoactive drugs

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Larvae of *Galleria mellonella*, also known as the "wax moth", have been widely used as an alternative model system for antimicrobial activity studies and in vivo toxicity tests. This insect has been highlighted for fulfilling the principles of the 3 Rs in research ethics: replacement, reduction, refinement. *Galleria mellonella* is also notable for the fact that its immune system shares functional and structural homology with the immune system of vertebrates. In view of the various possibilities for using this animal in experimentation, the aim of this work is to propose the use of *G. mellonella* as an experimental model for evaluating the effect of psychoactive drugs, such as cocaine. Seven groups (n= 10) of last instar larvae weighing between 180 and 260 mg were selected: i) no injection (negative injection control); ii) administration of PBS (negative toxicity control); iii) administration of DMSO (positive toxicity control), administration of cocaine at concentrations of iv) 5 mg/mL; v) 7.5 mg/mL; vi) 10 mg/mL and vii) 20 mg/mL. 10 µL were administered intra- hemocoelically into the last left proleg. These groups were observed for 120 minutes and 120 hours after the injection. The effects experienced by the larvae during the first 60 minutes were: immediate and intermittent hyperexcitation, followed by paralysis with sporadic spasms and finally complete paralysis. These behavioral disorders varied according to concentration: the higher the concentration, the longer they remained in the hyperexcitation phase. The larvae in the lowest concentration group (5 mg/mL) became active again after about 60 min and remained hyperactive for almost 60 min later. Five out of ten larvae in the 10 mg/mL and 20 mg/mL concentration groups died. All the larvae in the positive control (DMSO) died around 5 min after the injection. At the end of the experiment, it was possible to see the stimulating effect of cocaine and to conclude that this abuse drug had a dose-dependent effect on *G. mellonella* larvae and a toxic effect at concentrations of 10 mg/mL and 20 mg/mL. We would like to acknowledge the Goiás State Scientific Police for providing the cocaine sample and the UFG Nuclear Magnetic Resonance Laboratory for quantifying the sample.

Growth regulator flumetralin induces cytotoxicity in 3T3 cells

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Flumetralin is a synthetic plant growth regulator herbicide belonging to the 2,6- dinitroaniline class, widely used in tobacco cultivation to control the growth of axillary buds in the tobacco plant. This study aimed to assess the toxicity of flumetralin in murine fibroblast cells (3T3). Additionally, the effects of flumetralin on the production of free radicals and mitochondrial membrane potential were evaluated. Cytotoxicity was assessed through MTT reduction and neutral red uptake assays after incubation with 0 to 1.0 mg/L of flumetralin for 24 hours. The production of reactive species was determined using DCFH-DA, and mitochondrial membrane potential was measured by fluorescent TMRE probe. It was observed that flumetralin induced concentration-dependent cytotoxicity in 3T3 cells, calculated EC50 values were 1.070 and 1.018 mg/L for MTT reduction and neutral red uptake assays, respectively. Flumetralin also induced the production of free radicals and a loss of mitochondrial membrane potential, which may contribute to the process of cell death. Therefore, it can be concluded that flumetralin exhibited cytotoxicity in 3T3 cells and that generations of reactive species and mitochondrial depolarization are part of the toxicity mechanism of the herbicide.

In vitro development of neonatal epidermis model (Boti Baby Skin®) for safety and efficacy assessment of cosmetics destined for the pediatric public

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Introduction: Neonatal skin remains in a state of tissue maturation for a period extending up to 34 weeks, rendering it more susceptible to chemical irritations and infections compared to adult skin, primarily due to the fragility of its cutaneous barrier. In this context, it is important that products designed for infant skincare are developed with gentle and safe formulations to mitigate the risks of allergies and irritations. In vitro evaluation methods are widely used to analyze the efficacy and safety of cosmetic products, due to their high reproducibility and control. However, even in an in vitro evaluation method, it is necessary to account for all structural, biological, and morphological differences between adult and infant skin. Consequently, the development of an in vitro model that simulates the fragility of neonatal skin is important to assess the safety and efficacy of cosmetic products for children. In this perspective, Boti Baby Skin® is being developed with the aim of providing a sensitive method to support safety and efficacy analysis that should be conducted prior to clinical studies in cosmetic formulations intended for the pediatric public. **Objective:** Development of a reconstructed epidermis model that mimics the morphological characteristics of neonatal skin for evaluating products and ingredients cosmetic designated for children. **Methodology:** A comparative study was carried out between the adult epidermis model and the developing neonatal epidermis model. The adult epidermis model was performed with adult keratinocytes over a 12-day differentiation period. The Baby model was created with neonatal keratinocytes with a reduction in the differentiation period. Comparative histological analyzes (HE staining) were performed to verify the structural difference between the tissues. Cutaneous tolerability trials using cell viability measurement (MTT) were conducted on neonatal skin to observe responses to treatment with the positive control (SDS) and a complex of cosmetic active ingredients intended for the Baby portfolio, with the objective of understanding tissue fragility. Bioinformatic analyzes using systemic biology techniques were carried out to understand the role of the flg, flg2, ivl, krt10, krt14, cdh1, vim and mki67 genes in biological processes related to the skin barrier for experimental validation of the biomarkers. **Results and Discussion:** The results obtained demonstrated that the cellular origin did not influence the sensitivity of the model, but that the differentiation period is directly related to the fragility of the tissue. In the skin tolerability analysis, we observed that with a difference of 24 hours in the differentiation period, viability was significantly reduced (~40%) compared to treatment with the positive control, corroborating the choice of the differentiation period with greater fragility to mimic neonatal skin. For the active complex, viability remained at 100% even in the most fragile tissue. For bioinformatic analyses, the set of genes used as input demonstrated that these biomarkers are related to biological processes, molecular functions and cellular components important for skin development, such as: keratinocyte differentiation, epithelium development, structural activity and correlation with maintenance of keratin filaments. As next steps, we will continue to evaluate the difference in the modulation of these genes between adult and neonatal skin in order to determine the structural differences between the models at a molecular level. **Conclusion:** The implementation of the baby epidermis in vitro corroborates the critical safety analysis that must be carried out before clinical studies with baby cosmetic formulations, especially when evaluating the main differences in the skin barrier between the adult and neonatal model. **Acknowledgments:** Grupo Boticário.

In vitro pharmacokinetics, in vitro – in vivo extrapolations and metabolites identification of eutylone using rat liver microsomes

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Background/Introduction: The determination of pharmacokinetic parameters allows the characterization of the toxicological profile of a wide range of substances. In vitro approaches have gained prominence in recent years, due to their large robustness and lower financial and time investment. Among them, liver microsomes model stands out due to its simple handling, easy obtaining and high throughput assessments of kinetic parameters. Eutylone is considered a synthetic cathinones, being one of the tier one substances for monitoring reported by CSFRE in its latest guidance of 2023. Also, in Brazil, Da Cunha et al. reported the prevalence of New Psychoactive Substances (NPS) in the context of parties and electronic music festivals, which eutylone was highlighted as the sixth synthetic substance most detected. **Objective:** Elucidate kinetic parameters of a long-term prevalent NPS through an in vitro approach using rat liver microsomes (RLM) and in vitro – in vivo (IVIV) extrapolations, in addition to elucidate its metabolites. **Methods:** To evaluate the pharmacokinetic of eutylone, the substance was incubated with NADPH regeneration system and RLM for different times of incubation, concentration of microsomal proteins and initial concentration of eutylone. Reactions were stopped by adding ice-cold acetonitrile and the supernatants injected into a LC-MS/MS. For the determination of unbound fraction of eutylone to plasmatic and microsomal proteins, the substance was incubated in RLM, rat blood plasma and phosphate buffer solution. Solutions were then filtrated using ultrafiltration devices with molecular weight cut-off of 30,000 Da, which the ultrafiltrated were injected into a LC-MS/MS. For the elucidation of the metabolites, the same procedure was employed, considering only phase I (adding only NADPH regeneration system), only phase II (adding only UDPGA, PAPS and SAM) and phase I + phase II (adding both) reactions. Reactions were stopped by adding ice-cold acetonitrile and the supernatants injected into a LC-HRMS QToF. **Results:** Metabolic stability of eutylone allowed the calculation of an in vitro elimination half-life ($t_{1/2}$) of 4.16 min. The kinetic profile of eutylone in RLM presented a hyperbolic profile (Michaelis-Menten model). Through the kinetic curve, parameters such as maximum velocity (V_{max}), concentration at half maximum velocity (S_{50}) and Hill coefficient (H) could be extracted, being 19.40 µmol/mg/min, 4.78 µM and 1.21, respectively. In addition, it was also possible to calculate the in vitro maximum intrinsic clearance (Cl_{max}) and Michaelis-Menten constant (K_m), which presented values of 3.36 mL/min/mg and 3.66 µM, respectively. The unbound microsomal (f_{u-m}) and plasmatic (f_{u-p}) fractions were 0.93 and 0.15, respectively. Additionally, applying constants related to allomeric scale and the values calculated for unbound fractions, IVIV extrapolations were predicted, determining in vivo intrinsic clearance ($Cl_{int, in\ vivo}$) of 8.20 mL/min/kg, hepatic clearance (Cl_H) of 1.29 mL/min/kg and hepatic extraction rate (E_H) of 0.02. We identified eight different metabolites of eutylone, in which four are produced by phase I reactions and four by phase I + phase II reactions (Fig. 2). The molecular reactions were (a) N-dealkylation, (b) demethylenation; (c) beta-ketone reduction, (d) aliphatic hydroxylation, (e) O-methylation and (f) O-glucuronidation. No metabolites were found when only considering phase II reactions. **Discussion/Conclusion:** Eutylone presented a very low metabolic stability in RLM. 3,4- methylenedioxy analogues have been described as substances with brief $t_{1/2}$. Kinetic information demonstrated a Michaelian profile with a slight positive cooperativeness. IVIV extrapolations denotes a potential relevance of other organs in the metabolism of this substance as well as a limited pre-systemic elimination achieved by the first-pass metabolization. Also, for the first time, phase II metabolites of eutylone were reported in literature, which all of them were produced by O-methylation and glucuronide conjugation. **Acknowledgments:** Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (process number 2021/15172-8).

N-ethyl pentedrone (NEP) metabolites elucidation using two different in vivo approaches of zebrafish

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Background/Introduction: The use of animal models represents a powerful approach to study the metabolism of substances, presenting advantages such as a wider comprehensive understanding of the entire organism, as well as the assessment of physiological factors capable of modifying the kinetics and the production of metabolites. Plenty of in vivo models have been used for drugs metabolism evaluation, in which zebrafish demonstrates several advantages related to its easiness handling; anatomic, physiological, and genetic similarities concerning mammals; and the possibility of high-throughput experiments allowing the study of multiple substances simultaneously. N-ethyl pentedrone (NEP) is a New Psychoactive Substance (NPS) with stimulant activity in the central nervous system first identified in the illicit market in the mid-2010s. Anecdotal use reports widespread on the internet since 2016, which four intoxication cases reported in the literature to date, all presenting severe outcomes. **Objective:** Evaluate metabolites production in water and brain tissues of zebrafish after exposure to N-ethyl pentedrone through the Zebrafish Water Tank (ZWT) model. **Methods:** Six-months old male zebrafish (CEUA: 6253-1/2023) were divided into five tanks (8 animals per tank) containing 200 mL of reconstituted water at 30°C. In three tanks 0.5 µg/mL of NEP were added and at the other two only FET water. Additionally, one tank containing only NEP was also prepared to evaluate the NEP stability in FET water along the experiment. Over eight hours, aliquots of exposure water were removed each hour, in addition to an aliquot at the beginning of the procedure. After the 8-hours exposure, animals were euthanized by submerging them into a mixture of ice:water (5:1, m/m) for 40 minutes. Afterwards, zebrafish were decontaminated in ultrapurified water and had their brain tissues removed and organized into pools of five brains per sample. Exposure water metabolites were extracted using MTBE+IS (MDMA-d5 0.6 µg/mL) and TBS after an enzymatic hydrolysis procedure and brain metabolites were extracted in a tissue homogenizer with methanol+IS (methamphetamine-d5 50 ng/mL). The extracted metabolites were injected into a liquid chromatographer-tandem high resolution mass spectrometer (LC-HRMS). **Results:** In the tank without any zebrafish, it was not observed significant degradation of NEP along the entire experiment. However, a decreasing trend was observed in the tanks containing NEP-exposed animals, potentially indicating the kinetic processes of absorption and/or metabolization of substance by zebrafish. Four metabolites were found in exposure water being produced by N-dealkylation (metabolite 1 – M1), aromatic hydroxylation (metabolite 3 – M3), aliphatic hydroxylation (metabolite 6 – M6), and N-dealkylation followed by an aliphatic hydroxylation (metabolite 7 – M7). M1 was the most abundant metabolite found in exposure water. In zebrafish brain tissues, seven metabolites were found which three were also found in exposure water (M1, M3 and M6). Four were exclusively found in the central nervous system and were produced by beta-ketone reduction (metabolite 2 – M2), N-dealkylation followed by beta-ketone reduction (metabolite 4 – M4), aromatic hydroxylation followed by O-glucuronidation (metabolite 9 – M9), and N-dealkylation followed by aromatic hydroxylation and O-glucuronidation (metabolite 11 – M11). **Discussion/Conclusion:** Overall, eight different metabolites were characterized through the two zebrafish approaches. Among the structures found, one was exclusively found in the exposure water and four exclusively found in zebrafish brain tissues. Despite the differences observed, M1 was the most abundant metabolite in both approaches, demonstrating the importance of the N-dealkylation reaction in the metabolization of NEP. Additionally, the abundance of NEP metabolites was determined in brain tissue for the first time in the literature, highlighting the influence and specificities of central metabolism by brain cytochrome P450 (CYP450) enzymes in NEP metabolism. **Acknowledgments:** FAPESP (process number 2021/15172-8).

Use of a 3D model of toxicity shows sensitivity of HepG2 cells to Triclopyr

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Introduction: Two-dimensional (2D) cell culture models have been well established for decades. However, the main obstacle to the complete efficiency of these methods in toxicology remains their partial lack of physiological relevance. Such limitations have led to the development of several technologies for cell cultivation in 3D environments. Cell culture methodologies in three-dimensional models, such as 3D CellFate® matrices made from bacterial nanocellulose hydrogels, can contribute to elucidating possible key toxicological mechanisms and assist in the construction of a conceptual framework ("Adverse Outcome Pathways, AOPs)" of environmental contaminants of emerging concerns. In particular, the herbicide triclopyr, which is already considered an environmental problem due to the high concentrations found, resulting from its incorrect disposal, leaching and aerial dispersion. **Objective:** this work aims to generate data from the culture of HepG2 cells in the 3D CellFate® matrix, produced by the company Biocelltis Biotechnology. In addition to evaluating cell death, nuclear abnormalities and mitochondrial organization induced by triclopyr in vitro using liver cells. **Methods:** HepG2 cells were expanded in monolayers and seeded at a density of 105 cells/mL onto CellFate® 3D matrix in 24-well plates. After cells adaptation to the new environment, they were exposed to triclopyr at concentrations of 5, 50 and 500 µM for 24 hours. Cell viability analysis was performed using the using Live/Dead Cell Imaging kit (Invitrogen, Nº R37601). The cells were stained with Hoechst 33342 (1 µM) and 100 nM MitoTracker Green (Molecular Probes, ThermoFisher Scientific, Cat No. M7514) to assessing nuclear abnormalities and mitochondrial mass organization. An analysis of metabolic capacity by MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5- DiphenyltetrazoliumBromide] was also performed using the same initial procedure, treated with triclopyr, but only with monolayer cells. **Results:** cell morphology in the CellFate® matrix differed from 2D culture. In the hydrogel, the cells formed cellular aggregates linked to the matrix fibers. It is easily notice more elongated cells performing cell-cell and cell-matrix interactions, in addition to high cell viability. In the Live/Dead assay with triclopyr, significance was observed at the highest concentration of 500 µM (average of 73% viability), which was not reflected in the 2D MTT test. Thus, the data suggest greater sensitivity in the 3D model, even because the quantification of cell viability by fluorescence intensity did not accurately reflect what was notice in the micrographs of 50 and 500 µM, indicating much more cell death. The confocal microscopy results suggest nuclear abnormalities at the concentration of 500 µM. Furthermore, it is possible to observe a difference in the organization of mitochondrial network. As doses increase, the mitochondrial network positions itself closer to the positive control, with mitochondria more clustered close to the nucleus. **Conclusion:** high viability, confluence and differentiated morphology were observed when HepG2 cells were cultured in 3D CellFate® matrix. As there are few results in the literature describing the toxicity mechanisms of triclopyr in vitro, it was observed that the compound induced cell damage and death at a concentration of 500 µM, in addition to nuclear abnormalities and mitochondrial dynamics when using the 3D matrix. Although no effect on the ability to metabolize MTT dye was observed in 2D assays. The cell death pathway can occur through nuclear and mitochondrial damage, as was seen with increasing dose. Therefore, the 3D matrix used in this study can open new perspectives as a promising and innovative technology in the development of toxicological models, prospecting for new drugs and biomedical research. **Acknowledgments:** to Biocelltis Biotechnology S.A., biotechnology company focused on the production of biomaterials and human tissues reconstituted in the laboratory, to the TOXICAM laboratory, to the Electronic Microscopy Center – CME and to FAPESP (2020/11128-1 and 2022/03045-4).

08
NEUROTOXICOLOGIA

Acute effects of levamisole, a cocaine adulterant, on neurotransmitters levels in rat brain

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Introduction: Levamisole (LVS) is widely used as a cocaine adulterant and has shown a lot of clinical complications due to this combination with cocaine effects. One of the hypotheses for its use as adulterant is because it seems to change the neurotransmitters levels, specifically of dopamine and serotonin, in some brain regions, such as cerebellum, stem and hippocampus. However, these effects were not evaluated in other brain regions related to the reward system. **Objective:** The aim of this study was to measure the neurotransmitters (NT) dopamine (DA), serotonin (5-HT), gamma-aminobutyric acid (GABA), acetylcholine (ACh) and glutamate (GLU) in prefrontal cortex and striatum of rats after acute exposure to LVS. **Methods:** (Approved CEUA/UFRGS number 34357). Twenty male adult Wistar rats were divided into four groups (n=5/group) and received saline (control group) or LVS by i.p. at the doses of 12 mg/kg, 24 mg/kg and 36 mg/kg (adapted from a protocol OECD 420). Twenty-four hours after the administration all the animals were euthanized and the prefrontal cortex and striatum were dissected for analysis by liquid chromatography/tandem mass spectrometry (LC-MS-MS). **Results:** There was an increase (ANOVA/Bonferroni) in dopamine (DA) levels in the prefrontal cortex of the LVS 12 mg/kg group ($p<0.05$) and in the striatum of the LVS 36 mg/kg ($p<0.001$). However, it is important to highlight that 80% of animals with LVS 36mg/kg died 15 minutes after administration and this result refers to only one animal in the group (survivor). Nevertheless, no significant changes were observed in the levels of 5-HT, GABA, ACh and GLU in these structures. **Conclusion:** Acute exposure to LVS increased the levels of dopamine in prefrontal cortex and striatum of rats and this effect may contribute to this cocaine-related effects. However, more studies are necessary to investigate the relation between levamisole and dopamine in brain. **Acknowledgments:** CREAL-UFRGS, CNPq, CAPES and PROPG/UFRGS for the support.

Assessing the acute toxicity of the synthetic cathinone 4'-Fluoro-α-PHP and its impact on the neural and energetic metabolome in rats

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Background: Synthetic cathinones are an emerging class of psychostimulant drugs used for their rewarding properties that are similar to cocaine and amphetamine-like molecules. The extent and patterns of its use are still unclear and are probably being underestimated. Additionally, studies covering the effects, toxicity, and pathways through which these substances act in the body are still scarce. **Objective:** Therefore, the purpose of this work was to study the mechanism of acute toxicity of the synthetic cathinone 4'-fluoro-α- pyrrolidinohexanephenone (4'-Fluoro-α-PHP) in an animal model. **Methods:** Acute oral toxicity assessments were performed on male Wistar rats. The animals received a single dose (300 mg/kg) of the 4'-Fluoro-α-PHP and were euthanized by decapitation without anesthesia 1, 6, and 24 hours after treatment. Brain regions were dissected, frozen in liquid nitrogen, and stored at -80 °C before sample preparation and analyses. The plasma samples were used for the evaluation of toxicokinetics during the studied times. The neurochemical modulation in rats was measured in brain tissue in an analytical system constituted by a Nexera-i LC-2040C Plus system coupled to an LCMS-8045 triple quadrupole mass spectrometer. The experimental data were statistically analyzed with SPSS 28.0. **Results:** Considering the results, it was possible to determine the plasma concentrations of the drug over time. In the control group, the concentration was zero, consistent with the evaluated group. In the 1-hour group, the peak plasma concentration occurred (0.36 µg/mL) after the exposure time had elapsed; in the 6-hour group, there was a reduction in concentration to 0.11 µg/mL. After 24 hours of exposure, the concentration found was 0.03 µg/mL.In a comprehensive evaluation of brain tissue analysis, the neurotransmitters dopamine (DA), serotonin (5-HT), γ-aminobutyric acid (GABA), and glutamate exhibited decreased levels one hour after oral administration compared to untreated controls. However, in the group evaluated 24 hours later, both DA and 5-HT levels were higher than those observed in the control group. Regarding the DA metabolites, 3-4-dyhydroxyphenilacetic acid consistently displayed lower levels than DA, while maintaining a proportional relationship across the different time points evaluated. In comparison, homovanillic acid did not exhibit lower levels in the groups evaluated at 1 hour and 6 hours; instead, its levels remained proportionally higher than the control group throughout all evaluated time periods. After the analysis the post-test showed that the values of dopamine were higher in the animals of the 24-hour group when compared to the control group, demonstrating a dopaminergic action of the drug. The data for serotonin showed that 5-HT concentrations were different between the groups, and this difference is mainly evidenced in the striatum and in the cerebellum, with serotonin concentrations being more pronounced between the control and 24-hour groups. Data on the acetylcholine, GABA, and glutamate were also analyzed. We only verified a difference for the levels of acetylcholine in the prefrontal cortex (PC), with its concentrations decreasing significantly over time when compared to the control and 24-hour groups. In addition, GABA showed a difference in its concentrations in the hippocampus, cerebellum, PC, and in the ventral tegmental area regions, and its concentrations were decreased when compared to the control and 24 hours groups. **Discussion/Conclusion:** Our results indicate that a single administration of the synthetic cathinone 4-fluoro-PHP is sufficient to alter the concentration of neurotransmitters in rats, resulting in a substantial danger of exposure to these synthetic cathinones that are typically consumed in repeated doses, at higher dosages, and for prolonged periods in humans. Therefore, we considered that the neurochemical modulation that we pointed out after the acute administration of 4'- Fluoro-α-PHP may contribute to the neurotoxicity induced by this cathinone.

Dimethyltryptamine and harmine, components of ayahuasca, isolated and in combination, partially prevented cocaine-induced neurotoxicity in SH-SY5Y cells

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Introduction: Ayahuasca is a psychoactive tea used by the indigenous population of the Amazon in shamanic rituals and by religious groups such as the União do Vegetal and Santo Daime. It has visual and auditory effects, inducts an introspective state, and increases the speed of thought. The tea is prepared by the infusion of *Psychotria viridis* – which contains dimethyltryptamine (DMT) – and *Banisteriopsis caapi* – which contains harmine. DMT is the substance responsible for the psychoactive effects of the tea and it is biotransformed by the intestinal monoamine oxidase. Harmine inhibits this enzyme and allows DMT to interact with serotonin receptors 5-HT_{2A} and 5-HT_{1A} in the central nervous system, acting as an agonist. Several studies investigate the pharmacological potential of ayahuasca in different scenarios, including substance use disorders, such as cocaine. However, the literature lacks information about its neurotoxicity, as well as its neuroprotective potential, which is very relevant when considering ayahuasca's application against substance use disorders. **Objective:** This study aims to evaluate the *in vitro* neurotoxicity of DMT and harmine, isolated and in combination; determine cocaine lethal concentration 50% (LC50); and investigate the neuroprotective potential of DMT and harmine, isolated and in combination, against cocaine-induced toxicity. **Methods:** The study was performed using SH-SY5Y human neuroblastoma cell culture. The concentration-response curves (CRC) for cocaine, harmine, and DMT (isolated and in combination) were determined after 48 hours of exposure. DMT and harmine concentrations tested were 0.1; 1; 10; 100 and 1000 µM, and the DMT:harmine concentrations were 10:10, 10:20, 10:50 and 10:100 µM. Cocaine concentrations tested were 0.5, 1, 2.5 and 5 mM. The LC50 of cocaine was used to verify the neuroprotective potential of the tea substances. All CRCs were evaluated by means of the MTT cell viability assay. Results were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons *post hoc* test. A p-value less than 0.05 was considered statistically significant. **Discussion and Conclusion:** DMT, harmine, and DMT:harmine groups showed no significant toxicity at concentrations ranging from 0.1 to 100, 0.1 to 10, and 10:10 to 10:50 µM, respectively. Cocaine concentration closest to LC50 was 2.5 mM. Non-toxic concentrations of DMT and harmine (10 µM) and their combination (10:20 µM) were used to assess their neuroprotective potential against cocaine-induced toxicity. The DMT+cocaine, harmine+cocaine, and DMT:harmine+cocaine groups showed significant increase in cell viability compared to the cocaine group (69.73%, 74.02% and 67.98%, respectively, *vs.* 45,87%%). Further studies are required to confirm this neuroprotective effect, such as flow cytometry to determine if these substances can prevent apoptosis pathways involved in cocaine cytotoxicity. **Keywords:** dimethyltryptamine, harmine, ayahuasca, cocaine, neurotoxicity, SH-SY5Y. **Acknowledgements:** FAPESP.

Evaluation of acetylcholinesterase inhibition in aqueous extract of flowers and stems of *Miconia albicans*

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Introduction: Acetylcholine is a neurotransmitter widely spread throughout the central and peripheral nervous system, playing a main role in cognitive functions. The acetylcholinesterase is an enzyme responsible for hydrolyzing acetylcholine into acetate and choline, therefore inactivating the action of the neurotransmitter. Studies have been carried out with plant extracts to evaluate the inhibitory potential of acetylcholinesterase (AchE). However, until now, there are no studies about the Miconia albicans AchE inhibition potential. This species is a Cerrado plant used in traditional medicine to treat inflammation, infection, and arthritis due to its anti-inflammatory action. **Objective:** Identify the main compounds and evaluate the AchE inhibitory potential of M. albicans aqueous extract of flowers and steams. **Methods**: Aqueous extract of flowers and stems (AEFS) was obtained by decoction. Chromatographic analyzes were carried out on a LaChrom Elite® Hitachi high-performance liquid chromatograph coupled to a diode array detector (HPLC/DAD), using C18 column (5 µm, 150 x 4.6 mm, LiChroCART® Purospher Star® RP-18 end-capped) after a guard column with the same physicochemical characteristics, kept at 25°C. The elution was performed under a mobile phase composed of 1% of phosphoric acid and acetonitrile in gradient (90:10 until 40 minutes then 70:30 for 10 minutes, 50:50 for 1 min and 4 min back to 90:10, total 55 min), in a flow rate of 0.6 mL/min. Identification analysis were carried out after extracting the 280 and 354 nm chromatograms. The sample was solubilized at 2 mg/mL in water: methanol (1:1) and compared with the standards available in the equipment library constructed in-house. The in vitro assay to evaluate the inhibitory activity of the enzyme was carried out using the method developed by Elman (1961) and modified by Lopez (2002). Galantamine standard was used as positive control at concentration of 0.16 to 5 µg/mL. The sample was evaluated at concentrations of 15.6 to 1000 µg/mL. The reaction was monitored at 405 nm. The results were calculated as the percentages of enzyme inhibition. IC_{50} was calculated using the GraphPad Prism® 8.0 program. **Results**: AEFS inhibited AChE up to 21.9% at the highest tested concentration. The HPLC analyses shows the presence of gallic acid (Rt: 5.27), ellagic acid (Rt: 28.57) and myricitrin (Rt: 32.49), among other non-identified phenolic compounds. **Discussion/Conclusion**: The peak that appeared at 5.27 min (280 nm) was identified as gallic acid with 0.9214 standard similarity index (SSI). At 28.57 min (354 nm), the peak was positively confirmed as ellagic acid with 0.9971 SSI. At 32.49 min (354 nm), myricitrin was found with 0.9919 SSI. Other peaks still need further analysis to identify the unknown compounds. The dose-response AchE inhibition curve was preformed using galantamine as a positive control (IC_{50} = 0.38 µg/mL). AEFS inhibited AchE activity (statistically predicted IC_{50} value of 1400 µg/mL) in a dose-dependent way. The IC_{50} found can be considered a low inhibitory activity. To the best of our knowledge, this is the first study reporting this inhibition of *M. albicans* extract on AchE activity. Therefore, more studies with this and other extracts of this species are needed to better understand this biological property. **Acknowledgments**: University of Brasilia, Graduate Program in Pharmaceutical Sciences, Coordination of Superior Level Staff Improvement – CAPES, Foundation of the Research Support of the Federal District – FAPDF.

MDMA does not elicit behavioral and developmental abnormalities in zebrafish embryo-larvae at low concentrations

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Introduction MDMA (3,4-methylenedioxymethamphetamine) is an entactogen widely used in raves and music festivals owing to its effects on empathy and affiliative social behavior. It has shown promising therapeutic potential notably in post-traumatic stress disorder (PTSD), but there is still concern regarding its toxicity, as most pre-clinical studies have assessed high doses, and little is known about its developmental toxicity. Thus, it is yet to be determined whether MDMA impairs offspring's motor activity and/or elicits abnormalities during development under therapeutic and non-abusive recreational doses. In this context, zebrafish (Danio rerio) emerges as suitable model since it mirrors human's pregnancy, allows for clear visualization from fertilization to hatching and later larvae behavioral assessment in less than a week. **Objective** The aim of this study was to evaluate whether MDMA were to elicit behavioral and/or developmental abnormalities in zebrafish embryo- larvae at low concentrations. **Methods** For eggs harvesting fish were kept overnight in a breeding tank (2:1 ratio male:female) and, in the following morning, fertilized eggs that had reached blastula stage were selected for the experiments. Fish Embryo Acute Toxicity Test (FET) was carried out according to the OECD Guideline No. 236. Briefly, 20 eggs were individually placed in a 24-well plate and exposed to MDMA's solutions prepared in embryo medium (EM) ranging from 50 to 500 ng/mL (negative control: EM, as for all following assays). Embryo-larvae's development was monitored daily from 24 to 96 hours post- fertilization (hpf). Hatching rate was evaluated at 72 hpf and occurrence of coagulation, non-detachment of the tail, lack of somite formation and heartbeat were used to assess lethality. On the fourth day of exposure larvae were transferred to a concave slide, anesthetized with 0,02% MS-222 and immobilized with 1,2% agarose prior to image acquisition using a camera coupled to a stereomicroscope. Measurements of body length, pericardial and eye area were made using DanioScope. Embryonic activity was measured at 26 hpf transferring 20 embryos to a concave slide and recording a 3-minute video after a 5- minute acclimation period. Files were then analyzed using DanioScope to determine tail coiling activity. For the larval photomotor response assay, larvae were transferred to a 96- well plate at 144 hpf and placed inside the chamber of Zebrabox's tracking system. The stimuli configuration consisted of 10 min of acclimation (5:5 light/dark) followed by four 10- minute alternating light-dark cycles. Larvae's photomotor response is here described as total distance travelled and swim speed. All assays were conducted in triplicates. This study was approved by the Faculty of Pharmaceutical Sciences at Ribeirão Preto Animal Use Ethics Commission (Protocol n. 22.1.606.60.3). **Results** MDMA had no effect on hatching time, malformations and lethality under the concentration range tested in the FET test. It increased the pericardial area of larvae from 200 ng/mL to 500 ng/mL but did not alter the body length or eye area. Tail coiling activity of embryos were significantly higher in both 250 and 500 ng/mL but not 50 ng/mL. In the photomotor response assay, larvae exposed to 50 ng/mL of MDMA showed decreased total distance traveled in the light cycles, whereas those exposed to 500 ng/mL swam faster than the control group in the dark cycles. **Discussion/ Conclusion** Embryonic hyperactivity might suggest neurotoxicity, but this feature did not seem to impair the later overall motor activity of larvae. MDMA seems to elicit an anxiolytic-like effect at 50 ng/mL. The obtained results suggest that at low concentrations MDMA does not elicit any significant alterations in development and does not impair embryo-larvae locomotor activity. Further studies should investigate a potential anxiolytic effect under this concentration range. **Acknowledgements** This study was funded by FAPESP (Process n. 2021/14980-3).

Pyriproxyfen affects subcellular structures and reduces neural differentiation during embryonic development

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Background: Pyriproxyfen (PPF) is a larvicide added to drinking water to control arbovirus vectors. However, some studies have already associated the use of PPF with the impairment of the central nervous system in developing vertebrate organisms. Although some effects of PPF on non-target organisms have been described, the impacts on neural cells of developing embryos have not yet been fully elucidated. **Objective:** The aim of this research was to investigate the impact of PPF on subcellular structures and neural differentiation in the forebrain of chicken embryos, which were used as an experimental model. **Methods:** The fertilized eggs were incubated at 37.5ºC temperature and 65% humidity (CEUA-UFSC protocol nº 5843231018). Afterward, the embryos were exposed in ovo at embryonic age E1 (24 h of incubation) at concentrations of 0.01 and 10 mg/L PPF. Control embryos received exclusively DMSO + saline solution (0.00 mg/L PPF). Analyzes were performed at embryonic age E10 (10 days of incubation). The dissected brain was submitted to routine histology for analysis of subcellular structures by transmission electron microscopy and for analysis of protein content related to neuronal (anti-NeuN antibody) and glial (anti-Vimentin antibody) differentiation by immunohistochemistry. The brain was also submitted to analysis of transcript levels related to neuronal (fox3) and glial (vim) differentiation by RT-qPCR. **Results:** Subcellular alterations were observed in embryos exposed to both concentrations of PPF. The alterations observed were rupture of the cell membrane, cytoplasmic vacuolation, loss of mitochondrial cristae, and dilatation of the cell membrane, mitochondrial membranes, perinuclear space and, Golgi and endoplasmic reticulum cisternae. The frequency of alterations in the subcellular compartments of neural cells increased by 47% in the group exposed to 0.01 mg/L PPF and 56% in the group exposed to 10 mg/L, when compared to the control. In the groups exposed to both concentrations of PPF, there was a reduction in the transcript levels related to neuronal and glial differentiation. The content of proteins related to neuronal differentiation was reduced in embryos exposed to the highest concentration of PPF. **Discussion and Conclusion:** Based on the integrative analysis of the results, it is demonstrated that PPF impacts subcellular compartments, potentially influencing cellular processes related to neuronal and glial differentiation. Taken together, these findings reveal that PPF causes damage to the cellular architecture of the brain, and consequently, it may potentially interfere with central nervous system development. **Acknowledgments:** This work was funded by CAPES and CNPq grants. Special thanks to the LAMEB, and LCME multiuser laboratories and the Laboratory of Poultry Science of UFSC/Brazil.

Time-dependent activation of the intrinsic and extrinsic apoptosis pathways in SH-SY5Y cells exposed to ketamine, ethanol, and ketamine-ethanol combination

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Introduction: Ketamine is a drug with analgesic and anesthetic properties used by young adults mainly for its hallucinogenic side effects. It is commonly used in combination with ethanol, which can cause irreversible damage to the central nervous system. Previous results from our research group show an additive neurotoxic effect between ketamine (1 mM – K1) and ethanol (100 mM – ET100). However, the literature still lacks studies on the mechanisms involved in cell apoptosis. **Objective:** This study aimed to evaluate the proteins of the intrinsic (Bax and Bcl-2) and extrinsic pathways (caspase-8) of apoptosis, as well as the levels of both reduced (GSH) and oxidized glutathione (GSSG) in SH-SY5Y cells exposed to K1, E100, and their combination (K1E100) in periods preceding cell death – 3h and 6h. **Methods:** After the exposure periods, cell viability was analyzed using trypan blue dye; Bax, Bcl-2, and caspase-8 were evaluated using the western blotting technique (n=3–5); and GSH and GSSG were analyzed by ELISA. **Results:** Trypan blue assay revealed no significant cell death after 3h and 6h of exposure for all groups. The K1E100 group showed an increase in caspase-8 (239%) and in Bax (194%) after 3 and 6 hours of exposure, respectively. The isolated groups showed no significant differences. Glutathione, GSH, and GSSG levels presented no significant changes during the 3-hour exposure. However, total glutathione and GSH levels decreased after 6h of exposure to both E100 and K1E100. **Discussion/Conclusion:** The K1E100 combination activates the extrinsic apoptosis pathway after 3 hours of incubation and the intrinsic apoptosis pathway after 6 hours. Moreover, the imbalance of GSH levels caused by ethanol exposure suggests that oxidative stress precedes cell death by apoptosis. **Acknowledgments:** PIBIC and FAPESP.

09
SEGURANÇA ALIMENTAR

5-Hydroxymethylfurfural in organic and conventional honey from different municipalities of Paraná-Brazil

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Introduction: Honey is a food that has accompanied and helped humans throughout their historical evolution, serving as a source of food, due to its high energy and pharmaceutical capacity through traditional knowledge. Due to the increasing consumption of honey, either as a sweetener or for medicinal purposes, production has grown. Brazil is the 11th country in honey production, with an average of 42,300 tons in 2018, and has product quality requirements, regulated by the Ministry of Agriculture, Livestock and Supply (MAPA), which imposes quality requirements that ensure that honey is marketed without any change in its natural form. predisposed in Brazilian national standard and by the International standard Codex Alimentarius. These quality requirements serve to ensure that no level of substance is added to honey, such as food additives, but it can often be compromised as limited availability and high cost in the market have increased the risks of adulteration. **Objective:** To investigate the presence of food additives, such as 5-hydroxymethylfurfural, in organic and conventional honey in different municipalities of Paraná-Brazil by gas chromatography coupled to mass spectrometry (GC-MS). **Methods:** Honey samples were collected in different cities of Paraná (Altamira, Marialva, Maringá, Paranacity and Pitanga) in local fairs, small producers and markets, with a total of 7 samples. The collected honey samples were stored in a 50 mL falcon tube at -20 ºC until the analysis of food additive residues. After the extraction of the samples by the QuEChERS method, the identification of 5- hydroxymethylfurfural in GC-MS was performed. **Results and Discussion:** Honey is a natural compound produced by bees, and its composition consists of enzymes, amino acids, organic acids, carotenoids, vitamins, minerals, aromatic substances, flavonoids and phenolic acids. In view of the results found, in samples 3 (conventional) and 4 (organic), the substance 5- hydroxymethylfurfural was detected, which, despite being a compound commonly found in honey, is indicative of adulteration caused by long-term storage in inadequate conditions, alteration in pH levels, overheating or even by the intentional addition of invert sugar for adulteration. Although quantification is ongoing, increased levels of 5-hydroxymethylfurfural are directly related to heating honey to eliminate microorganisms that cause contamination and to decrease viscosity and crystallization that are factors that increase with shelf life. **Conclusion:** However, despite the detection of the compound, it cannot be said that the honey samples from this study were adulterated, since the Codex Alimentarius allows levels of up to 80 mg.kg-1 of 5-hydroxymethylfurfural in honey. The methods for detecting adulterants are complex, and since the consumption of honey is increasing, it is necessary to inspect and evaluate the quality of this product of both small and large beekeepers, aiming at food safety and product quality. **Acknowledgments:** The State University of Maringá (UEM); To the Toxicology Laboratory (LATOX) and to Cesumar University (UniCesumar).

Copper content assessment in samples of sweet commercialized in Minas Gerais, between the years of 2016 and 2023

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Introduction: The production of home made sweets, usually produced in copper pans, are very common in the up-country of Minas Gerais. The copper (Cu) is an essential element for mental and physical health that doesn't present toxicity in your metallic state but some of their salts are toxic to humans. When acidic food is cooked in copper pans without a coating or with a worn coating, toxic concentrations of copper can infiltrate the food. The normative instruction nº 160/2022, stipulates the maximum tolerable limit in fruits jams or sweets in syrup of 10 mg · kg-1. In partnership with the state Sanitary Vigilance, the Ezequiel Dias Foundation implemented an assessment program of food and drinks, where the commercial samples are analyzed, on a fiscal basis, in order to assess compliance with the current legislation, being one important tool for evaluating the quality of the food. **Objective:** Evaluate the quality of the sweets commercialized in Minas Gerais between the years of 2016 and 2023. **Methods:** The samples were weighed, burned in a muffle furnace, digested with nitric acid and quantified in a flame atomic absorption spectrometer (Perkin Elmer, model AANALYST 100). A calibration curve was used in the range of 0.05 to 5.00 mg·L-1, reagents with low metal content and ultra-pure water. **Results:** Between 2016 and 2023, 139 sweets in paste and syrup were analyzed; no unsatisfactory results were obtained among sweets made from pineapple, banana, guava, orange, papaya with cider and peach, with the maximum copper content obtained being 5.7 ·kg-1 referring to papaya jam with cider in 2017. Among the samples with unsatisfactory results, 04 from cider sweets, 65 from fig sweets, 08 from papaya sweets and 23 from rapadura were observed, with 25%, 3.1%, 12.9% and 4.35% of unsatisfactory results, in addition to maximum levels of copper equal to 86.9 mg·kg-1, 19.7 mg·kg-1, 25 mg·kg-1and 4.35 mg·kg-1, respectively. The methodology's quantification limit (QL) is 0.25 mg·kg-1 and for results lower than the QL, for statistical purposes, the value of 0.5 QL was considered. The distribution of copper content in sweets observed was presented in table 01, hereafter. The methodology's quantification limit (LQ) is 0.25 mg·kg-1 and for results lower than the LQ, for statistical purposes, the value of 0.5 LQ was considered. **Discussion/Conclusion:** Between 2016 and 2023, 139 samples of various sweets were analyzed, with 3.6% of unsatisfactory results observed. Among these results, samples of rapadura and cider, figs and papaya sweets showed high levels and/or above the limit recommended by legislation. It is highlighted that the number of samples analyzed decreased over the years in contrast to the maximum copper content. The copper content in sweets can be related to the acceptance of the final product by the consumer. In the case of greenish sweets, a different color from the fresh fruits is not well accepted. Adulteration of the product, through the addition of cupric salts, results in a greenish color that favors its acceptance. It is necessary to monitor these foods, given the widespread consumption and the possibility of adulteration, taking into account the toxic nature of copper salts for human health. **Acknowledgments:** We thank those who, directly or indirectly, contributed to the execution of this work, the MG Health Surveillance and the other sectors of FUNED.

In vitro mycotoxigenic profile of a fungus isolated from Averrhoa carambola

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Introduction: Among the already known risks of star fruit consumption, such as the caramboxin toxin, responsible for causing nephrotoxicity and neurotoxicity in patients with chronic kidney disease, there is also a need to identify secondary metabolites caused by deteriorative and mycotoxigenic fungi. The toxicity of these metabolites has been affecting human and animal life daily due to their acute and chronic exposure through food. **Objective:** The present study aimed to evaluate the mycotoxigenic profile of a deriorant fungus isolated from Averrhoa carambola fruits in vitro by UHPLC-HMRS. **Methods:** After isolating the fungus from the epidermis of fruits incubated for 15 days at room temperature until rot appeared, the isolated fungus was purified and cultivated in potato dextrose medium for 7 – 15 days at 18ºC. After this step, 3 plates with the isolated fungus were cut (1 x 1 cm2), transferred to a falcon tube and kept for 3 h at -80ºC. After stirring for 60 minutes in a homogenizer, 60 minutes in a sonicator with acetonitrile-water-formic acid (84:16:1) and filtered through 0.45 µm PTFE, the filtrate was analyzed in UHPLC-HRMS. The ion chromatogram and MS and MS/MS spectra were analyzed and compared with the literature and open access database, Human Metabolome Database (HMDB). **Results:** Two mycotoxins characteristic of the Aspergillus genus were detected, fumargillin and epoxy-fumitremorgin C, probably produced by Aspergillus fumigatus (in the molecular characterization phase). **Discussion and Conclusion:** Of these mycotoxins, epoxy-fumitremorgin C is a heteropentacyclic organic compound, an indole alkaloid with anti-tumorogenic action. The presence of these mycotoxins poses a risk to animal and human health, but also the fungal isolate, and in particular the isolation of the epoxy-fumitrermorgin C mycotoxin, could be used in the future for pharmacological and toxicological research. The antimicrobial action of fumargillin is well known. The mycotoxins detected were fumargillin and epoxy-fumitrermogin C from the in vitro fungal isolate from star fruit, both with pharmacological and toxicological potential. **Acknowledgments:** funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (Grant# 313035/2022-9), PIBIC/CNPq scholarship for the first author and second author was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. The Laboratório de Toxicologia (LATOX) and Complexo de Centrais de Apoio à Pesquisa (COMCAP), also contributed to the realization of this study.

Mycotoxigenic profile of the fungus isolated from passion fruit (Passiflora edulis flavicarpa) in vitro

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Introduction: Brazil is a country whose climate favors the growth of numerous species of mycotoxigenic fungi such as Fusarium oxysporum. Among the crops most affected by this fungus is passion fruit, which becomes more susceptible in the post-harvest period. Fusarium oxysporum is a natural soil fungus that affects the radical development of the plant leading to rot through the secretion of enzymes that lead to cell invasion, and consequently, apoptosis. There are four main groups of toxins produced by fungi of the genus Fusarium: deoxynivaline, zearalene, fumosins and trichothecenes, which cause mild and severe poisoning in humans and animals, such as: neurotoxicity, hepatotoxicity and carcinogenicity. **Objective:** To investigate the mycotoxigenic profile of the fungus isolated from passion fruit fruits in vitro. **Methods:** For the extraction, 3 plates of potato dextrose agar were used to cultivate the fungus for 15 days at 28ºC, cut (1 x 1 cm2), transferred to a falcon tube and kept for 3 hours at -80°C. Afterwards, stirred for 60 minutes in a shaker and 60 minutes in a sonicator with acetonitrile: ultrapure water: formic acid (84:16:1), filtered with vacuum pump system, and 0.45 µm PTFE filter. To determine the secondary metabolites in vitro, aliquot of the extract was analyzed in UHPLC-HRMS. The ion chromatogram, MS and MS/MS spectra were analyzed and compared with the literature and the Human Metabolome Database (HMDB). **Results and Discussion:** In total, thirteen mycotoxins characteristic of the genus Fusarium were evaluated, namely: fusaric acid, deoxynylvalenol (DON), diacetoxyscirpenol (DAS), fumonisins (A1, A2, B1, B2, B3 and B4), moniliformin, T2 trichothecenes, zearalenone and 15-monoacetoxyscirpenol (MAS). At the end of the analysis, only fusaric acid (C10H13NO2) was identified in the sample. Fuusaric acid can cause alteration of cell growth, cell membrane permeability, inhibition of ATP synthesis and chelation of important cofactors such as iron and zinc. In addition, it can trigger plant defense reactions and programmed cell death, in addition to potentiating the effect of other mycotoxins. In humans, it increases the concentration of serotonin and tryptophan in the brain, which can lead to problems in the individual's health, such as: changes in mental status, anxiety, tachycardia, vomiting, diarrhea, neuromuscular hyperactivity, among other symptoms. **Conclusion:** The mycotoxins produced by the genus Fusarium are varied and act in different ways in the body. From the analysis carried out with the fungus isolated from the passion fruit fruit, fusaric acid was identified, which is a secondary metabolite that potentiates the effect of other mycotoxins and is considered an indicator of contamination in grains and food; They cause harm to human and animal health and to the industry, as it has chemical stability and resistance to boiling, pasteurization and freezing. It is extremely important to be careful and control of fungal contamination at all stages of fruit cultivation, so that the ingestion of contaminated food is avoided, since mycotoxins can cause adverse health effects. **Acknowledgments:** The State University of Maringá (UEM); To the Toxicology Laboratory (LATOX) and to the Complex of Research Support Centers (COMCAP) of UE

Presence and content of cadmium in cocoa and derivatives

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Introduction: Cadmium (Cd) is a non-essential metal whose accumulation in the soil may be related to several natural factors, such as geogenic origin, physical-chemical properties, soil variations, and volcanic activities. Anthropogenic activities, such as the use of fertilizers and pesticides, can also contribute to this accumulation. From the soil, cadmium can be absorbed by plants and, therefore, can be present in different plant structures, including edible portions. In cocoa, cadmium is easily accumulated in the edible parts of the fruit (beans and pulp). **Objective:** This study aimed to assess global cadmium levels in cocoa- derived products. **Methods:** Data on cadmium content in cocoa-based products were obtained through the Global Environmental Monitoring System Database - Food Contamination Monitoring and Assessment Program (GEMS/Food) of the World Health Organization (WHO), obtained from different regions of the world, between 2015 and 2022. Data on sugars, cake mixes, pies, chocolates, candies, and data expressed on a dry basis were excluded. The results were expressed in mg Cd/kg of product, and non-detected values were considered zero. Data were analyzed by descriptive statistics at mean and 95th percentile (p95). **Results:** 15,344 data were extracted from the GEMS/Food database. However, only data on cocoa derivatives were considered from the beginning of the production chain to ready-to-eat products: cocoa beans (n = 1,136), cocoa liquor (n = 106), cocoa butter (n = 15) and cocoa powder (n = 1,958). Cadmium contents in the five types of cocoa-based products ranged from 0.0 to 49.0 mg of Cd/kg of product, with the most significant variation found in cocoa powder. The highest averages of cadmium content were found in cocoa beans and cocoa powder (32.08 and 24.22 mg Cd/kg, respectively). The lowest average levels were in liquor and cocoa butter, being 1.25 and 0.0 mg Cd/kg, respectively. Observing the 95th percentile data, the behavior was similar to the average, with the highest values being for beans and cocoa powder (49.00 and 43.00 mg Cd/kg, respectively) and the lowest p95 values found in liquor and cocoa butter (3.55 and 0.0 mg Cd/kg, respectively). **Discussion/Conclusion:** The processing of beans and cocoa powder can explain the higher cadmium levels in these products compared to liquor and cocoa butter. The reduction of cadmium content in liquor may be related to sorting and blending of cocoa from different producers. During production of cocoa butter and cocoa powder, cadmium tends to be maintained in non-fat cocoa solids, which consequently reduces the amount of cadmium in cocoa butter and increases the levels of cadmium in cocoa powder. Variations in cadmium content for the same cocoa-based product can be explained by differences in cultivars or genotypes that imply the absorption and translocation of cadmium to the cocoa fruit. Furthermore, different cultivation locations, such as in areas whose geological formation is the result of volcanic activities and with consequent deposits of cadmium, can result in greater absorption of the element by the plant. Considering current Brazilian legislation, all products analyzed were above the maximum tolerated limit for cocoa-based products, with a higher value of 0.40 mg Cd/kg of product. Therefore, despite all the health benefits highlighted in recent years, cocoa consumption must be considered due to the presence and levels of metals with the potential to cause toxic effects, such as cadmium, which can lead to kidney failure, bone problems, and reproductive difficulties.
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Results of survaillance pesticide residues in rice and beans in 2023

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Introduction: The high demand for food production on a global scale from 1960 onwards led to the so-called Green Revolution. This process initiated a change in agricultural cultivation aimed at greater productive gains with the introduction of technological devices and the expansion of the use of pesticides in food production. Since 2008, Brazil has become the world's largest consumer of pesticides, and according to IBGE, rice (131.4 g/day) and beans (142.2 g/day) represent 2 of the 3 most consumed foods per capita (POF-2017-2018). Considering the toxicological potential of these substances, monitoring their residues aims to guarantee the population's food security. **Objective:** the objective of this work is to present the results of the analysis of pesticide residues in rice and beans monitored in 2023. **Methodology:** 10 rice and 9 bean samples collected in Minas Gerais in October/2023 were analyzed. Extraction was carried out using the modified QuEChERS method and analysis of 306 pesticides by liquid chromatography with sequential mass spectrometry (LC-MS/MS) and gas chromatography with sequential mass spectrometry (GC-MS/MS). **Result:** Two rice samples were considered unsatisfactory. The presence of pesticides was detected in all samples, with the quantity in each sample varying between 1 and 6 active ingredients. Acetamiprid and Tebuconazole were the substances detected most frequently, 5 and 8 samples respectively, and in higher concentrations, 0.03 mg/kg and 0.04 mg/kg respectively. In the bean samples, 1 sample was considered unsatisfactory. In only 1 sample was the presence of pesticides not detected. The amount of pesticides per sample varied between 1 and 7 active ingredients, with Carbendazim and Procymidone appearing more frequently (4 and 5 samples) and in maximum concentrations of 0.04 mg/kg and 0.36 mg/kg respectively. **Discussion/ Conclusion:** The monitoring purpose is to quantify the residues of regular pesticides and detect the presence of irregular active ingredients. Clothianidin and Chlorpyrifos were detected in the two unsatisfactory rice samples and both are not among the 131 pesticides permitted for this crop. Considering those that appeared in higher concentration and higher frequency, none of the samples exceeded the Maximum Residue Limit (MRL), which is 3.0 mg/kg for Acetamiprid and 6.0 mg/kg for Tebuconazole. Among them, Tebuconazole is considered extremely toxic, possibly non-carcinogenic, but can cause damage to important human organs. In the bean crop, 0.02 mg/kg of Fipronil was detected in a sample exceeding its MRL of 0.01 mg/kg. This substance is considered highly toxic, bioaccumulative, carcinogenic and possible cause of neurological damage. Carbendazim, Procymidone and Imidacloprid were found in higher concentrations, but within the MRL. Among these, Carbendazim has the greatest harmful potential as it is considered carcinogenic, its use was banned in 2022, but its residue will be considered irregular from December 2024. No substance outside the list of 143 permitted pesticides was found. Even though not all pesticides permitted for crops were analyzed, several other active ingredients were analyzed, as the introduction of new substances onto the market changes the use profile. The samples presented unsatisfactory results both due to the amount of residue greater than their MRL, and due to the detection of irregular pesticide residues. This shows that monitoring is an important tool to provide support for assessing dietary risk, therefore it should be reinforced with a goal to expanding the number of pesticides researched in inspected crops, especially when dealing with widely consumed foods.

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SEGURANÇA E SAÚDE AMBIENTAL

A methodological framework for comprehensive assessment of cosmetic product biodegradability

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The discharge of rinsable products can pose a significant threat to effluent pollution, requiring the development of products characterized by a minimized environmental footprint. Furthermore, the rapid biodegradability of cosmetics ingredients assumes paramount importance in this context. This study aimed to establish a standardized methodology for evaluating the biodegradability of cosmetics formulations, leveraging available data on the biodegradability of cosmetic ingredients. The proposed methodology adheres to the criteria set forth by the Organization for Economic Co-operation and Development (OECD) and Regulation (EC) No 1272/2008. To assess the formulation, biodegradability data for each cosmetic ingredient are systematically compiled. Ingredients demonstrating biodegradability exceeding 70% within a 28-day period are deemed biodegradable. The biodegradability profile of each ingredient is weighted against its percentage composition in the overall formulation. A formulation is classified as biodegradable only if at least 70% of its organic composition meets the biodegradability threshold according OECD 301A/E, and no more than 10% of the formulation lacks available data or contains non-biodegradable ingredients. Notably, inorganic ingredients such as water are exempt from this calculation. Through the implementation of this methodology, the study successfully identified cosmetic ingredients with lower biodegradability, thereby revealing opportunities to enhance the biodegradability of cosmetic products. Noteworthy advancements were observed in 2023, with 54% of newly launched rinse-off products exhibiting elevated biodegradability profiles. In conclusion, this research underscores the efficacy of employing a biodegradability assessment methodology as a potent approach for the comprehensive monitoring of Grupo Boticário's rinsable product portfolio. These methodologies provide a robust framework for mitigating environmental impact and steering the organization toward increasingly sustainable practices.

Analysis of four metals toxic to human health related to exposure through mining activities - Brumadinho Minas Gerais

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On January 25, 2019, the BI dam broke at the Córrego do Feijão mine in the municipality of Brumadinho Minas Gerais. The waste sludge from the disaster exposed residents to chemical risks, with the metals arsenic, cadmium, mercury and lead being detected by FioCruz. This work aims to address the health effects of these metals, with guidance on how to reduce exposure to them. A literature review of the four metals will be carried out, addressing their chemical characteristics, sources of exposure and health effects caused by them and ways of mitigating damage and reducing exposure will be proposed. Arsenic is a natural element widely distributed in the Earth's crust and can form a series of poisonous compounds. Arsenic poisoning causes keratosis, skin, lung, prostate, kidney, bladder and liver cancer, gastrointestinal disorders and heart problems. Non-occupational human exposure to arsenic occurs through ingestion of food and water. Therefore, the means of preventing exposure is to subsidize environmental preservation authorities to protect human health and food quality. Cadmium is usually found as a mineral combined with other elements. All soils and rocks, including coal and mineral fertilizers, contain cadmium. Exposure to cadmium produces a wide variety of adverse effects involving many organs and systems. Detecting adverse effects on the kidneys is of fundamental importance in order to prevent more serious effects on the lungs or bones. In acute exposure to humans, the most common effects are inhalation, which affects the lungs, and causes chronic damage to the kidneys, which, after prolonged exposure, are considered critical organs. In the recommendations for human exposure, we find the appropriate management and disposal of cadmium-nickel batteries, fungicides and herbicides and other products with cadmium. A balanced diet is always a preventive measure to avoid ingesting a contaminant through food. Mercury is a highly toxic metal. It penetrates the body through different routes, depending on its form of presentation, and has been used in the manufacture of dental and medical equipment, fertilizers and pesticides. Exposure occurs through fume inhalation, ingestion or contact. Metallic mercury can cause acute poisoning, where respiratory signs and symptoms predominate, and subacute and chronic poisoning, where effects appear on the nervous system, kidneys and skin. In the case of organic mercury compounds, their chronic effects stand out, mainly on the nervous system and teratogenic action. Exposure recommendations address the health aspects of mercury compounds in the context of the healthcare sector, including phasing out mercury-containing medical devices, phasing out dental amalgam, and developing public health strategies to address mercury use in gold extraction. Lead is a widely distributed metal found free and in association with other elements. In contact with the body, it does not undergo metabolism. Contamination routes can be inhalation of fumes, dust and ingestion. Metallic lead compromises several physiological systems, such as the central nervous, hematopoietic, renal, gastrointestinal, cardiovascular, musculoskeletal and reproductive systems. Measures to prevent exposure to lead are within the scope of primary prevention, that is, measures that seek to eliminate or reduce excessive exposure to this metal. The Brazilian mining industry is important, bringing great financial gains, but promoting the pollution of biomes with heavy metals and affecting the quality of life of neighboring communities. These metals are dangerous and can affect health, with many of them having neurotoxic, nephrotoxic and carcinogenic effects. The construction of effective government strategies, at a health level, in the active search for individuals exposed to these metals, thus promoting their identification, specific treatment and monitoring of the sources of exposure to which these individuals are exposed, is of crucial importance.

Chemical composition of total precipitation in an urban and preserved area in the State of Rio de Janeiro

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Airbone particles are considered one of the most important substances in studies of atmospheric processes. The properties and dynamics of clouds, for example, are altered by different types and quantities of particles. As a result, total precipitation has the power to reveal the physical and chemical mechanisms of the atmosphere, identify sources that emit pollutants, and transport and remove them. Therefore, it is possible to assess the safety and health of the environment and, consequently of individuals, based on studies of the composition of total precipitation. The objective of this work is study the chemical composition of total precipitation in two distinct regions of the state of Rio de Janeiro (Gávea and PARNASO), in order to evaluate the impact of anthropogenic activities on the environment (environmental health). Total precipitation sampling was carried out monthly for a period of 12 months. Analysis of ions proceeded by ion chromatography, while metals by ICP-MS.In the Gávea region, the volume-weighted average concentration of ions presented the following distribution in ascending order: $C_2O_4^{-2}$<PO_4^{3-}<F^-<$CH_2(COO)_2^{2-}$<NO_2^-<Br^-<<CH_3COO^-<$CHOO^-$<SO_4^{2-}<K^+<Mg^{2+}<NH_4^+<Ca^{2+}<NO_3^-<Na^+<Cl^-. The lowest concentration was 0.037±0.056 μeq L^{-1} and the highest was 81.4±74.3 μeq L^{-1}. The metals presented the following distribution in ascending order: Cr=Ni=Sn<Mn<Cu<Fe<Pb<Zn. The highest concentration was 0.044±0.12 mg L^{-1} and the lowest was 0.0010±0.0006 mg L^{-1}. In the PARNASO region, the volume- weighted average ion concentration presented the following distribution in ascending order: F^-<NH_4^+<$CH_2(COO)_2^{2-}$<Br^-<$C_2O_4^{2-}$<$CHOO^-$<NO_2^-<CH_3COO^-<Ca^{2+}<Mg^{2+}<SO_4^{2-}<NO_3^-<PO_4^{3-}<K^+<Na^+<Cl^-. The highest concentration was 43±51μeqL^{-1}. Fluoride was not detected. The metals presented the following distribution in ascending order: Cr<Mn<Ni<Fe<Sn<Pb<Cu<Zn. The hightest concentration was 2.9±4.9 mg L^{-1} and the lowest was 0.0010±0.0001 mg L^{-1}. The average acidity in Gávea was 5.73±0.45, while in PARNASO it was 6.05±0.53. The average electrical conductivity was 21.9±1.7 μS cm^{-1} in Gávea and 21.4±0.9 μS cm^{-1} in PARNASO. The concentrations of Na^+ and Cl^- in the Gávea region were much higher than those found in PARNASO. In conjunction with the ratio of these species, the direct contribution of sea spray has been proven. On the other hand, the high concentrations of NO_3^-, SO_4^{2-} and NH_4^+ confirm the greater direct impact of anthropogenic emissions in Gávea, an urban area. The high records of K^+ and PO_4^{3-} in PARNASO are consistent with the natural sources present, mainly the extensive vegetation and soil. Organic acids, even in tiny quantities, contributed to the acidity of the Gávea samples in a more significant way, precisely due to the different and abundant human contributions in the region. Chromium was the only trace element that presented the same concentration in both locations, being naturally present in the soil/rocks and emitted by the resuspension of road dust. Manganese, too, was the only one with a higher average concentration in Gávea compared PARNASO, thus showing the impacts of burning gasoline. The other metals were higher in concentration in PARNASO, especially iron, whose origin is natural. Nickel was associated with the consumption of liquid fuels and coal, while zinc with geological plant materials and micronutrients. Despite being a marker for aerosols, the origin of copper is still quite varied. Tin and lead are linked to the wear of vehicle components, traffic, and fuel additives. Therefore, it can be concluded that the PARNASO region suffered a much smaller impact from human sources compared to Gávea, which consequently implies better air quality and greater environmental safety. Thanks are extended to CBTOX, CNPq, CAPES and FAPERJ.

Hematological profile of individuals exposed to pesticides used in soybean and corn monoculture in the municipality of Belterra in the State of Pará

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Brazil is the largest consumer of pesticides in the world. In the North region, the commercialization of active ingredients with a marked dispersion in different environmental compartments is increasing, some considered highly toxic to the human body. In the state of Pará, the most used classes are insecticides, fungicides and mainly the herbicide glyphosate, widely used in the monoculture of oil palm, soybeans and corn. The penetration routes of pesticides are generally respiratory, dermal and through food ingestion. The objective is to evaluate the complete blood count using the Erythrogram, Leukogram and Platelet indicators in individuals exposed to pesticides in rural communities in the municipality of Belterra in the state of Pará. Ethical opinion 5.452.896, CAAE: 56800122.2.0000.0019 of the descriptive cross-sectional study with n – 284 individuals of both genders, over 18 years of age, with 12 months of living in the territory of rural communities in the municipality of Belterra, located in the mesoregion of lower Amazonas and Microregion of Santarém in the state of Pará. The complete blood count was used as an indicator of exposure and descriptive statistics were performed. Result: Study participants n – 284 (100%), man n – 103 (36.3%) and woman n – 181 (63.70%). The age group of the population 18 – 30 years (n- 30 – 10.65%), 31 – 40 years (n- 37 – 13.02%), 41 – 50 (n- 55 – 19.36%), 51 - 60 (n- 76 – 26.76%), Over 60 years old (n- 85 – 29.92%) and Undetermined (n- 1 – 0.35%). The reference value of red blood cells was used (4.00 to 5.50 million/mm3), blood count below 4 million red blood cells/mm 3 in the age group of 18 – 30 years (n- 7 -2.46%), 31 – 40 years (n- 17 -5.99%), 41 – 50 (n- 14 – 4.93%), 51 - 60 (n- 24 – 8.45%), 60 - 69 years (n- 12 – 4.23%), 70 and More (n- 12 – 4.23%); Blood count above 6 million red cells/mm 3 age group 60 - 69 years (n- 1 – 0.35%). Total leukocytes whose reference value (5,000 to 10,000/mm^3), leukopenia (below 5,000/mm^3) 18 – 30 years (n- 2 -2.11%), 31 – 40 years (n- 4-1.41%), 41 – 50 (n- 4 – 1.41%), 51 - 60 (n- 3 – 1.06%), 60 - 69 years (n- 10 – 3.52%), 70 and More (n - 6 – 2.11%); leukocytosis (above 10,000/mm^3) 18 – 30 years (n- 1 -0.35%), 31 – 40 years (n- 4 -1.41%), 41 – 50 (n- 9 – 3.17%), 51 - 60 (n- 14 – 4.93%), 60 - 69 years (n- 1 – 0.35%), 70 and More (n- 1 – 0.35%); Platelet count with reference value (150,000 to 450,000/mm^3), thrombocytopenia (below 150,000/mm^3) 18 – 30 years (n- 0 -0.0%), 31 – 40 years (n- 0 -0.00 %), 41 – 50 (n- 3 – 1.06%), 51 - 60 (n- 2 – 0.70%), 60 - 69 years (n- 3 – 1.06%), 70 and More (n- 9 – 3.17%); thrombocytosis (above 450,000/mm^3) 18 – 30 years (n- 0 -0.0%), 31 – 40 years (n- 1 -0.35%), 41 – 50 (n- 0 – 0.00%), 51 - 60 (n- 1 – 0.35%), 60 - 69 years (n- 1 – 0.35%), 70 and More (n- 1 – 0.35%). Studies suggest hematological changes, with laboratory changes of erythropenia, leukocytosis and leukopenia, in addition to thrombocytopenia and thrombocytosis. Individuals from rural communities in the municipality of Belterra in the state of Pará are environmentally exposed to organophosphate pesticides in the region, imposing a dangerous coexistence between households and soybean and corn plantations.

Impact of temperature increase on air pollutants - a case study in a small city in southern Brazil

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Background: Approximately 6.7 million deaths worldwide per year may be linked to air pollution. Despite recent studies suggesting that, overall, the levels of atmospheric pollutants are decreasing worldwide, there are specific locations with characteristics that contribute to the increase in these levels. Additionally, climate change has also been a subject of discussion for decades within the environmental context, especially where its impacts are felt most intensely. Brazil has a limited air quality monitoring network, primarily focused on major metropolises. The environmental landscape regarding air quality, pollutant levels, their seasonal patterns, and health implications in small and medium-d cities, as well as rural areas, remains largely unexplored. Small and medium-d cities are infrequently studied in this context. **Objective:** This study aimed to assess the impact of temperature increase on air pollutants, and deaths attributed to air pollution in Dom Pedrito, a small city in the southernmost of Brazil. **Methods:** The city under study was Dom Pedrito, located in the state of Rio Grande do Sul, Brazil. The municipality has a total population of 38,222 inhabitants and a population density of 7.49 inhabitants per square kilometer. The data for air pollutants (O3, PM2.5, and PM10) were manually collected using The Weather Channel application, which is powered by satellite data from the Copernicus Atmospheric Monitoring Service. Meteorological data (temperature, precipitation, wind speed, humidity, atmospheric pressure, and UV index) were obtained from the database of the Instituto Nacional de Meteorologia (INMET). To calculate the number of deaths from cardiovascular diseases attributable to PM2.5, the Sistema Único de Saúde database (DATASUS) was used. All data were for the year 2022. Additionally, scenarios with temperature increases (+2 and +4 °C) were simulated using machine learning to assess the impact of climate change on air pollutants and human health **Results:** The main findings of our study showed that the highest average concentrations of air pollutants are observed in the summer, with 55.58, 5.84, and 8.69 µg/m3 for O3, PM2.5, and PM10, respectively. Moreover, a positive correlation between temperature and O3, PM2.5, and PM10 was identified ($r = 0.59$, $r = 0.18$, $r = 0.18$, respectively, with $p < 0.001$ for all). In the simulated scenarios of temperature increase, the concentration of pollutants O3, PM2.5, and PM10 showed, respectively, an increase of 7.2%, 14.2%, and 12.0% with a 2 °C temperature rise and an increase of 12.0%, 18.4%, and 15.5% with a 4 °C temperature rise. For a 2 °C increase (PM2.5 = 5.58 µg/m3), 1.64 deaths will be attributed to pollution, equivalent to 1.41% of deaths from cardiovascular diseases in adults over 30 years. For a 4 °C increase (PM2.5 equal to 5.78 µg/m3), 2.18 deaths will be attributed to PM2.5 pollution, equivalent to 1.88% of cardiovascular deaths among adults over 30 years. **Discussion/Conclusion:** Summer was the most critical period regarding pollution levels, and the association between temperature and air pollutants was significant, especially for O3. This occurs because ozone is formed from a photochemical process and its concentration in the atmosphere is increased since temperature increase events bring with them greater incidences of solar radiation, increasing its concentration in the environment. In this study, the temperature increase scenario influenced by climate change revealed that PM2.5 pollution could progressively influence cardiovascular mortality, even in a small municipality. **Acknowledgments:** The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) (001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (310856/2020-5) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (310856/2020-5) for promoting this research.

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TOXICOLOGIA ANALÍTICA

A green approach for dispersive solid phase microextraction in the determination of antidepressants in urine employing malt residue as sorbent phase

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Background: Over the last few years, there has been a concern about the development of sustainable analytical methodologies. This is related to the concept of Green Analytical Chemistry, which aims to reduce waste, time of analysis, and exposure of the operator to toxic compounds. Therefore, one strategy employed for the development of greener methods is the use of natural sorbents for solid-based extraction techniques. One natural sorbent of particular interest is malt residue, which is a by-product of beer fabrication. However, its application as an extraction phase in analytical methods has not yet been explored. **Objective:** This study aimed to develop, optimize, and validate a green methodology employing malt residue for the determination of antidepressants in urine samples. **Methods:** Sample preparation consisted of a mixture of 500 µL of urine with 15 mg of malt residue, 150 µL of a NaOH 1 M solution, and 25 µL of the internal standard solution (maprotiline 10 µg/mL). This mixture was agitated and centrifuged for 6 minutes at 9000 rpm. After centrifugation, the supernatant was removed and 500 µL of the desorption solvent (MTBE and ethyl acetate, 1:1, v/v) was added. The mixture was again agitated and centrifuged for 6 minutes at 9000 rpm. Then, 450 µL of the solvent supernatant was removed, transferred to another tube, and evaporated using N2. The extract was reconstituted in 40 µL of acetonitrile and transferred to a vial for injection of 2 µL in the analytical system. Parameters capable of influencing the extraction were optimized through multivariate approaches such as a simplex-centroid design for solvent type and a central composite design for malt residue mass, NaOH, and solvent volumes. Analyses were performed using gas chromatography coupled to mass spectrometry with a total run time of 5 minutes. The method was validated according to the ANSI/ASB Standard 036. In order to prove the applicability of the method, 109 urine samples were analyzed from intoxication cases attended by the Toxicological Information Center of Rio Grande do Sul. The samples were evaluated for the following antidepressants: amitriptyline, desipramine, desvenlafaxine, fluoxetine, imipramine, nortriptyline, paroxetine, sertraline, and venlafaxine. **Results:** The best parameters encountered for sample preparation are the ones described in the previous session. Regarding validation, LLOQs were set as 50 ng/mL for amitriptyline, desipramine, desvenlafaxine, fluoxetine, imipramine, nortriptyline, and venlafaxine and 200 ng/mL for sertraline and paroxetine. Calibration curves were linear between the LLOQ and 5000 ng/mL. Accuracy and intra and inter-day precision coefficients of variation were within the requested limits (< 20%). These conditions were maintained when using dilution ratios of 1:10 and 1:30. Recoveries were satisfactory, ranging between 60.2 % and 133.5 %, except for desvenlafaxine, with 20.6 %. Of the total analyzed samples, 76 were positive for at least one antidepressant. Only imipramine was not detected. The prevalence of substances is as follows: nortriptyline (30.3 %), amitriptyline (28.4 %), fluoxetine (22.0 %), desvenlafaxine (11.9 %), venlafaxine (3.7 %), sertraline (3.7 %), desipramine (2.8 %) and paroxetine (0.9 %). **Discussion/ Conclusion:** The method is proven to be reliable due to validation achievements. This is the first report of malt residue being employed as an extraction phase for a solid-based microextraction, which represents an advance in the replacement of commercial products with more renewable alternatives. Additionally, the applicability of the method was attested by the analysis of urine samples from intoxication cases. The higher prevalence of fluoxetine and amitriptyline and its metabolite nortriptyline are in accordance with data from intoxications monitored the Toxicological Information Center. **Acknowledgments:** The authors would like to acknowledge the financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

A LC-LC-MS/MS method for the monitoring of N-nitrosamines in rifampicin

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In 2020, the presence of N-nitrosamine, specifically 1-methyl-4-nitroso piperazine (MNP), was initially detected in rifampicin capsules. Rifampicin, an antibiotic widely utilized in treating bacterial diseases like tuberculosis, leprosy, and brucellosis, is considered a critical medication, particularly in tuberculosis treatment. Owing to the carcinogenic properties associated with N-nitrosamines, even at minute concentrations (parts per billion levels), global regulatory agencies have directed their attention towards monitoring these contaminants across various pharmaceutical formulations. The United States Food and Drug Administration (FDA) has set a permissible intake threshold of 0.16 parts per million (ppm) for MNP in rifampin, allowing manufacturers to distribute rifampicin containing MNP at concentrations below 5 ppm. Given this scenario, the development of analytical methods capable of precisely quantifying various N-nitrosamines at the low concentrations (ppb) at which they occur in the Active Pharmaceutical Ingredient (API) of the medication becomes crucial. The aim of this study was to design a versatile bidimensional liquid chromatography coupled to tandem mass spectrometry (LC-LC-MS/MS) method for the quantification of N- nitrosamines in pharmaceutical products. Ten prevalent N-nitrosamines were considered: MNP, N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosomethylethylamine(NMEA),N-nitrosomorpholine (NMOR), N-nitrosopiperidine (NPIP), N-nitrosodi-n-butylamine (NDBA), N-nitrosopyrrolidine (NPYR), N-nitroso-di-n- propylamine (NDPLA), and N-nitrosodiethanolamine (NDELA), along with three representative APIs: rifampicin, omeprazole, and prednisolone. The strategy involved optimizing a chromatographic method (LC-LC, heart-cut) to isolate an enriched fraction of N-nitrosamines from the APIs in the first chromatographic dimension, followed by the separation of N-nitrosamines on the second dimension column, and ultimately quantifying them through mass spectrometry. In the first dimension, an OASIS HLB column (2.1 x 30 mm, 20 µm) with a loading solvent of water:methanol 97:3 v/v was employed. A correlation between the logP values of N-nitrosamines and APIs with the experimental retention times were observed. This correlation provided a predictive capability to identify APIs that could be effectively separated from the target N-nitrosamine, guiding the development of an optimal separation strategy. Subsequently, conditions for the second dimension were optimized to ensure the separation of the target N-nitrosamines with subsequent quantification. The most favorable results were achieved using an HSS T3 column (2.1 x 50 mm, 1.8 µm) and a mobile phase with 0.01% formic acid:methanol 80:20 v/v with, employing a gradient elution. The interface utilized was an APCI source operating in positive mode, and quantification in MS/MS was conducted in selected reaction monitoring (SRM) mode. The method was validated and successfully applied for the quantification of MNP and NDMA in rifampicin. The samples were prepared through liquid-liquid extraction using a mixture of water and methanol (95:5 v/v). The validation parameters assessed under the established experimental conditions included linearity (r> 0.999), linear range (3.3 – 6667 ng g-1), quantification limit (3.3 ng g-1), inter-day precision (< 4.5%), and accuracy (92 – 96%). Two rifampicin-containing medications were analyzed, revealing concentrations (mean ± t sc) of MNP at 0.44 ± 0.05 and 2.1 ± 0.3 µg g-1. NDMA was not detected. This method provides several advantages, including analyte concentration and matrix cleanup, reducing sample preparation steps. Moreover, it enables the determination of various N-nitrosamines in diverse APIs beyond rifampicin, guided by the consideration of logP values. **Acknowledgments:** This work was financially by the São Paulo State Research Foundation (grant 2021/03239-0) and CNPq (grants #465768/2014-8 and #304584/2021-5).

A simple and quick method for determination of anorexiants in phytotherapeutics using paper spray ionization mass spectrometry

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Background/Introduction: The issue of drug adulteration extends beyond synthetic formulations and includes herbal medicines (also known as phytotherapeutics), particularly those used for weight loss. The presence of anorectics in adulterated products can lead to serious health risks to users, who may believe that the product is entirely natural. Therefore, they can increase their consumption to suppress their appetite and achieve the desired weight loss effects. As such, identifying these adulterations through forensic methods is crucial. **Objective:** Aim to develop a simple and quick method for identifying and quantifying anorectics in phytotherapeutics. **Methods:** Samples of Aloe vera, Garcinia cambogia, and Spirulina were purchased from local stores and fortified with known concentrations of amfepramone, fenproporex, and sibutramine for optimization and validation of the method. Hence, factorial designs were used to optimize the paper spray ionization mass spectrometry (PS-MS) conditions (temperature and voltage of the ionization source and sample volume) and extraction (type of solvent, percentage of formic acid, and use of ultrasonic energy). After optimization, for the qualitative method, selectivity and LOD (limit of detection) were evaluated and for the quantitative method, the validation parameters were linearity, limit of quantification (LOQ), precision, and accuracy. The method was applied to 38 commercial samples. **Results:** Through the optimization of analysis conditions in PS-MS, it was discovered that temperature and voltage played a significant role in increasing analyte signals. The optimal conditions were determined to be the capillary temperature of 320°C, a paper spray voltage of 3.0 kV, and a sample volume on paper of 18.0 µL. For extraction, all variables were found to significantly affect the analytical signal, with the best condition being the addition of 10 mg of sample, 2.0 mL of acetonitrile containing 2.0 mg/L of the internal standard, and vortexing for 1 minute before collecting the supernatant for PS-MS analysis. The LOD for all analytes in the 3 herbal medicines studied was 10 µg/g, except for sibutramine in spirulina samples, which had an LOD of 25 µg/g. The linear range for quantification was 0 to 5 mg/L in the extract (0 to 2.5 mg/g) with LOQs ranging from 6 to 27.5 µg/g. The method showed good performance with accuracy ranging from 77% to 105% and precision from 3.07 to 5.10%. **Discussion/Conclusion:** Among the commercial samples analyzed, one contained sibutramine. As a result, a simple and quick method (which demands less than 10 minutes) has been developed with appropriate analytical performance for the identification and quantification of 3 anorexiants in phytotherapeutics.
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Analysis of the innovative methods of biological sample preparation for analysis of endocannabinoids and phytocannabinoids

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Background/Introduction: Cannabinoids are being researched worldwide as a prominent treatment of various diseases, such as cancer, pain, inflammation, and neurodegenerative diseases. Phytocannabinoids are already used in the control and treatment of epilepsy, pain, and other conditions, while it has been shown that endocannabinoid levels are altered in a sick state. On the other hand, Cannabis is the recreational drug most used in the world. Thus, the quantifying of these compounds in the biological samples should be in the routine of an analytical lab for different applications, such as therapeutic monitoring, diagnosis of acute poisoning, or forensic purposes. **Objective:** Summarize the recent biological sample preparation developed for the quantification of endocannabinoids and phytocannabinoids. **Methods:** The terms "biological sample" or similar such as "blood sample" or "urine sample" and "cannabinoids" were screened in the databases PubMed, Embase (Elsevier), CINAHL (EBSCO), Cochrane Library, Scopus (Elsevier), Web of Science (Clarivate Analytics), LILACS and SciELO. Articles from 2018-2023 were selected and the including criteria were analyzing endocannabinoids and/or phytocannabinoids, use of a chromatographic method, and report method validation. **Results:** 2518 studies were found and 38 of them were selected, with the including criteria. Articles were excluded if they involved in vitro studies or synthetic cannabinoids. Of all 35 analytes analyzed, THC was the most researched phytocannabinoid, and anandamide was the most researched endocannabinoid. Several studies also researched cannabinoid metabolites, of those, 11-COOH-THC was the most studied. The most utilized biological samples were human (94,7%) and of the conventional types (urine, blood, and its compounds serum and plasma), but some articles (31,6%) used non-conventional samples, like hair and oral fluid. The extraction methods reported were solid phase extraction (SPE) and its variations (SPME, online SPME, IT-SPME, pipette tip SPME, online micro SPE) and liquid-liquid extraction (LLE) and its variations (SALLE and SLE), with SPE being the most utilized. Some of the extraction solvents used were hexane and methanol, with acetonitrile the most utilized of all, and their volume varied greatly, from 0.01 to 7 mL. Liquid chromatography (LC), specifically UHLPC-MS/MS, was the most utilized method, followed by gas chromatography (GC). **Discussion:** Both extractions, SPE and LLE have a wide range of possible procedures, which impacts the final limit of detection of analytes. Selecting the right protocol for the intended analysis requires great research in order to enhance the efficacy of the quantification. In addition, each analysis should be custom-made according to each analyte, equipment and quantification goal. New tendencies should be explored to achieve better results, such as the combination of techniques, the use of microextraction techniques, automatic procedures, and the use of eutectic 'green' solvents, to minimize the environmental impact of analytical labs. **Acknowledgments:** CAPES, CNPq and the Federal University of Santa Catarina for the funding and materials required to make this study.

Application of diffent analytical methods in the identification of hallucinogenic mushrooms

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Introduction: The hallucinogenic mushrooms, popularly known as magic mushrooms, are species of fungus capable of altering an individual's perception of the world. The primary representative group of these mushrooms is the Psilocybe genus, in which the psychotropic effect is caused by the compounds psilocin and psilocibine. These mushroom are usually trafficked dried, making their identification a challenging task. Traditionally, gas chromatography coupled with mass spectrometry (GC-MS) is considered the standard technique for forensic analyses. However, analysis psilocin and psilocibine by GC-MS is difficult as these compounds are thermolabile and are usually in very low concentration on dried samples. Recently, the direct analysis in real time coupled to high resolution mass spectrometry (DART-HRMS) has show great potential in forensic analysis, as it can analyze samples in their native form, and do not require any derivatization step for the analysis of themolabile compounds, as occurs in the GC-MS. **Objective:** Evaluate the applicability of three analytical methods (GC-MS, electrospray-HRMS, and DART-HRMS) in the analysis and differentiation of edible and hallucinogenic mushrooms. **Methods:** Two mushroom samples were analyzed: (1) seized by PCERJ, and (2) a sample of Lyophyllum shimeji. Both samples were evaluated by DART, in which the sample (without pre-treatment) was positioned in front of the ionization source, operating with helium gas at 300°C. In the analysis by GC-MS and electrospray-HRMS, the samples were extracted in two ways: (A) with methanol solvent and assisted by ultrasound, and (B) performed similarly to the second, with the addition of the acidification process using acetic acid. GC-MS was carried out using a DB-5MS column and an ionization energy of 70eV. Before GC-MS analysis, samples were derivatized with MSTFA. For ESI-HRMS analysis by direct injection, an energy of 10eV was used for full scan and 20eV for the fragmentation mode of the target ions. **Results:** Analyses through GC-MS and ESI-HRMS, with or without acidification, did not show the presence of psilocin in any of the samples. DART-HRMS enabled the detection of psilocin (precursor ion m/z 205.1337 and fragment ions m/z 160.1333 and m/z 58.0659) only in sample 1, with an error of less than 5.0 ppm for both ions. **Discussion/Conclusions:** Only DART-HRMS was capable of distinguish the different types of mushrooms evaluated. Such fact can be associate with several factors, such as the low concentration of the psychoactive compounds or even systematic errors associated with the extraction method. As both solvents used in the extraction procedure were successfully applied in the extraction of psilocin by other authors in the literature, it can be hypothed that psilocin concentration in the sample was the decisive factor influencing the different result among the analytical methods. It is worth mentioning that sample 1 was deteriorated when seized. DART ionization enabled the detection of the psychoactive substance, along with high accuracy using coupled HRMS. Therefore, DART- HRMS analysis proved to be a unique technique in addressing the issue of analyzing samples of highly degraded mushrooms.

Assessing the reliability of a urine immunoassay for cocaine detection in plasma: implications for emergency toxicological protocols

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Background: The qualitative analysis of cocaine in urine constitutes a routine element in emergency toxicological protocols. However, the limitations of urine screening tests in detecting recent poisonings or addictions necessitate a more robust approach. In contrast, plasma analysis allows for a nuanced correlation between measured substance levels and the clinical manifestations of substance exposure. Consequently, the imperative of rapid tests for plasma samples in clinical-emergency contexts becomes apparent. Furthermore, it is imperative to underscore the importance of evaluating the feasibility of urine tests in alternative biological matrices, ensuring the broad applicability of the test across diverse clinical and emergency scenarios. This verification process enhances the overall utility and relevance of rapid detection methods for toxic substances. **Objective:** The principal aim of this investigation is to assess the reliability of a urine immunoassay in detecting cocaine and its metabolites within plasma samples obtained from individuals exhibiting signs of intoxication. **Methods:** Plasma samples derived from suspected poisoning cases, attended by the Toxicological Information Center of Rio Grande Sul, underwent screening utilizing the Assure Tech Multi Drug 7 Rapid Test (Zhejiang, P.R. China). This lateral flow immunochromatography-type assay was subjected to statistical determination of various *cut-off* values (5, 20, 40, 80, 125, 200 ng/mL) to ascertain optimal results for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall efficiency. The results obtained from the immunoassay were systematically compared with those derived from Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). The sample preparation for mass spectrometry analyses involved protein precipitation using 50 µL of plasma and 240 µL of methanol:acetonitrile (3:1, v/v), followed by vortex, centrifugation, and injection into the analytical system. Analytes evaluated included cocaine (COC), benzoylecgonine (BZE), and ecgonine methyl ester (EME). The lower limit of quantification for all substances was established at 5 ng/mL. **Results:** A total of 412 samples were incorporated into the study, with 52.4% comprising male individuals and an average age of 18 years (ranging from 0 to 73 years old). Suicide attempts represented the most prevalent exposure circumstance. Optimal sensitivity and specificity were achieved with a 40 ng/mL *cut-off* for BZE as the target analyte, yielding 96.3% sensitivity and 97.9% specificity. However, the results for COC as the target analyte demonstrated unsatisfactory sensitivity, registering 0% from 20 ng/mL onwards. For EME, sensitivity and specificity consistently surpassed 90% across all evaluated *cut-offs*. Nevertheless, the PPV values remained below 40% for all *cut-offs* examined. **Discussion/ Conclusion:** The data unveiled satisfactory reliability for the rapid test when considering BZE as the target analyte in plasma samples, aligning with the assay's design for the detection of BZE, the most prevalent metabolite in plasma. Conversely, the study indicates the unsuitability of utilizing this device for detecting COC, potentially attributable to the limited number of positive samples for COC, representing a notable study limitation. Despite achieving excellent sensitivity and specificity results for EME, the low PPV values underscore that satisfactory outcomes are contingent upon the concurrent presence of BZE in the samples.

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Bidimensional chromatography-tandem mass spectrometry for the determination of N-nitrosamine impurities in losartan

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The contamination of pharmaceutical products by N-nitrosamines, specifically within the 'sartan' family in 2018 (e.g., valsartan and losartan), has raised significant concerns among global regulatory agencies and the pharmaceutical industry. Sartans belong to the class of angiotensin II receptor antagonists, widely used for the treatment of hypertension. N- nitrosamines are recognized for their mutagenic, teratogenic, and carcinogenic properties. The principal N-nitrosamines identified in sartans include N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitroso-N-methyl-4-aminobutyric acid (NMBA), and N-nitrosoethylisopropylamine (NEIPA). In response, regulatory agencies and scientists worldwide have devoted efforts to monitor these compounds. This study aimed to develop and validate a method for determining N-nitrosamines in medicines containing the API losartan. The proposed method utilizes bidimensional liquid chromatography coupled with tandem mass spectrometry (LC-LC-MS/MS). The approach involved isolating an enriched fraction of N-nitrosamines in the first chromatographic dimension concurrently with the retention of the API. For the method development, stationary phases of the chromatographic columns, loading solvent, volume of loading solvent, volume to transfer the N-nitrosamine fraction from the first chromatographic dimension to the second, mobile phase, and column regeneration were evaluated. The optimal conditions were: 1D column: Atlantis T3 column (3.0 x 100 mm, 3 µm), loading solvent: water:methanol 95:5 v/v, loading volume: 1.125 mL, 2D column: HSS T3 column (2.1 x 50 mm, 1.8 µm); volume to transfer the N-nitrosamine fraction from 1D to 2D: 1.215 mL of water added to 0.1% formic acid:methanol, and volume of column regeneration with water:methanol: 0.54 mL. The injection volume was 200 µL. The sample preparation involved diluting 500 mg of the medication in 5 mL of water:methanol 90:10 (v/v), providing a practical and efficient approach for the rapid quantification of N-nitrosamines in medications. The utilized interface was an APCI source functioning in positive mode, and the quantification process occurred through selected reaction monitoring (SRM) mode using internal standardization with isotopically labeled N- nitrosamines. The method was validated for the following N-nitrosamines: NDMA, NDEA, NMBA, and NEIPA. The validation parameters assessed under the established experimental conditions were as follows: linearity ($r > 0.99$), linear range (NDMA and NMBA, 160 – 962 ng g^{-1}, and NDEA and NEIPA, 160 – 256 ng g^{-1}), quantification limit (160 ng g^{-1}), inter-day precision at the LOQ (<5.8%, n=12), and accuracy at the LOQ (NDMA: 94.3%, NMBA: 103.7%, NDEA: 105.6%, NEIPA: 103.3%). The method was suitable for the quantification of NDMA, NDEA, NEIPA, and NMBA, demonstrating selectivity, linearity, precision, and accuracy in compliance with regulatory standards. Furthermore, the analytical technique facilitated the determination of N-nitrosamine concentrations in pharmaceuticals that adhered to the stipulated limits set by the National Health Surveillance Agency (ANVISA). Notably, the quantification levels for NDEA and NEIPA were found to be 1.6 times lower, while those for NDMA and NMBA were approximately six times lower, as elucidated in accordance with Guide No. 50/2021.

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Comprehensive LC-MS/MS analysis of amphetamine- type stimulants and synthetic cathinones in oral fluid samples from electronic music festival attendees

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Background/Introduction: Amphetamine-type stimulants (ATS) and new psychoactive substances, including synthetic cathinone (SC), are among the most consumed illicit substances globally. The main effects of ATS and SC are cardiovascular and neurological neuropsychic disorders. It is extremely important to correctly identify the substances to which an individual is exposed, with the primary objective of minimizing harm and concurrently yielding trustworthy epidemiological data. To achieve this, the application of techniques such as liquid chromatography coupled with tandem mass spectrometry (LC- MS/MS) has demonstrated considerable efficacy, exhibiting notable sensitivity in substance identification. **Objective:** This work aims to develop, validate, and apply a sensitive method for the analysis of amphetamine-type stimulants and synthetic cathinones in oral fluid samples by LC-MS/MS. **Methods:** Oral fluid samples were collected using Salivette® devices. Initially, the extraction was optimized using a multivariate experimental design. A simplex-centroid design was used to select the type of extractor solvent, and the Doehlert design was used to optimize the sample and extractor solvent volume. The final protocol was based on the addition of 200 µL of oral fluid, 20 µL of a solution of MDEA-d5 and amphetamine-d5, 50 µL of NaOH 1M, and 500 µL of MTBE:ethyl acetate (1:1, v/v) into a microtube. The mixture was vortexed for 10 seconds and centrifuged at 9000 rpm for 6 min. A 470 µL aliquot of the organic phase was transferred to a new tube and dried under N2 flow. The dried extract was reconstituted with 30 µL of acetonitrile and 1 µL was injected into the analytical system. The analyses were performed using a LC-MS/MS system (LCMS 8045; Shimadzu, Japan). Chromatographic separation was carried out using a Raptor Biphenyl column (50 mm × 3.0 mm, 2.7 µm), with a total run time of 7 minutes. The mobile phases were water (A) and acetonitrile both supplemented with formic acid 0.1% (B). The method was validated according to ANSI/ASB Standard 036 recommendations. The evaluated parameters were limits of detection (LOD) and quantification (LOQ), linearity, precision, bias, matrix effect, recovery, and integrity dilution. After the full validation, 26 oral fluid samples collected from electronic music festival participants were submitted for analysis. **Results:** The method was optimized and validated for 16 substances (amphetamine, dibutylone, ephedrine, ethylone, eutylone, HMMA, MDA, MDEA, MDMA, mephedrone, methamphetamine, methylone, N-butylpentylone, N-ethyl heptedrone, N- ethylpentylone, and pentylone). The LOD varied between 0.1 ng/mL and 5 ng/mL, while the LOQ varied between 1 ng/mL and 5 ng/mL). The method proved to be linear, obtaining r2 > 0.99 for all substances. Within-run precision ranged from 2.5% (high quality control (QC) of amphetamine) to 12% (medium QC of N-ethyl heptedrone), and inter-run precision ranged from 2% (medium QC ephedrine) to 8.4% (low QC of MDMA). The bias varied from -12.5% (medium QC of HMMA) to 19.6% (medium QC of N-ethyl heptedrone). No significant matrix effect was observed (91.2% to 119.3%). Extraction recovery was greater than 80% for all substances, except for ephedrine (76.8%) and HMMA (43.9%). In the sample analysis, the most detected analytes were MDMA (n=15, 53.8%), MDA (n=9; 34.6%), methamphetamine (n=5; 19.2%), MDEA (n=3; 11.5%), and HMMA (n=2; 7.7%). **Discussion/Conclusion:** A sensitive and simple method was effectively developed and validated. All validation parameters yielded satisfactory results, in accordance with the ANSI/ASB guideline limits. Sample analysis verified a high prevalence of ATS in the recreational scenario, including substances not commonly reported by users, such as methamphetamine and MDEA. **Acknowledgments:** The authors would like to acknowledge the financial support from the Coordination of Improvement of Personal Higher Education, Brazil.

Detection and quantification of CBD, THC and metabolites in oral fluid by LC-MS/MS

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Background/Introduction: Derivatives of *Cannabis sativa L.* have a history of more than 5.000 years of consumption for medicinal, recreational and spiritual purposes globally. Currently, distinguishing between different patterns of cannabinoid utilization requires advances in laboratories. Quantitative analysis of these compounds in oral fluid is emerging as a field of growing interest in laboratory toxicology. Oral fluid has gained recognition in the last decade as an alternative biological matrix for drug detection in forensic and clinical settings. Its sample collection is simple, non-invasive and easily observed, as well as offering resistance to tampering. This matrix eliminates the need for specialized collection facilities or same-sex collectors. This work has the potential to significantly improve clinical and forensic assessment by offering a robust tool to detect and monitor cannabinoid consumption. **Objective:** The aim of this research was to develop and validate an analytical method for detection and quantification of Cannabidiol (CBD), Δ9-tetrahydrocannabinol (Δ9 -THC), and their respective metabolites 7-hydroxycannabidiol (7-OH-CBD), 7-carboxy-cannabidiol (7- COOH-CBD), 11-hydroxy-Δ9 -tetrahydrocannabinol (11-OH-Δ9-THC), 11-nor-9-carboxy-Δ9 - tetrahydrocannabinol (Δ9-THCCOOH), and Cannabinol (CBN) in oral fluid using liquid chromatography-tandem mass spectrometry (LC-MS/MS). **Methods:** The sample extraction was performed of a 500 µL solution of oral fluid elution buffer-Quantisal™ fortified with 50 µL of the working solution containing the internal standard (CBD-d3 and THC-d3 at 500 ng/mL), along with 500 µL of saturated solution of sodium tetraborate and 2 mL of methyl tert-butyl ether (MTBE). The samples were vortexed for 30 seconds and then centrifuged at 4500 rpm for 5 minutes. Subsequently, 1700 µL of the extract was transferred to vials and subjected to drying in TurboVap for approximately 15 minutes. Resuspension was carried out using 100 µL of acetonitrile. Chromatographic separation occurred on a bifenyl Raptor™ column (100 × 2.1 mm ID, 2.7 µm), with a total run time of 7 minutes. **Results/Discussion:** The method showed linearity in the concentration range of 0.5 to 200 ng/mL for all analytes, except for CBN, which showed 0.5 to 100 ng/mL. The calibration curve demonstrated robust linearity, with the coefficient of variation for each concentration not exceeding 10%. The detection limits were established at 0.5 ng/mL for all analytes , sensitive value for detection in authentic oral fluid samples. **Conclusion:** A methodology is under development for the analysis of cannabinoids in oral fluid, employing liquid-liquid extraction and LC-MS/MS. This approach has it significant potential for broader applications in analytical science, showcasing remarkable efficiency in terms of both low sample volume and detection limits. The ongoing development of this methodology indicates promise in enhancing the accuracy and reliability of cannabinoid analysis in oral fluids. **Acknowledgements:** This work was developed with the support of the Coordination for the Improvement of Higher Education Personnel (CAPES) - Brazil through the granting of a master's scholarship, process number: 88887.711761/2022-00 , São Paulo Research Foundation (FAPESP) and National Council for Scientific and Technological Development (CNPq).

Determination of acetylcholinesterase activity in cases of acute poisoning between 2021 and 2023

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Introduction: The mechanism of action of organophosphate and carbamate-based pesticides is to inhibit the activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), enzymes responsible for the hydrolysis of acetylcholine (Ach). Thus, there is an accumulation of Ach which, at high levels, can trigger intoxication. In cases of acute intoxication, the test most often used for diagnosis is the activity of BChE, while AChE is more related to chronic intoxications. However, since 2016, the determination of AChE activity in cases of acute intoxication has been carried out at the Toxicology Research Laboratory of the University Hospital of the Federal University of Santa Catarina (LPTox) as support for cases guided by the Santa Catarina Toxicological Information and Assistance Center (CIATox/SC). **Objective:** The objective was to evaluate the results of acetylcholinesterase activity carried out by LPTox between 2021 and 2023, together with data recorded by CIATox/SC. **Materials and Methods:** A retrospective descriptive study based on the results of toxicological analyses recorded in the laboratory between 2021 and 2023 and data taken from DATATOX. The analyses were carried out using a spectrophotometric method, validated at LPTox, using an adaptation of the one proposed by WOREK et al. The results were tabulated and organized in such a way as to present a descriptive analysis. **Results:** 85 analyses of 72 patients related to cases of acute intoxication were recorded by the LPTox. Of these, 14 were in 2021, 30 in 2022 and 41 in 2023. Values below the reference range were observed in 40 determinations, representing 47%. As for epidemiological aspects, according to CIATox/SC data, 499 cases of poisoning involving exposure to pesticides were recorded in 2021, 443 in 2022 and 717 in 2023 (preliminary data). Of the 72 samples taken, 54 patients were monitored by CIATox/SC and had their data recorded in DATATOX, making it possible to collect more detailed information. Of these, 30 were men and 24 women. In terms of age group, the largest number involved adults over the age of 20, corresponding to 79.6%. Among the circumstances recorded, 68.5% were suicide attempts, 9.25% were accidental, 12.9% were undetermined and 9.25% were other forms. Exposures involved lead in 18 cases, organophosphates in 17 cases, carbamates in three, organophosphates and carbamates in three, and other substances in 13. The outcome of the cases was 39 cured, 6 died and 9 had other outcomes. **Discussion/Conclusion:** There was an increase in the demand for analysis over the three years, demonstrating the applicability of this analysis in emergency cases. AChE inhibition was observed in almost half of the cases, showing that this analysis is applicable to cases of acute intoxication. Of the patients registered, there were more male patients aged between 30 and 39, most of whom had attempted suicide. It is worth noting that all the intoxications of patients under the age of 10 were accidental. There are no confirmatory methods for stating which pesticide is involved; if there is no information on the product ingested, suspicion is based on the cholinergic symptoms caused by the inhibition of Ach and through enzyme activity results. As such, this is a diagnostic confirmation analysis, and any follow-up is carried out when there is no clinical progression. However, it is important to identify the specific agent by chromatographic methods in order to contribute to the patient's expected prognosis and to the development of public policies aimed at reducing access and, consequently, cases of poisoning by these compounds. **Acknowledgments:** HU- UFSC/EBSERH, CIATox/SC and collaborators.

Determination of cortisol and cortisone in urine and saliva by disposable pipette extraction (DPX) and ultra-efficient liquid chromatography tandem-mass spectrometry (UHLPC-MS/MS)

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Higher levels of serum cortisol (primary stress hormone) appear to be associated with anxiety, sleep, and depressive symptoms, which are common non-motor symptoms of Parkinson's disease (PD). However, little is known about the levels of cortisone and cortisol themselves in 24-hour urine samples obtained from PD patients. Additionally, the chemical structures of these substances are similar, which limits their analysis by immunoassay techniques based on specificity. To overcome these analytical difficulties, we have developed and validated a DPX method associated with UHPLC-MS/MS for simultaneously determining cortisol and cortisone (as potential endocrine biomarkers for PD) in 24-hour urine and saliva samples collected before sleep, upon waking, half an hour after waking, and 1 hour after waking from the same PD patient. For urine analysis, 29 PD patients (18 with anxiety and 11 without anxiety) were selected; for saliva analysis, 14 of these 29 patients agreed to participate. For urine samples, the proposed method was linear from 0.5 (LLOQ) to 500 ng/mL (R = 0.9983) for cortisol and from 3 (LLOQ) to 500 ng/mL (R = 0.9972) for cortisone. For saliva samples, the method was linear from 0.5 (LLOQ) to 50 ng/mL (R = 0.9983) for cortisol and from 0.5 (LLOQ) to 50 ng/mL (R = 0.9934) for cortisone. PD patients presented higher urinary cortisone concentration than the control group (healthy volunteers, n = 22), but the same urinary cortisol concentration. Saliva collected from PD patients 1 hour after waking had higher cortisone concentration compared to the control. PD patients with and without anxiety had similar cortisol and cortisone levels in urine and saliva. The method was sensitive and robust for determining cortisol and cortisone in urine and saliva samples obtained from PD patients. The elevated cortisone in these patients may provide relevant information in the context of Parkinson's disease.

Determination of imatinib in capillary plasma spots by LC-MS/MS

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Background/Introduction: Imatinib mesylate (IM), a primary treatment for Chronic Myeloid Leukemia (CML), necessitates careful monitoring due to variations in patient responses resulting from individual differences in pharmacokinetics. Sustaining plasma IM levels above 1,000 ng/ml is associated with achieving major and complete molecular responses, underscoring the importance of therapeutic drug monitoring (TDM). While conventional TDM relies on plasma samples, the utilization of capillary dried plasma spots (DPS) enables at-home collection and presents distinct advantages, including enhanced stability and easier transportation. **Objective:** The objective of this study is to develop and validate an LC- MS/MS method for the quantification of IM in DPS. **Methods:** Three 6 mm DPS discs underwent a two-step extraction protocol. Initially, 150 µL of 0.1% albumin was added, followed by incubation for 30 minutes at 45 °C and 1,500 rpm. The resulting supernatant was transferred to a new microtube and precipitated with 20 µL of trichloroacetic acid 25%. The content was centrifuged and the supernatant reserved aside. A second extraction with organic solvent was performed. In the microtubes containing the imatinib discs, 500 µL of methanol and 10 µL of the internal standard (Imatinibe D-8 2.5 µg/mL) were added. The samples were incubated at room temperature at 500 rpm for 20 minutes, followed by ultrasonication for 10 minutes. After, the organic solvent was transferred to new microtube and dried at 60 °C for 40 minutes. The dried extract was resuspended with 100 µL of MeOH and 100 µL of the supernatant from the first extraction precipitated with trichloroacetic acid. Following homogenization, the extracts were filtered with hydrophilic membrane filter and transferred to insert containing vials. The analysis was performed in a LC-MS/MS Xevo® TQD-micro triple quadrupole mass spectrometer with electrospray ionization in positive mode. The chromatographic separation was performed in a reversed phase C18 column (5 x 2.1 mm, 1.7 um), maintained at 40 °C, eluted with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) eluted at 0.4 mL/min in gradient mode initial 15% B with linear increase to 50% B at 2.0 min and 90% at 3.0 min, kept for 1 min, returning to 15% at 4.5 min. Ionization condition were: capillary voltage 2.50 kV, cone voltage 50 V, dessolvation temperature 500 °C. Dessolvation gas 1000 L/hr and cone 50 L/hr. The MRM transitions for quantification were m/z 494.2 < 394.2 for IM and m/z 502.2 < 394.2 for Imatinib D8, collision energy were 25 V for both analytes, respectively. The method was validated according to FDA, to date specificity linearity, precision/accuracy, sensitivity and selectivity were performed. **Results:** Total run time was 6 min, retention times were 1.2 min for IM and 1.18 min for internal standard. No interferent peak was identified in blank samples. Intra and inter- assay precision results were coefficient of variation (CV%) ranged from 1.8% to 13.0%. Accuracy was 89% to 114.5%. The method was linear from 100 to 3,000 ng/mL with weighted linear 1/x equation y = 0.0027x + 1.265 with r=0.99. **Conclusion:** The method demonstrated satisfactory analytical performance in determining the concentrations of IM in DPS. After concluding the validation tests for matrix effect and extraction yield, the method will be applied in a clinical study comparing IM levels in venous fresh plasma and DPS. **Financial support:** CNPq, CAPES

Development and application of a new sorbent phase derived from hydroxyapatite nanoparticles for the determination of cocaine and related substances in postmortem blood

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Introduction: The consumption and dependence on psychoactive substances are identified as significant modulators of public health parameters, contributing to changes in morbidity and mortality patterns and reflecting an increase in occurrences of pathologies and injuries. In this context, cocaine is included, one of the most abused drugs worldwide, a potent local anesthetic and powerful sympathomimetic agent with stimulating properties on the central nervous system. The determination of cocaine and related products in biological samples plays a prominent role in the field of forensic and clinical toxicology, as it assists in verifying the incidence and circumstances of exposure. Consequently, the development of innovative analytical methods that facilitate the routine work of toxicology services and adhere to the principles of green chemistry becomes an important tool. The use of nanoparticles has been explored in many research fields. Hydroxyapatites are calcium phosphate nanoparticles known for their high adsorption potential due to their high superficial area and unique surface properties that provide interactions with organic compounds. **Objective:** This study aims to develop a dispersive solid-phase extraction technique using hydroxyapatite nanoparticles as an adsorptive phase for the determination of cocaine, benzoylecgonine, cocaethylene, and methyl ester ecgonine (EME) in postmortem blood, combined with LC- MS/MS analysis. **Methods:** The optimized extraction parameters included the desorption solvent, the influence of the pH sample, protein precipitation, and the volume of nanoparticles. Univariate experimental design was conducted for each parameter to determine the best extraction conditions. For the determination of the best desorption solvent, methanol, acetonitrile, and isopropanol were tested in different proportions. A Nexera UFLC system coupled to an LCMS-8045 triple-quadrupole mass spectrometer (Shimadzu, Japan) was used for the analysis and the results were plotted with Statistica® software. The validation process proceeded accordingly with the ANSI/ASB 036 Standard Practices for Method Validation in Forensic Toxicology guide. The parameters validated were the lowest limit of quantitation (LLOQ), linearity, precision, accuracy, and interferences. After validation, the method applicability was tested in discarded postmortem samples provided by the *Instituto Geral de Perícias do Rio Grande do Sul (IGP-RS)*. **Results and conclusions:** The final extraction method involves adding 100 µL of the sample to 20 mg of hydroxyapatite nanoparticles, followed by agitation and centrifugation. The supernatant was discarded, and the desorption solvent, 300 µL of a mixture of acetonitrile and methanol in a 1:1 ratio (v/v), was added. The sample is then agitated and centrifuged again, and the supernatant is injected into the LC-MS/MS. The LLOQ achieved was 10 ng/ml for all analytes. The developed method proved to be linear, where calibration curves have a coefficient of correlation, r^2 above 0.99, applying a weighted least square model. The coefficient of variation of bias and precision was inferior to 20%. Matrix effect results show enhancement in ionization for all analytes except for EME, where ionization has been suppressed. In the majority of analytes, the effects were normalized by the addition of internal standard. The method was applied to 88 pre-tested postmortem samples, in which 100% were positive for cocaine, 89% positive for EME, 96% positive for benzoylecgonine, and 51% positive for cocaethylene. In conclusion, these results indicate that hydroxyapatite nanoparticles have excellent adsorptive properties in the extraction of cocaine and its derivatives in postmortem blood samples, and the method was successfully applied to real case samples. **Acknowledgments:** The authors would like to acknowledge the financial support from the Coordination of Improvement of Personal Higher Education, Brazil.

Development and validation of a bioanalytical method to determine rifapentine in dried-blood spots using high performance liquid chromatography coupled to the ultraviolet detector

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Background/Introduction: According to the World Health Organization, Tuberculosis is one of the leading causes of death around the globe. More recently, the latent tuberculosis infection (LTBI) has caught the attention of researchers due its importance in this scenario and, therefore, measures have been taken worldwide to address this issue. In the context, the drug rifapentine was recently introduced in the Brazilian public health care system. On the other hand, there are few methods available in the current literature to monitor this therapy. Considering this issue, the use of less invasive, painful, and traumatic methods is desirable to apply routinely. **Objective:** Therefore, the aim of this study was to develop and validate a bioanalytical method for the determination of rifapentine in blood samples. **Methods:** The first step was to carry out the ultraviolet spectrum of rifapentine to get the wavelength of maximum absorption. After that, the separation of rifapentine was accomplished using high-performance liquid chromatography (HPLC) coupled to an ultraviolet detector. All assays were carried out using a Shimadzu LC-system equipped with an LC-10ATV pump, SPD-10AVP detector, DGU-12A degasser and a SCL-10AVP system controller. Injections were performed manually employing a Rheodyne injector and the separations were monitored by the LC-Solution software. A RP-18-column (Zorbax Eclipse Plus, 150 mm x 4.6 mm; 5µm) was used to accomplish analyte separation. The dried blood spot (DBS) technique was used for sample collection, which consisted of the adsorption of the blood sample fortified with rifapentine on filter paper. The drug extraction from DBS was fully optimized including extraction solvent and its volume, as well as the stirring speed of the equipment and the time of extraction. Finally, the developed method was validated according to the Brazilian regulatory guideline (RDC 27/2012). **Results:** The separation of rifapentine was carried out in the reverse mode of elution, using methanol:water (60:40, v/v) as mobile phase and ultraviolet detection at 336nm. Under these conditions, total run time was less than 8 minutes. The optimized method extraction included using 1mL of methanol as extraction solvent, 60min of extraction and stirring speed of 1500rpm. Finally, the method was validated in the concentration range of 0.1 to 1.4 µg of rifapentine per mL of blood, which achieved satisfactory results in terms of linearity, residual effect, and dilution test, and displayed good responses in terms of precision, accuracy, and stability test. The coefficients of determination (r2) obtained was > 0.98 and the relative standard deviation (RSD) and the relative error values achieved were lower than 15%. **Discussion/Conclusion:** An affordable bioanalytical method to assay rifapentine in DBS samples was developed and validated, which may be used to evaluate drug levels on routine basis.
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Development and validation of an analytical method with pharmacokinetic application in the light of chemometrics and green chemistry

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Green analytical chemistry (GAC) is an innovative approach that aims to develop analytical methods and techniques that are more sustainable and environmentally friendly. By combining traditional analytical chemistry principles with new, more sustainable aspects, GAC strives to minimize the use of toxic substances, reduce the amount of waste generation, and promote energy efficiency during chemical analyses. The application of chemometrics and experimental design is crucial in the development of more sustainable analytical methods, seeking optimization and adaptation to green chemistry parameters. Through the use of advanced statistical tools, chemometrics allows the efficient analysis of large data sets, identifying patterns and relationships between variables, enabling the precise identification of key variables, allowing a smaller number of experiments to be carried out, thus minimizing production waste. Combining these approaches not only optimizes the development process, but also represents a significant step forward towards more responsible and environmentally friendly analytical practices. This work aimed to develop and validate a bioanalytical method for quantifying simvastatin lactone (SVL) and simvastatin β-hydroxyacid (SVA) in the plasma of patients applying chemometrics through experimental design (DoE) in the optimization of a liquid-liquid extraction (LLE) and assessment of the degree of sustainability of the method using the Analytical Greenness calculator (AGREEprep) software. The method was applied to a pharmacokinetic study designed to assess the effect of a gastric bypass surgery on the exposure to SV and its active metabolite SVA. A LC-MS/MS methodology was developed using an Ascentis Express C18 column (150 × 4.6 mm, 2.7 um), mobile phase in isocratic elution mode (10: 90) water + 0.2% formic acid and methanol, with a flow rate of 0.7 mL/min and a temperature of 40°C. The identification was carried out using a triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source with multiple reaction monitoring (MRM) where two ionization modes were used in two different runs with fluvastatin as the internal standard (IP) in both methods: ESI + for SVL (m/z 441à325) and PI (412à266); ESI - for SVA (m/z 435à315) and PI (410à210). The extraction condition was defined through the use of chemometrics in order to do a smaller number of experiments and minimize the generation of residues. The experimental design was applied to a liquid-liquid extraction (LLE) method, where initially, a fractional factorial design was carried out with 4 factors: ethyl acetate volume, centrifugation time, buffer solution volume and buffer solution pH, in two levels (+ and -) followed by a central composite design with the most significant factors: ethyl acetate volume and buffer solution volume. To maximize the recovery of the analytes SV and SVA in a simultaneous extraction, a desirability test was conducted. The following condition was defined as an optimal extraction condition: 1 mL of ethyl acetate, 173 µL of ammonium acetate buffer solution pH 5 (10mM) and an extraction time of 15 minutes. To assess method sustaintability, the Analytical Greenness calculator software (AGREEprep) determined a substantial sustaitability score of 0.62, considering factors such as solvent toxicity, waste generation, energy efficiency and human safety. While ackowledging analytical limitations, such as energy-intensive equipment and high-cost solvents, the method aligns with green chemistry principles. The method demonstrated selectivity, precision and linearity, with a linear range of 0.3 ng/mL - 40 ng/mL for both SVL and SVA, as defined by the European Medicines Agency guidelines (ICH M10). The validated method was then successfully applied to patient samples, the plasma values analyzed remained within the linearity range of the method and corroborated previous results in the literature.

Development of a green chemistry technique of µQuEChERS for determination of anticonvulsants and antipsychotics in human breast milk employing liquid chromatography with diode array detector (LC-DAD)

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Introduction: Psychoactive drugs are the main agents involved in cases of poisoning according to the Rio Grande do Toxicological Information Center South (CIT-RS) and the National Toxic-Pharmacological Institute Information System (SINITOX). Within the classes of medications involved, anticonvulsants (ACVs) and antipsychotics (APCs) stand out, generating serious effects on cases of poisoning, mainly in neonates and infants via human breast milk (HBM). Globally used for analysis of analytes for pharmaceutical purposes, liquid chromatography with diode array detector (LC-DAD) is the chromatographic technique most widely used in the literature due to its simplicity and low analysis value, facilitating the reproducibility of these methods in any laboratory. **Objective:** The objective is to develop an analytical method by µQuEChERS, using rice husk as a biosorbent in the clean-up stage, to determine anticonvulsants and antipsychotics in human breast milk using LC-DAD. **Methodology:** The extraction technique was based on µQuEChERS. HBM was the biological matrix of choice for the determination of anticonvulsants carbamazepine and lamotrigine and the antipsychotics aripiprazole, chlorpromazine, haloperidol and quetiapine. LC-DAD, the wavelength for analysis, analyzed in the samples was 220 nm for analytes and internal standard (medazepam). In addition, the µQuEChERS was evaluated using the superabsorbent polymer (PSA) and rice husk (RH) as sorbent in the clean-up phase. In addition, the method was optimized by Design of Experiments (DoE) employing 500 ng/mL per analyte considering the amount of natural sorbent, time and speed of the centrifuge. RH was previously standardized physicochemically and morphologically. The chromatographic method used consists of a mobile phase consisting of acid water (H2O + 0.1% trifluoroacetic acid) and methanol (80:20) with a gradient system, with a flow rate of 0.7 mL/min, oven temperature of 45ºC, analytical execution of 12 minutes using chromatographic column (50mmx2,1mm, 3,5µm). **Results:** Results showed that the RH was higher than the PSA of the commercial kit. The data for DoE optimization and validation will be presented in the e-poster as they are currently in development. **Discussion and Conclusion:** The µQuEChERS developed in HBM for the determination of ACVs and APCs is a simple, reliable, robust and reproducible method that corroborates its prosperity in multi-analyte analysis in biological samples for toxicological purposes, among others. Furthermore, the µQuEChERS using RH as biosorbent was more efficient than the commercial kit that uses PSA as sorbent. In addition, the method promises even more advantages if combined with procedures that cause less damage to nature, becoming a technique of "Green Chemistry" because it eliminates or reduces solvents and reagents used in organic syntheses. **Acknowledgments:** CAPES

Development of a methodology for the quantification of aminoglycoside antibiotics in microsamples of capillary plasma using UPLC-MS/MS

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Introduction: The aminoglycosides amikacin and gentamicin are widely used for treating severe infections in critical, septic, and burn patients. Given their concentration-dependent action and a narrow therapeutic range, personalized pharmacokinetic (PK) considerations become crucial. Monitoring the dosing regimen is therefore essential to avoid complications such as ototoxicity and nephrotoxicity. Recognizing the significance of Therapeutic Drug Monitoring (TDM) in these patients, there is a demand for innovative sample collection techniques, like capillary microsampling. In this context, the Tasso-SST®, when attached to the upper limb, facilitates blood collection from capillaries of the skin. Recognizing that hospitalized patients are already debilitated, less invasive collection methods alleviate potential discomfort. **Objective:** This study aims to validate an assay for the determination of amikacin, tobramycin, and gentamicin in small samples of plasma for future use in TDM after collection of capillary blood with the Tasso-SST® device. **Methods:** The analytes were extracted from 100 µL of plasma by adding 50 µL of internal standard solution (kanamycin at 25 µg/mL in acetonitrile), 250 µL of 1% formic acid in water, and 400 µL of acetonitrile. After homogenization for 5 minutes, samples were centrifuged at 12,500 rpm for 30 minutes. An aliquot of 20 µL of the supernatant was injected into the UPLC Acquity® I-Class coupled with a triple quadrupole mass spectrometer Xevo® TQD with positive electrospray ionization. Chromatographic separation occurred on a CORTECS® T3 column (2.7 µm, 3 x 150 mm) at 30 °C. The mobile phase comprised water with 2 mM ammonium acetate and 0.3% formic acid (elution A) and acetonitrile with 0.3% formic acid (elution B). It eluted in a gradient mode with a flow rate of 0.5 mL/min. Quantitation MRM transitions were: m/z 478.3 → 322.2 for gentamicin C1, m/z 450.3 → 322.2 for gentamicin C1a, m/z, 464.3 → 322.2 for gentamicin C2, and m/z 586.3 → 163.1 for amikacin. For kanamycin, the transition was m/z 484.2 → 163.0. The method was validated according to FDA guidelines. **Results:** The analytical runtime was 6 minutes with retention times of 1.0 minute for gentamicin C1, 1.01 minutes for gentamicin C1a, 0.99 minutes for gentamicin C2, and 1.0 minute for amikacin. The method demonstrated selectivity with no interference peaks at the analyte retention times. The lowest limit of quantification was 0.50 µg/mL for total gentamicin and 1.0 µg/mL for amikacin, with accuracy ranging from 94.9% to 112.6%, inter-assay CV% of 1.60% to 2.78%, and intra-assay CV% of 2.74% to 4.02%. The method was linear from 0.5-50 µg/mL ($r > 0.999$) for total gentamicin and 1.0-100 µg/mL ($r > 0.999$) for amikacin. The average extraction yield was 103% and 105% for gentamicin and amikacin, respectively. Matrix effects were compensated by the internal standard and present CV% lower than 7%. Extracts were stable in the autosampler for up to 12 hours. **Conclusion:** The method proved suitable for determining aminoglycoside concentrations in plasma samples. Upon completion of validation tests, it will be applied in a clinical study. **Acknowledgments:** We express gratitude for the financial support from CNPq.

Development of an analytical method: quantification of Acetanimophen in plasma through an innovative smartphone application

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Background/Introduction: Paracetamol (acetaminophen) is an over-the-counter drug widely used for its analgesic and antipyretic properties. However, paracetamol poisoning is one of the most common types of intoxication reported by Poison Control Centers around the world. The most common treatment is the infusion of the antidote N- acetylcysteine (NAC). Routinely in Brazil, paracetamol quantification is done using a UV-Vis spectrophotometer after a colorimetric reaction with the patient's plasma. PhotoMetrix PRO is an application that utilizes the smartphone camera to analyze captured images and to determine the concentration of substances in samples based on their coloration. The application can perform univariate or multivariate analysis and is a useful tool combined with colorimetric toxicological analysis. **Objective:** To develop a low-cost and reliable method to quantify paracetamol concentration in human plasma using the smartphone application PhotoMetrix PRO. **Methods:** The method was validated based on INMETRO guidelines with adaptations. Parameters as limit of detection (LOD), limit of quantification (LOQ), linearity, within- day and between-day imprecision, and bias were evaluated. Calibrators were prepared at concentrations ranging from 25 to 300 µg/mL (LOQ = 25 µg/mL) for the calibration curve and low, medium, and high-quality controls at 40 (LQC), 150 (MQC) and 275 (HQC)µg/mL were monitored in triplicates every day, for five consecutive days. Sample preparation was based on the Glynn and Kendal colorimetric method (1975), but with reduced reagents and samples volumes. This colorimetric reaction changes sample color to yellow. For sample preparation, 250 µL of plasma were transferred to a 2 mL polypropylene tube and 500 µL of trichloroacetic acid were added. The tube was vortexed for 10 seconds, centrifuged for 5 minutes at 4000 rpm, and 500 µL of the supernatant were transferred to a test tube. Then, 250 µL of HCl 6 N and 250 µL of NaNO2 10% was added, followed by vortex agitation for 10 seconds, and resting for 2 minutes. Subsequently, 500 µL of sulfamic acid 10% were added, followed by vortex agitation, and then 1.25 mL of NaOH were added and vortexed. Finally, samples were analyzed on PhotoMetrix PRO in a Galaxy S21 FE smartphone. The application was operated on Vector RGB mode. The region of interest chosen was 32x32, Flash mode Off, Exposure 0, Focus Mode on infinity, auto White-Balance, and resolution of 640x480. A ring light was also used to improve illumination in a wood box to provide homogenous photography. The results were compared later with those obtained from a UV- Vis spectrophotometer (DU-8200 Drawell®) operating in a wavelength of 420 nm. **Results:** Linearity was obtained from 25 to 300 µg/mL (r > 0.99) with limit of detection (LOD) and lower limit of quantification (LLOQ) at 25 µg/mL. Imprecision (% relative standard deviation) and bias (%) were less than 18.5% and 7.2%, respectively. For spectrophotometer analysis, imprecision and bias were better than 13.5% and 8%, respectively. **Discussion/Conclusion:** The developed methodology was successfully validated for paracetamol quantification in plasma using PhotoMetrix PRO. For the next steps, statistical analysis will be performed to compare both methodologies and then, real samples from intoxicated patients will be analyzed.

Acknowledgements: This project is supported by PIBIC (grant number 122962/2023-0). Materials and reagents were also financed by CAPES, CNPq and FAPESP.

Dispersive magnetic solid phase extraction for preparation of biological samples from patients suspected of *Cannabis sativa* poisoning

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Introduction: Cannabis sativa is the most consumed illicit drug globally according to the 2023 World Drug Report from the United Nations Office on Drugs and Crime. In Brazil, its consumption follows this trend. Among its main constituents are the phytocannabinoids tetrahydrocannabinol (THC) and cannabidiol (CBD). A rapid and cost-effective analytical and confirmatory method for the recent detection of cannabinoids in biological samples is necessary for acute poisoning. In this context, the application of magnetic separation technology has proven to be notable for simplifying the procedures involved in sample preparation. Dispersive magnetic solid phase extraction (DMSPE) is based on the manipulation of magnetic nanoparticles to promote separation of the phases, allowing for easier phase separation through simple magnetic decantation. **Objective:** This study aimed the development an analytical method for identifying and quantifying cannabinoids in urine samples from patients with acute Cannabis sativa poisoning through DMSPE and high performance liquid chromatography coupled to diode array detector (HPLC-DAD). **Methods:** Chromatographic conditions were evaluated in a univariate manner and determined by chromatographic resolution and peak symmetry through mathematical calculations. Urine samples were prepared using the DMSPE technique with 200 ng/mL and 20 ng/mL of CBD and THC analytical standards, respectively. Sorptive phases (C18-E, DSC-MCAX, X-AW, and silica) were evaluated by dispersion measurements according to the peak areas measured. Magnetic nanoparticles (MNPs) were synthed from ferric chloride hexahydrate (FeCl3.6H2O) and ferrous chloride tetrahydrate (FeCl2.4H2O), covered by the previously determined sorptive phase. **Results:** The column with stationary phase C18 (250 mm x 4.6 mm x 5 µm) was defined as the best column to separate the studied analytes, since its resolution was 1.42, indicating complete separation. Isocratic elution consisted of the mobile phase A (water) and B (acetonitrile) (10%A:90%B). It was determined that the C18-E sorptive phase had the best THC and CBD extraction. It presented the best THC peak area with a coefficient of variance (CV) of 11.68% and 6.41% for CBD. Satisfactory results were obtained from an extraction performed using synthed MNPs functionalized with C18-E, optimizing the extraction in approximately 30 minutes. **Discussion/Conclusion:** C18-E consists of reverse phase C18 with silica-based end cap providing robust hydrophobic retention while minimizing secondary polar interactions arising from active silanol groups. This sorbent is particularly effective for extracting hydrophobic or polar organic analytes from aqueous matrices, such as urine. Given that both THC and CBD are nonpolar compounds with an affinity for lipophilic substances, it is safe to use a sorbent with strong hydrophobic retention during removal or purification processes. Furthermore, the use of magnetic properties in DMSPE eliminates the need for centrifuges or filtration systems during the extraction process, by separating the extractor phase from the analytical matrix. This results in minimizing sample preparation time and reducing analyte losses. Furthermore, the use of MNPs brings other advantages, such as the need for a reduced amount of extractor phase, and low consumption of organic solvent. These benefits highlight the efficiency and versatility of the DMSPE technique. It was concluded that the DMSPE technique with MNPs coated with C18-E was effective in extracting the cannabinoids under study and it was possible to detect them with the chromatographic technique described. Future perspectives consist of further optimizing extraction to obtain higher peaks in less time and with the smallest amount of sorbent and solvent. **Acknowledgments:** CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CIATox/SC (Centro de Informação e Assistência Toxicológica de Santa Catarina), UFSC (Universidade Federal de Santa Catarina), LPTox II (Laboratório de Pesquisas Toxicológicas) and the PPGFMC (Programa de Pós Graduação em Farmacologia).

Emergency Toxicological Analysis: comparison of a Chromatographic method for screening toxic substances in urine using dispersive Solid-Phase Microextraction (d-SPE) and competitive immunoassay

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Introduction: the medications when used irrationally can be considered toxic agents to humans. In this context, benzodiazepines and tricyclic antidepressants, such as amitriptyline, are listed among the main 10 drugs reported in human cases in the Information and Toxicological Assistance Center of Santa Catarina (CIATox/SC). Regarding drugs of abuse, cocaine is the second most reported substance from this group in CIATox/SC cases. With the increasing cases of intoxication, emergency toxicological analysis is important for diagnosis and prognosis of acute poisonings. **Objective:** compare an analytical method for the identification of toxic substances in urine samples using dispersive Solid-Phase Microextraction (d-SPE) with High-Performance Liquid Chromatography coupled to a Diode Array Detector (HPLC-DAD) (method validated for cocaine, benzoylecgonine, and amitryptiline) and competitive immunoassay (commercial test that detects 12 classes of substances, including cocaine and tricyclic antidepressants). **Method:** 1 mL of collected urine (pH 3.0) was added to a Falcon tube containing DSC-MCAX sorbent phase (60 mg). The tubes were vortexed and then agitated for 5 minutes. The sample was centrifuged for 10 minutes, and the supernatant was discarded. Eluation solvent acetonitrile with 2% ammonium hydroxide (1 mL) was added, followed by vortex and agitation for 5 minutes. After agitation, it was centrifuged, and the supernatant was transferred to an Eppendorf tube for drying. After drying, 100 µL of acetonitrile was added for resuspend and then injected into the HPLC-DAD. Qualitative validation was performed using the UNODC guide for evaluating parameters such as precision, residual effect, stability, and selectivity. The method was applied in duplicate to 20 urine samples from patients with suspected poisoning attended by CIATox/SC for comparison with the d-SPE/HPLC-DAD technique and lateral flow competitive immunoassay tests. **Results:** The residual effect test showed no residues from previous analyses, moreover, satisfactory results were obtained, demonstrating the stability of the analytes. The comparison results with the immunoassay showed a limit of detection (LOD) lower than the immunoassay (100 ng/mL in d-SPE/HPLC- DAD versus 150 ng/mL for benzoylecgonine and 1000 ng/mL for nortriptyline in immunoassay). 13 cases showed results compatible to those observed in the rapid test, while 7 cases detected cocaine and benzoylecgonine that were not previously detected by the immunoassay (false-negative). Moreover, when comparing the clinical condition of patients with the results obtained by the d-SPE/HPLC-DAD method, coherence is observed. **Conclusion:** It is a quick and sensitive method, considering that the LOD values for cocaine and amitriptyline were below those reported in the immunoassay package insert. It is low cost and uses minimal sample and organic solvent, contributing to green chemistry. It allows the simultaneous preparation of multiple samples from different patients. Moreover, other substances can be included in the technique if they have similar physicochemical characteristics to the presented analytes. **Acknowledgments:** To PIBIC/CNPq, CIATox/SC, LPTox, and UFSC.

Enhancing NPS information: screening of urine samples obtained at an electronic music party using new technologies

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Background/Introduction: Given the cooperation agreement with the National Drug Board of Uruguay – Faculty of Chemistry, Udelar and continuing with the drug monitoring program, the Environment, Drugs and Doping Unit (UMADD) of the Technological Pole Institute of Pando in 2019, carried out the third edition of the study "Screening of new psychoactive substances, THC and cocaine in urine samples obtained at a rave party in the metropolitan area." To improve the information obtained, it is necessary to seize the potential of the new technologies to have different technologies that allow the identification of new psychoactive substances which might represent a potential danger for public health. Furthermore, the relationship and communication with colleagues in the region as well as having exchange programs that facilitated the research were crucial. **Objective:** The objective was to expand and improve the information, using new technologies, on the circulation in the Uruguayan territory of New Psychoactive Substances and other drugs of abuse which are consumed at these events, as well as to begin an active cooperation with colleagues in the region. **Methods:** 28 samples were analysed by three extraction techniques, in different physicochemical conditions allowing the extraction of drugs of diverse nature, considering neutral, basic, acidic substances and those excreted as metabolites and/or conjugates. Each of the extractions was then analysed by LC-MS/MS (LC8045, Shimadzu) and LC-HRMS (microOTOF-QIII, Bruker). For LC-MS/MS, the injected sample volume was 1 µL, while for HRMS, it was 5 µL. The chromatographic method was the same for both instruments, with the use of a Shimadzu Velox column (2.1 x 5mm, 2.7 µm) eluted with (A) water and (B) acetonitrile, both fortified with 0.1 % of formic acid. The flow was maintained at 0.3 L/min in gradient mode as it follows: 0-10 min: 2-35% of B; 10 to 16 min: 35-95% of B; 16 to 18 min: 95% of B; 18 to 18.5 min: 95-2% of B; 18.5-25: 2% of B. Triple quadrupole analyses were performed in multiple reaction monitoring mode (MRM) with electrospray ionization parameters of: nebulizing gas flow: 3 L/min; interface temperature: 300º C; heating gas flow: 10 L/min; DL temperature: 250º C. HRMS analyses were performed in positive mode and mass range of 50 to 1000 Da, with electrospray parameters of: nebulizing gas flow: 3L/min, capillary voltage: 4500V; dry temperature: 250º C; dry gas: 4 L/min. **Results:** As notable results in the screening, MDMA, MDA, MDEA, Cocaine, Ketamine were found in 100% of the samples. In addition, it was possible to identify the presence of Dipentylone, Methylone, Eutylone, Pentylone and Pentedrone. **Discussion/Conclusion:** This study allowed us to compare the results obtained by GC/MS against LC/MS/MS and LC-HRMS. Although there is a high percentage of coincidences in them, it was possible to identify new substances that later gave rise to poisoning in Uruguay. Also, MDMA and synthetic cathinones are highly correlated to poisonings in this scenario in Brazil. We also could evaluate the presence of eutylone, which was a rising drug by the year of 2019 in Brazil, according to apprehension data. This information is of utmost relevance for public health Public Health, both in Brazil and Uruguay, since they are substances that are not always seized and are the cause of poisoning. **Acknowledgments:** It is necessary to express my gratitude to the Universidade Federal de Ciências da Saúde of Porto Alegre, Department of Pharmacosciences, and to the Faculty of Chemistry of the University of the Republic of Uruguay, that allowed this research to be carried out through the teaching exchange program (720).

Green Analytical Toxicology procedure for determination of ketamine, its metabolites and analogues in oral fluid samples using dispersive liquid- liquid microextraction (DLLME)

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Background/Introduction: New psychoactive substances (NPS) are synthed via small changes in the molecular structure, producing drugs whose effect and potency are not yet fully known. Ketamine is one of the oldest NPS, with therapeutic use authorized in several countries, being metabolized mainly into norketamine and 6-hydroxy-norketamine. Furthermore, two structural analogues of ketamine have recently been identified, deschloroketamine and 2-fluorodeschloroketamine, marketed as drugs of abuse. To comply with Green Analytical Toxicology (GAT) fundamentals, miniaturized techniques such as dispersive liquid-liquid microextraction (DLLME) are employed to determine toxicants in biological fluids. **Objective:** This work aimed to develop an analytical method and validate it to determine ketamine, its metabolites (norketamine and 6-hydroxy-norketamine), and its analogues (2-fluorodeschloroketamine and deschloroketamine), using DLLME and liquid chromatography-tandem mass spectrometry (LC-MS/MS). **Methods:** To reach out the best extraction conditions for DLLME, the type of solvent used was optimized by a complete factorial design in two levels and two parameters (2^2). To determine the amount of each solvent, an optimization by a 2^3 rotational central composite design was performed. DLLME extraction consisted of two hundred microliters of oral fluid samples transferred to 1.5 mL polypropylene conical tube, followed by 20 µL of the IS solution and 100 µL of sodium tetraborate saturated aqueous solution. The tube was closed and stirred in a vortex for 10 s for homogenization. To promote DLLME, 100 µL of the dispersive solvent was added, followed by brief vortex agitation for 10 s. Then, using a glass syringe, 50 µL of the extracting solvent were rapidly added. This content was then stirred in vortex for 30 s and centrifuged at 18,407 g for 5 min. After centrifugation, 45 µL of the bottom phase were transferred to a glass vial and the contents were dried up under nitrogen flux at 30 °C. The residue was reconstituted in 100 µL of methanol, stirred in vortex for 10 s, and transferred to conical glass insert, and 0.5 µL were injected into LC- MS/MS. The method has been validated in agreement with the recommendations of the Standard Practices for Method Validation in Forensic Toxicology. Authentic oral fluid samples (n=29) were collected from individuals present at festivals and parties held in Brazil, between September 2018 and January 2020, and analyzed by the present methodology. **Results/Discussion:** Regarding method optimization, the best conditions were achieved with 200 µL of sample, 100 µL of methanol as dispersive solvent, and 50 µL of chloroform as extractor solvent. Linearity was obtained from 10 to 1,000 ng/mL, with limit of detection (LOD) and lower limit of quantification (LLOQ) at 10 ng/mL. Imprecision and bias (%) were less than 8.2% and 9.5%, respectively. The matrix effect did not exceed 10.6%, and the recovery values varied from 24% to 42%. No matrix interference and good selectivity in the evaluation of ten different sources of oral fluid and 42 drugs at 500 ng/mL, respectively, were observed. The 29 oral fluid samples were positive to at least one analyte from this study. The method also had its green characteristic evaluated by three different tools. **Conclusion:** A fast extraction method by DLLME for quantification of ketamine, its metabolites and analogues was developed, optimized, validated, and applied to biological samples. Following the GAT principles, several advantages were obtained, related to the miniaturization of the extraction by DLLME, low volume of organic and sample solvents, low cost, few steps, and little time required for extraction. This method was evaluated based on three tools to evaluate its green characteristics, with satisfactory results. **Acknowledgments:** FAPESP (process number 2020/07470-6 and 2020/10809-5) and CNPq (process number 315640/2021-9).

Identification of alcohols in oral fluid by rapid colorimetric test

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Introduction: According to the World Health Organization (WHO), ethanol is responsible for the world's highest mortality rate in traffic accidents. With the increasing consumption of alcoholic beverages, which may cause cases of ethyl alcohol intoxication, in addition to the voluntary or accidental intake of methanol, isopropyl alcohol and ethylene glycol, it is necessary to identify these alcohols through the analysis of biological matrices. Blood collection is obtained invasively, therefore, the use of oral fluid as an alternative matrix is recommended, especially because it is non-invasive and there is a good ratio of ethanol dosage in blood and oral fluid. With the exponential increase of intoxications by the alcohols cited and the tests available for their identification in matrices are high cost and not fast, it is of paramount importance to use a simple test, low cost, which can be performed on site and with quick presentation of its result. **Objective:** To determine ethanol, methanol, isopropyl alcohol and ethylene glycol in oral fluid samples in order to verify the exposure to such alcohols. **Methods:** Oral fluid samples were collected without any device, based on the free release of oral fluid in a falcon tube. The method involves a rapid colorimetric test with the addition of 1% potassium dichromate solution in 6M sulfuric acid inside microtubes with oral fluid containing alcohols. To improve the method and obtain quicker results, equipment microwave time was tested, ranging from 5 to 10 seconds. The aim was to catalyse the reaction and obtain results in a short period of time. The microwave time was used at high power. **Results:** In synthesis, the rapid colorimetric test was performed with 200 µL with 1% potassium dichromate solution in 6M sulfuric acid, added in microtubes, with approximately 200 µL of oral fluid fortified with the alcohols separately, each alcohol in a microtube and a blank sample with the acid solution and oral fluid as the negative reference sample for comparison. The concentrations used for each alcohol ranged from 0.18 g/dL to 4.5 g/dL, until the cut off was found. The cut off of ethanol was 0.6 g/dL, methanol 0.5 g/dL, isopropyl alcohol 1.7 g/dL and ethylene glycol 4.5 g/dL. The samples were vortexed and placed in a microwave equipment for 10 seconds at 10 watts, then left to cool to room temperature for 5 minutes and placed again for 10 seconds and left to cool to room temperature for 10 minutes. After the reaction between the sample collected and the solution, in addition, with the heating, in a few minutes, it was possible to identify each alcohol, if the individual has performed such intake. When the result was positive, the color in the sample was blue and when negative, it was orange. Possible interferents such as acetonitrile, acetone, lactic acid, ethyl ether, chloroform, formaldehyde were evaluated and no one interference was observed. **Discussion and Conclusion:** A technique involving a rapid colorimetric test showed that the concentration of potassium dichromate in 6M sulfuric acid, followed by heating in equipment microwave proved to be efficient in the identification of ethanol, methanol, isopropyl alcohol and ethylene glycol, generating quick, results with low cost. The proposed technique has not yet been validated according to validation guidelines. However, validation of method is the next step to guarantee future data from this methodology, comparing to GC-FID analysis to confirm alcohol presence. Furthermore, the obtained results will serve as a basis for possible intoxication cases. **Acknowledgments:** CAPES and PIBIC-CNPQ.

Multielementar methodology for the quantification of metals in brazilian sage incense smoke using ICP-MS

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Incense smoke can be regarded as a gas-solid colloidal dispersion, containing fine and ultrafine particles, as well as toxic gases such as HCN, CO2, CO, H2S, and compounds with acute mutagenic capacity, such as heavy metals, benzo(a)pyrene and PAHs. Numerous studies in the literature underscore the toxic effects of metals, predominantly through respiratory tract exposure. Consequently, the presence of toxic metals in incense smoke emphas the imperative to formulate robust methodologies for the detection and quantification of these elements. The objective of this study was to establish a comprehensive methodology for quantifying metals in Brazilian Sage incense smoke through ICP-MS. Four distinct types of incense sticks with a Sage fragrance were assessed in this investigation. An apparatus was devised for collecting incense smoke based on the Health Canada T207 method with modifications. In this system, a glass column was employed, connected to two glass bubblers containing a diluted HCl:HNO3 solution (40 and 50 mL, respectively) under a strong airflow. A vacuum pump was affixed to the system's end, responsible for directing the airflow into the system, thereby bubbling the smoke into the digestion solution contained in the bubblers. The resultant extract was subsequently transferred to a plastic bottle, where an aliquot of 2,5 mL of HF and 2,5 mL of H2O2 were added. Digestion was conducted in an ultrasonic bath at 80 ºC for 40 minutes; the obtained extracts were then filtered, diluted and analyzed by an Agilent 7800 ICP-MS integrated with Mass Hunter® 4.3. The digestion solution was fortified with an Au197 standard to monitor potential losses during the collection process. Forty-seven elements were evaluated in collisional He mode through external calibration using an analytical curve and in semi- quantitative mode with calibration by a correction factor. Out of the forty-seven elements analyzed, thirty-five elements achieved recoveries between 40% and 120% in quantitative mode through external calibration with an analytical curve, and twenty-three elements in semi-quantitative mode. The metals Mn55, Cu63, Ga71, Ba137, Ce140 and Nd146 could be quantified in quantitative mode, while in semi-quantitative mode, Ti47, V51, Co59, Ni60, Cu63, Sr88, Ag107, Cd111, Ce140, Pr141, Nd146, Sm147, Eu153, Gb157, Tb159, Dy163, Ho165, Er166, Tm169, Yb175, Lu175, Bi209 and Th232 were quantified with concentrations exceeding the limit of quantification. The presence of these metals in the studied incense smoke may be associated with cross-contamination during the production process or the quality of the raw materials used, given that the production process is typically artisanal. While this study may provide pertinent data for future investigations into metal determination in Brazilian incense smoke, certain parameters necessitate further examination, such as applied airflow, extractive solution volume, and matrix effects for analytes like Ge72, As75, Se78, Se80, Mo95, and Rh103.

Multivariate optimization of a bioanalytical method following a modified QuEChERS procedure associated with LC-MS/MS analysis for levamisole determination in fish meat

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Aquaculture stands out as the fastest-expanding sector in global food production. Nevertheless, the spread of diseases poses a significant challenge to this system, mainly attributed to the limited availability of regulated drugs. In this context, the exploration of levamisole, a drug with anthelmintic and immunostimulant properties, emerges as a promising alternative for enhancing fish farming. Investigations into the depletion of drug residues in edible tissues of animals intended for food production are essential for drugs designed for such purposes. Therefore, analytical methods with high sensitivity and selectivity are crucial for identifying residues in fish meat (muscle and skin in their natural proportion). This requirement becomes especially significant when dealing with complex matrices, such as fish fillets. Thus, this study aimed to optimize a bioanalytical method following a modified QuEChERS procedure as a sample preparation step associated with analysis by high-performance liquid chromatography-tandem mass spectrometry (LC- MS/MS) for the determination of levamisole residues in fish meat. A fractional factorial design was applied to investigate the variables that affected the extraction of levamisole by the QuEChERS method. At this stage, three factors were found to be statistically significant at the evaluated levels, namely: the addition of ammonium hydroxide (NH_4OH) in the extraction phase, and the amount of sodium chloride (NaCl) and primary secondary amine (PSA) in the partition and cleanup steps, respectively. Next, a final optimization of these significant variables was performed using a central composite design (CCD). The optimum conditions for levamisole extraction by the modified QuEChERS were achieved by using 1.0 g of sample and 2.0 mL of acetonitrile containing 1% NH^4OH. In the partition step, 0.5 g of magnesium sulfate ($MgSO_4$) and 0.7 g of NaCl were added, followed by the cleanup in which 150 mg of $MgSO_4$ and 125 mg of PSA were used. The chromatographic separation was carried out using a C18 reverse phase column and a mobile phase was a mixture of methanol with 0.5% formic acid (A) and water with 0.5% formic acid (B). The ratio of A:B phases was 85:15 (v/v) used in an isocratic mode. The electrospray interface source was set to operate in positive mode. Therefore, the multivariate optimization of sample preparation allowed for obtaining an analytical method with an extraction efficiency greater than 90% with adequate selectivity and detectability (LOQ = 1 ng/g) for levamisole monitoring in fish meat.

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New approach for derivatization of glyphosate and aminomethylphosphonic acid with p-toluenesulfonyl chloride and liquid chromatography-ultraviolet detector (LC- UV) analysis

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Introduction: Glyphosate (GLY) is a highly utilized chemical substance as a pesticide in Brazilian agriculture and gardening. This compound is one of the most popular and effective herbicides globally, also serving as the active ingredient in various commercially available weed control products. Its primary action involves inhibiting the enzyme responsible for amino acid production. It is noteworthy that GLY's usage began in the mid-1970s and has become increasingly common in Brazilian agricultural production. Consequently, accidental ingestion, occupational exposure, and cases of suicide or homicide involving this pesticide can lead to varying degrees of intoxication, potentially causing liver and kidney damage. Post-ingestion symptoms include nausea, vomiting, oropharyngeal ulceration, and respiratory discomfort. Fatal cases may exhibit cardiopulmonary arrest, hemodynamic disturbances, disseminated intravascular coagulation, and multiple organ failure. When metabolized, GLY, being a chemical molecule lacking chromophore groups, poses challenges in detection using UV equipment. Derivatization of the compound is necessary for effective detection. **Objective:** The technique aims to detect GLY and its main metabolite, aminomethylphosphonic acid (AMPA) after derivatization using para-toluenesulfonyl chloride (PTSCl) for liquid chromatography-ultraviolet detector (LC-UV) analysis. **Methodology:** The method involves derivatization with PTSCl 10 mg/mLfollowed by LC-UV detection. To improve derivatization conditions, factors such as power and equipament microwave time were tested, ranging from 3 to 7 watts and 10 to 30 seconds, respectively. The aim was also to match these conditions with those of the classical derivatization method, which involves 15 minutes at 50°C in an ultrasonic water bath. For chromatographic conditions, it was employed a C18 column 4,6mm x 150mm, a wavelength of 240 nm, a flow rate of 1.5 ml/min, and a mobile phase consisting of ultrapure water and acetonitrile (86:14) (v/v). **Results:** In summary, the derivatization was performed with 450 µL of ultrapure water, 250 µL of phosphate buffer, 100 µL of derivatizing agent, and 50 µL of GLY tested at concentrations of 10 µg/ml, 100 µg/ml, and 1000 µg/ml. The samples were vortexed and placed in a microwave equipment for 30 seconds at 5 watts to facilitate the derivatization reaction. Subsequently, 20 µL of the sample was injected into the LC-UV. Retention times for AMPA and GLYwere determined to be 6 minutes and 8 minutes respectively.Furthermore, the new microwave-assisted derivatization approach yielded results close to those obtained with the classical derivatization method (15 minutes at 50°C in an ultrasonic bath). **Conclusion and Discussion:** A technique involving derivatization with PTSCl followed by LC-UV analysis proved to be efficient compared to the traditional derivatization methodology because it took less time, has low cost, and still yielded the similar results.The absence of chromophoric groups in GLY and AMPA did not pose problems, as the compounds were detected through PTSCl derivatization. Moreover, the obtained results will serve as a basis for possible intoxication cases and future investigations. **Acknowledgments:** Fundo de Incentivo a Extensão

Quality management system in a public institution - Implementation of the ABNT NBR ISO/IEC 17025:2017 standard in a clinical and forensic toxicology laboratory

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Introduction: Toxicological analysis has received notable attention due to its versatility of applications, not limited to the pharmaceutical, food and chemical industries, but reaching the area of clinical and forensic diagnosis and research, promoting assertiveness, trends and applications. With its constant growth and importance, toxicological analysis must be safe and metrologically traceable, which is achieved by implementing a quality management system. Furthermore, it is essential to empha the importance of a quality management system in the context of toxicological analysis in the clinical, forensic and search areas. The ability to respond promptly and accurately to emerging situations underlines the need for a robust quality control framework. This ensures that, even in urgent scenarios, the analyses carried out by the laboratory maintain the highest standards of accuracy, reliability and safety, thus reinforcing the institution's commitment to public health and safety. In line with the areas of activity of the Analytical Toxicology Laboratory of the Campinas Poison Control Center (LTA-CIATox) and its significant contribution to public health since its foundation in 1982, it is necessary to implement a quality management system, meeting the requirements of the ABNT NBR ISO/IEC 17025:2017 standard, giving this institution an indisputable standard for its analyses. **Objectives:** To implement the quality management system, covering the areas in which LTA-CIATox operates, in accordance with the ABNT NBR ISO/IEC 17025:2017 standard and to subsidize accreditation by the General Accreditation Coordination (CGCRE) of the National Metrology Institute (INMETRO). **Methods:** Exploratory, descriptive and bibliographic methodologies were used, which, in consensus, made it possible to cover the areas of activity of the LTA-CIATox, meeting the requirements of the ABNT NBR ISO/IEC 17025:2017 standard. **Results and Discussions:** The implementation of the quality management system based on the ABNT NBR ISO/IEC 17025:2017 standard included the proposed scope and the standardization of processes. The definition of the scope included the main toxicological analyses carried out at LTA- CIATox, comprising 09 (nine) quantitative methods and 01 (one) qualitative method, covering the main biological matrices (blood, urine, oral fluid and hair) and the main analytical techniques (GC-MS/MS, LC-MS/MS, HS-GC-FID). To make up the quality management system, 24 (twenty-four) quality management documents, 15 (fifteen) technical documents relating to laboratory activities, 39 (thirty-nine) equipment handling instructions and 10 (ten) method procedures were standardized. To implement and standardize these processes, 28 (twenty-eight) training sessions were held, which were monitored to verify their effectiveness. The challenges of implementing a quality management system in a public institution are diverse and can have a significant impact on activities, including the insertion of the quality policy into activities that already have an established routine for employees, administrative demands such as operational resources and purchasing processes, and turnover in the research area. These factors have been taken into account and will be evaluated periodically in risk analyses in order to mitigate the risk and not affect activities. **Conclusion:** The execution of this work achieved satisfactory results, and it is clear that the quality policy has been incorporated into the laboratory and management processes, covering the main areas of activity of the LTA-CIATox, with this stage of process standardization being successfully completed.The implementation of the quality management system has enabled the institution to achieve traceability of all laboratory activities, as well as promoting metrological traceability, covering the pre-analytical, analytical and post-analytical phases, providing security in the results generated. Therefore, this work is in the implementation phase, partially presenting the results, continuing with the execution of the next stages of the schedule, with completion scheduled for the second half of 2024 and achieving the objectives of accreditation with CGCRE-INMETRO. **Acknowledgements:** LTA-CIATox, Faculty of Pharmaceutical Sciences, CAPES and BACO Project.

Rapid chromatography-free confirmatory screening of stimulant and dissociative anesthetic drugs in human urine using DART-MS analysis

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Introduction: Immunoassays (IA) are commonly used as a screening method in Urine Drug Screening (UDS) tests for drugs of abuse in the field of forensic toxicology. However, IA results are considered presumptive and not confirmatory in their accuracy due to the high frequency of false positives attributed to cross-reactivities with other ubiquitous co-analytes. Due to the number of potential interferents in these assays, a positive IA result is generally confirmed by a chromatography-based method (e.g., GC-MS/LC-MS). Avoiding false positives is crucial as they require costly confirmation via chromatography-based approaches, which rely on expensive carrier gases and solvents are limited in throughput due to lengthy chromatography and sample preparation steps. In this work, we report the development of a chromatography-free screening method using direct analysis in real time- mass spectrometry (DART-MS) that is shown to accurately identify and measure five illicit drugs: amphetamine (1), methamphetamine (2), 3,4-Methylenedioxyamphetamine (MDA) (3), 3,4-Methylenedioxy methamphetamine (MDMA) (4), and Phenylcyclidine (PCP) (5). The optimized DART-MS based workflow achieves a throughput rate of 96 samples in 32 minutes. **Objective:** To develop a rapid, sensitive, and selective screening method for drugs of abuse in urine as an alternative to common IA based analyses.**Methods:** All wells of a 96 well ToxBox® plate were loaded with 100 µL certified drug-free urine, 100 µL solution A, and 300 µL DI water followed by agitation for 30 min. After agitation, 650 µL of Solution B was placed in each well followed by 10 aspiration-dispensation cycles using a multi-channel pipette to mix. Phases were allowed to separate for 10 min followed by removal of the aqueous layer (500 µL). The remaining organic layer was evaporated to dryness under constant flow N2 at 70 L/min and 45ºC and reconstituted in 100 µL of solution C. A 1 µL aliquot from each well was transferred to a Quick Strip® holder and dried under N2 at 45 ºC. Loaded HTS Quick Strip® plates were placed onto the HTS- XY- Transmission of a TQ- Plus (Bruker Daltonics) triple quadrupole mass spectrometer for DART-MS-MS analysis. **Results/ Discussion:** DART and MS parameters were optimized to maximize both sensitivity and precision for all analytes. Optimal DART desorption temperature and grid voltage for 1-5 was 250 °C and 50 V, respectively. DART was operated in Scanning Mode at a linear scan speed of 0.5 mm/sec. MS was operated in MRM mode with a cone temperature of 250 °C, a CID cell pressure of 1.25 mTorr, and a scan speed of 25 ms for all analytes. The resolution for the Q1 and Q3 were set to 0.7. Unique MS/MS transitions and optimal collision energies (CE) were identified for 1 (m/z 136→91, CE= 5 V), 2 (m/z 150→91, CE= 21 V), 3 (m/z 180→135, CE= 18 V), 4 (m/z 194→163, CE= 9 V), and 5 (m/z 244→86, CE= 12 V). The approach was calibrated and validated in triplicate using certified Confidentialdrug-free urine over range of 125-10,000 ng/L for 1-2, 125-5,000 for 3-4, and 6.5-250 ng/mL for 5 with an average %RSD of between 3 and 12%. Accuracy was evaluated by spiking analytes into certified drug-free urine at two levels with recovery values ranging 92% and 110%. The detection limits 1-5 ranged from 1.25 ng/L-16.3 µg/L in urine, well below the common screening cutoff value of 150 ng/mL. **Conclusion:** This work presents a validated rapid approach for the measurement of common stimulant drugs and PCP in urine matrices requiring <30 seconds per sample.

Simultaneous development and validation of ketamine, norcetamine, hydroxynorcetamine and dehydronorcetamine in human plasma BY LC-MS/MS with principles of green analytical chemistry

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Introduction: Ketamine (KT) is a drug widely used in veterinary and human medicine, primarily as anesthetic. However, in recent years, the use as a recreational drug has also observed and occasionally as a drug facilitating crime. In addition, the KT demonstrates properties similar to those of classic antidepressants, becoming an option to treat the larger depressive disorder. In a forensic aspect, the KT can be framed in the class of new psychoactive substances (NPS), and exemplifies the expansion of the synthetic drug market, which seek to circumvent legal regulations. KT determination in biological samples can be a relevant parameter for illicit use monitoring as well as application in pharmacokinetic studies and therapeutic monitoring. Moreover, in recent years, it has been a trend in toxicological analyzes the use of techniques that value simplicity, practicality, lower sample manipulation and the use of low organic solvent volume, principles of green analytical chemistry, GAC. The confidence limits were estimated according RDC ANVISA No. 27/2012. **Objective:** Optimize and validate liquid-liquid extraction technique, LLE and a bioanalytic technique based on GAC principles for simultaneous determination of ketamine and three of its biotransformation products (norketamine - NK, hydroxynorketamine - HNK and dehydronorketamine - DHNK) in human plasma samples by LC-MS/MS method. **Methods:** LLE technique was developed for KT, NK, HNK and DHNK determinations in human plasma, using ethyl acetate (EtoAc) as extractor solvent. The analytical technique was validated in HPLC Exion® coupled to Sciex QTRAP® 5400 spectrometer. A chromatographic column C-8 (150 x 4.6mm, 5µm) ACE was used, with 10 mm ammonium formiate mobile phase with 0.01% ammonium hydroxide (pH 8.0) in acetonitrile (65:35, v/v) and analysis in isocratic mode, 1 ml/min flow with total running time 13 min. **Results**: After infusion KT, NK, HNK, DHNK standard and NK-D4 (internal standard), at the concentration of 500 ng/ml in the LC-MS/MS, the peak profile was established for each analyte: chromatographic peaks, the retention time [KT (8.84 min), NK (5.73 min), HNK (2.53 min), DHNK (4.95 min) and NK-D4 (5.64)] and transitions (m/z) [KT (238), NK (224.1), HNK (240.2), DHNK (222.1) and NK-D4 (228.0)]. Intra and inter-assay accuracy and accuracy resulted in CV <15% for controls. The quantification limit (LOQ) was satisfactory, according to RDC No. 27/2012, admitting CV% smaller or equal to 20%. Stability: KT, NK and HNK have shown variations <15% of the average nominal concentrations. As for selectivity, the technique proved to be selective for NK, HNK and DHNK, with no interfering peaks of endogenous substances at the time of retention of the compound of interest. The KT presented satisfactory linearity (R = 0.9942). The matrix effect of each analyte was <15%. Additionally, no interfering peaks were detected during residual effect analysis. Subsequently, the method was applied to two post-mortem plasma samples and, as a result of the determination, it was obtained that sample 1 presented concentrations above LOQ for all evaluated analytics. Sample 2 presented concentrations above LOQ only for KT and NK and HNK and DHNK levels in the samples were below LOQ. **Discussion/Conclusion**: The developed and validated analytical technique had adequate parameters for use in plasma sample analysis that contains ketamine and metabolites at very low concentrations, good selectivity and reasonable running time. Additionally 5 of the 12 basic principles of GAC were observed in the extraction of plasma samples and analytical quantification in LC-MS/MS. In addition, simultaneous analysis of these analytes provides more efficient and economic assessment compared to individual analysis. **Acknowledgments:** CNPq; FAPEG and IPq-FMUSP.

Study of the stability of certified reference materials used in toxicological analyses by high-performance liquid chromatography with photodiode-array detection (HPLC-PDA)

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Background/Introduction: In toxicological analyses, the use of Reference Materials (RM) ensures the reliability and reproducibility of several analytical methods. However, acquiring them is a time-consuming and costly process. In this way, high-performance liquid chromatography with photodiode-array detection (HPLC-PDA) is presented as an efficient method to evaluate the stability of these substances. **Objective:** To evaluate the stability of RM used in the Laboratory of Analytical Toxicology of the Campinas Poison Control Center (CIATox), after opening and dilution of RM ampoules, through HPLC-PDA. In addition, to determine an appropriate period of use to ensure the maintenance of the quality of the analytical-laboratory processes. **Methods:** The analyses of 11 RM were performed through four replicates soon after the opening and/or dilution of the ampoules in an adequate solvent. Subsequently, the mean of the absolute areas (n=4) obtained was verified. Between 0 and 300 days, RM were reanalyzed in the same way, and the mean area obtained was compared with that obtained in the initial time (time zero) and the increase or decay were evaluated in percentage. Furthermore, on each day of analysis, the stability of a propylparaben solution was evaluated to ensure that the equipment worked correctly. Another three substances (N-desmethyl-clozapine, lamotrigine, and acetaminophen) have had their analysis started and will be evaluated for the same period described. **Results/Discussion:** For 6-acetylmorphine, clozapine, and olanzapine after a 300-day period, the mean variation (MV) were 11.0%, 9.9%, and 12.7%, respectively. For zolpidem and zolpidem-COOH, after a 60-day period, the MV were 0.7 and 17.9%. For chlorpromazine, desipramine, and imipramine, after a 100-day period, MV were 3.3%, 6.4%, and 6.3%, in that order. Benzoylecgonine, after a 120-day period, had a MV of 1.6%. Finally, for 7-COOH-CBD and 7-OH-CBD, after a 130-day period, the MV were 6,3% and 7.9%, for each one. For all the analyses performed for propylparaben, achieving a 300-day period, the MV was 6.5%. For N-desmethyl-clozapine, lamotrigine, and acetaminophen, the analyses were started with the first day of stability (time zero). All the results were concerning the initial mean area. **Conclusion:** With reanalysis of RM, it was possible to determine the highest MV of 17.9% (zolpidem-COOH, 60 day-period). Between all analytes tested so far, after the periods evaluated, there were acceptable stability of the solutions (±20%), guaranteeing that such substances could be used safely within this time interval. **Acknowledgements:** PIBIC (process number 121551/2023-7). Keywords: Reference materials, stability, high-performance liquid chromatography with photodiode-array detection (HPLC-PDA), toxicological analysis.

Switchable hidrophilicity solvents for microextraction of medications and drugs of abuse in urine and whole blood samples

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Introduction: Switchable hidrophilicity solvents (SHS) are chemical compounds with the property of switching their hydrophilicity in response to pH alterations, being hydrophilic in acidic conditions and hydrophobic in basic environments. They are very promising for the extraction of a wide range of organic compounds. **Objective:** Development of a switchable hidrophilicity solvents based liquid liquid microextraction (SHS-LLME) and Liquid Chromatography with Diode Array Detector (LC-DAD) for the determination of medications and drugs of abuse in whole blood and urine, for forensic toxicology purposes. **Methods:** Two tertiary amines, N,N-dimethylcyclohexylamine and triethylamine, were selected as SHS candidates. Solvents and seven additional variables (sample and solvent volumes, agitation after solvent and after base addition, base volume, resting time, and centrifugation) were evaluated through a fractional factorial design 2_{IV}^{8-4} to select the best solvent and the statistically significant variables for future optimization. In this stage, microextraction was performed using ultrapure water fortified with standards of substances at a concentration of 10 µg/mL, including desipramine, imipramine, nortriptyline, diazepam, flunitrazepam, flurazepam, lorazepam, nitrazepam, codeine, methamphetamine, MDA, MBDB, LSD, cocaine, benzoylecgonine, and cocaethylene. Subsequently, a 2^3 factorial design was conducted, along with a star design and quintuplicate of the central point. Optimization was carried out using ultrapure water fortified with the same standards mentioned earlier, at a concentration of 1 µg/mL. The proposed technique's application in biological matrices was evaluated in terms of peak intensity and matrix effect, employing compromise conditions. All extracts obtained in the previously described steps were analyzed using a liquid chromatography coupled with a diode array detector, both from Agilent Technologies®, model 1260 Infinity II. Chromatographic runs were performed using a mobile phase with elution gradient (composed of acetonitrile and phosphate buffer pH 2.3) and a Varian® RP18 octadecylsilane column – 5 µm, 150 mm x 4.6 mm, with a similar stationary phase pre- column. Peaks were identified using OpenLab EZ software version A.04.10 and processed in Origin® software to obtain peak area values for statistical calculations. The obtained areas were converted into desirability functions for each assay, and the overall desirability (D) of the assays were calculated for comparison. **Results:** The analytes were separated and detected via LC-DAD with satisfactory chromatographic resolution in a 20-minute run. Sixteen assays were performed for the fractional factorial design 2_{IV}^{8-4}, with two assays showing no separation between aqueous and organic phases, resulting in a D value of zero for them. Using the D results, the effects of the 8 studied variables were calculated, and a graph of calculated effects versus cumulative probability was plotted. This allowed the selection of Triethylamine as the most suitable SHS for this study and the variables to be optimized due to their statistical relevance: sample volume, solvent volume, and NaOH volume. Subsequently, 19 assays corresponding to the 2^3 factorial design, along with a star design and quintuplicate of the central point, were conducted, enabling the selection of optimal working conditions for the application of the developed method in biological matrices (whole blood and urine). **Conclusion:** The conducted assays demonstrated a promising method for liquid-liquid microextraction of substances with forensic interest in biological matrices (whole blood and urine) using triethylamine as a switchable hydrophilicity solvent. **Acknowledgments:** CNPq, CAPES, and Polícia Científica do Paraná.

Systematic emergency toxicological analysis for the detection of toxic agents in the urine of patients with suspected intoxication using gas chromatography coupled to mass spectrometry (CG-EM)

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Introduction: The challenge of emergency analytical toxicology is to carry out toxicological tests efficiently and quickly. As there is an increasing number of chemical compounds, it is important to develop methods that detect these compounds as quickly as they become available. In this sense, systematic toxicological analysis aims to detect analytes by excluding substances, mainly using analytical databases as libraries containing mass spectral information. **Objective:** To develop and validate a systematic toxicological analysis methodology for detecting toxic agents in urine samples from patients with suspected poisoning received by the Centro de Informações e Assistência Toxicológica de Santa Catarina (CIATox/SC) using GC-MS with on-column derivatization. **Method:** The sample was prepared by liquid-liquid extraction and the volume of sample that would yield the highest recovery was initially evaluated. In addition, the best pH, the form of adjustment, whether with solvents or buffer, and the most suitable volume of organic extracting solvent were tested. Once these parameters had been defined, the sample with organic solvent was shaken and centrifuged, and the supernatant (1900 µL) was dried in the sample concentrator at 50ºC for 15 minutes. At the end, the extracts were resuspended with 50 µL of ethyl acetate. The best method for derivatization using MTBSTFA was also evaluated, either by activating at 70ºC for 60 minutes or by injecting directly into the column. For chromatographic detection, a GC-QP2010 Ultra (Shimadzu®) gas chromatography equipment was used, coupled to a QP-2010 Ultra mass spectrometer, with an AOC-5000 Plus automatic sampler, a 5% diphenyl/95% dimethylpolysiloxane chromatography column, model RTx-5MS (30 m x 0.25 mm x 0.25 µm) and helium carrier gas (4mL/min). Elution in the GC-MS was carried out with a temperature ramp, starting at 60ºC, increasing by 10ºC every 2 minutes, reaching a final temperature of 310ºC. Analytical validation was carried out in accordance with the United Nations Office on Drugs and Crime (UNODC) Manual. **Results:** When using the standards cocaine, paracetamol, salicylic acid, aldicarb and malathion to evaluate the technique developed, better recoveries were obtained when using 1mL of urine sample than when using 200 µL. Adjusting the pH of the sample using extraction buffer was better than adjusting the pH of the urine just by adding NaoH or HCl. The best recovery percentages were obtained when acetic buffer was used at pH 4, instead of 3, and phosphate buffer at pH 12, instead of 10. It was possible to carry out an "on column" derivatization so that the derivatizer activation time was dispensable. The method developed showed a lower coefficient of variation (CV) when compared to the classic derivatization by maintaining it at 70ºC for 60 minutes. When testing the applicability of the method in 5 cases, it was noted that in all cases a greater number of compounds were detected when compared to immunoassay screening. In one of the cases, for example, which was a suspicion of poisoning, the immunoassay result was undetectable for the substances tested. In contrast, the method used in this study was able to detect two substances, propofol and lidocaine. **Discussion/Conclusion:** A major challenge for emergency toxicology analysis is to find a comprehensive method that can detect the greatest number of substances in the shortest possible time. In practice, it is not always possible to obtain the patient's history in order to clinically manage the case, and many procedures depend on the analytical result in order to provide clinical guidance. One advantage of the method was being able to reduce the activation time when performing the derivatization "on column". **Acknowledgments:** HU-UFSC/EBSERH and Multiprofessional Health Residency.

Tissue residues of florfenicol and metabolites in nile tilapia fillets during and after oral treatment with florfenicol

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The rapid expansion of aquaculture has given rise to various challenges, including the escalating incidence of bacterial diseases necessitating antimicrobial interventions. Simultaneously, mounting concerns about antimicrobial resistance underscore the urgent need for robust monitoring methodologies. Florfenicol (FFC), an approved veterinary drug for use in Nile tilapia (Oreochromis niloticus) farming, is of particular interest in this context. While earlier studies have successfully quantified the presence of FFC and its primary metabolite, florfenicol amine (FFA), during the depletion period, a notable knowledge gap persists regarding the formation of other metabolites and the kinetics of their evolution throughout and post-treatment. This gap can be partially attributed to the absence of commercially available analytical standards. Therefore, this study aimed to synthe and characterize the metabolites florfenicol alcohol (FFOH), monochloroflorfenicol (FFCl), and FFA, alongside quantifying FFC and these metabolites in tilapia fillets during and after the administration of florfenicol-medicated feed. The metabolites were synthed using florfenicol as the starting reagent through peptide coupling and were characterized using 1H and 13C Nuclear Magnetic Resonance, High-Resolution Mass Spectrometry, and quantitative NMR, ensuring a minimum purity level of 94%. A total of 180 male Nile tilapia, averaging 370 g in weight, were randomly placed in fifteen fiberglass 450-L water tanks (15 fish per tank) receiving well water in a continuously aerated open system. Following an eight-day acclimation period, the fish received florfenicol-medicated feed at a dose of 10 mg kg-1 of body weight for ten consecutive days, with a water temperature of 28 °C. Five fish were euthanized at each time point on days 1, 5, and 10 during treatment and on days 1, 2, 3, and 5 after treatment. Fillet analysis followed the QuEChERS approach for sample preparation. For residue quantitation, validated two-dimensional liquid chromatography coupled with tandem mass spectrometry (LC-UHPLC-MS/MS) method was employed. Quantitation utilized matrix-matched calibration curves and isotopically labeled internal standards (FFA-d3 and FFC-d3). The method's limit of quantitation was 50 ng g-1 for FFC and 20 ng g-1 for FFA, FFOH, and FFCl. Residual concentrations of FFC, FFOH, FFA, and FFCl in fillets were observed during the treatment period, reaching 2575 ng g-1, 890 ng g-1, 276 ng g-1, and 33 ng g-1 at Day 5, respectively. Subsequently, the concentration of FFC decreased during the post-treatment phase, indicating depletion and biotransformation. After 3 days post-dose, only FFOH and FFA were detected in fillets (209 ng g-1 and 25 ng g-1, respectively). These findings align with studies in mammals, suggesting that FFC is initially converted to FFOH and subsequently to FFA. Monochloroflorfenicol was detected only at very low concentrations and is a minor metabolite of florfenicol in tilapia. This study not only addresses critical research gaps but also provides valuable insights into the presence of FFC metabolites in tilapia fillets, especially in regions with varying climates. Such insights are crucial for safeguarding the health of animals, humans, and the environment in the context of aquaculture. **Acknowledgments:** This work was supported financially by the São Paulo State Research Foundation (FAPESP, grants #2021/08152-0 and #2022/08804-0).

Toxicological screening of human plasma employing automated solid phase extraction and liquid chromatography associated with mass spectrometry

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Introduction: In Brazil, drugs and illicit substances play a predominant role in reported cases of suicide attempts (SA). According to the most recent data from the Poison Control Center from Rio Grande do Sul State, these substances were implicated in 5,883 cases of SA reported in the state in 2021, underscoring the importance of conducting toxicological screenings when patients are admitted to emergency hospitals. **Objective:** The objective of this study is to develop and validate a method for identifying and quantifying compounds in patients following suicide attempts through toxicological screening. This involves utilizing plasma samples obtained via venous puncture, processed with solid phase extraction (SPE), and analyzed using UPLC/MS-MS. The target analytes include amitriptyline, amphetamine, benzoylecgonine, carbamazepine, cocaethylene, cocaine, codeine, diazepam, fluoxetine, MDA, MDEA, MDMA, methamphetamine, midazolam, morphine, nortriptyline, paroxetine, sertraline, and venlafaxine. **Methods:** Plasma samples (100 µL) were diluted with 875 µL of 4% phosphoric acid in water. Subsequently, 25 µL of an internal standard, containing amphetamine-d5, benzoylecgonine-d3, cocaine-d3, diazepam-d5, fluoxetine-d6, MDMA-d5, morphine-d3, and trimipramine-d3 at 100 µg/mL, was added. The extraction process utilized Oasis HLB® cartridges in an ASPEC system, comprising three steps: application, washing, and elution. The extracts were concentrated and analyzed using Acquity UPLC I-Class with a column ACQUITY UPLC HSS C18 (2.1 x 150 mm, 1.8 µm) in a 15-minute chromatographic run. The mobile phase consisted of ammonium formate 5 mM pH 3 (A) and acetonitrile with 0.1% formic acid (B), eluted in gradient mode. The acquisition method involved 180 different MRM transitions for the compounds under investigation. The method was validated following international guidelines from the Food and Drug Administration and the European Medicines Agency. **Results:** In the validation process, precision tests indicated intra-assay variation coefficients of 1.6-10.6% and inter-assays of 1.5-11.4%. Accuracy ranged from 94.4-106.6%. Extraction recovery for low-quality control ranged from 11.3-71.4%, and for high-quality control, it ranged from 22.9-84.7%. The matrix effect of low-quality control was -15.9-2.9%, and for high-quality control, it was -3.9-13.3%. Freeze-thaw stability for low- quality control ranged from -6.1-11.5%, and for high-quality control, it ranged from -1.8-4.7%. The 12-hour autosampler stability showed a variation for low-quality control from -10.4-9.8% and for high-quality control of -4.6-5.2%. **Discussion/Conclusion:** The results demonstrate the feasibility of using this method to rapidly identify a large number of compounds simultaneously. This capability enables targeted treatment of the specific compound causing poisoning, thereby enhancing the chances of patient recovery. Additionally, with epidemiological knowledge of the most commonly used xenobiotics in the region, public health planning and mitigating actions can be implemented to reduce the number of cases. **Acknowledgments:** We extend our gratitude to the study volunteer patients and acknowledge the financial support from the National Council of Scientific and Technological Development of Brazil (CNPq).

Use of Solid Phase Microextraction Tips (SPME-Tips) for analysis of gefitinib and its metabolite in plasma

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Background/Introduction: Gefitinib is a selective inhibitor of epidermal growth mediated by the factor receptor tyrosine kinase, which is frequently expressed in lung cancer of non- small cells. This medication is used as oral monotherapy in patients resistant to chemotherapy and is usually administered 250 mg once a day. Due to interindividual variations in plasma concentration, therapeutic monitoring of this medication becomes essential to improve pharmacotherapy, monitor patient adherence, and minimize the risk of toxicity. Currently, in (green) analytical toxicology (GAT), there is a growing interest in the use of new sample preparation, such as Solid Phase Microextraction (SPME) Tips, a method of miniaturizing of solid phase extraction. The SPME-Tips technique was recently developed, comprising a microfiber coated with an extractor phase, which is situated at the tip of the microtip. In SPME-Tips, the removal of analytes occurs in an extractor phase, allowing the direct inclusion of the tip in biological fluids, causing the analytes to be adsorbed on the fiber, and allowing resorption to be carried out using a small volume of solvents. **Objective:** The aim of this work was to develop and validate an analytical method for determination of gefitinib and its metabolite (O-desmethyl gefitinib) using SPME-Tips and liquid chromatography tandem mass spectrometry (LC-MS/MS). **Methods:** The SPME-Tips procedure was optimized by a Placket-Burmann and a Rotational Central Composite Design (RCCD), studying parameters such as salting-out effect, extraction time, sample agitation speed, elution solvent volume, and elution solvent constitution. Before each extraction day, all Tips were conditioned with methanol (10 min, 500 rpm) and ultrapure water (10 min, 500 rpm), followed by sample extraction (2 min, 500 rpm) and analyte elution (30 min, 500 rpm). For sample extraction, 100 µL of plasma was transferred to a 2 mL polypropylene tube, followed by the addition of 20 µL of gefitinib-d3 (internal standard) and 50 µL of TBS. The content were vortexed for 10 seconds and transferred to a well in a 96-well plate for Tips extraction. At the final stage, elution was performed with 125 µL of acetonitrile and 2 µL was injected into LC-MS/MS. All the procedures were approved by the Research Ethics Committee of UNICAMP-CEP (process number 17328619.9.0000.5404). **Results/Discussion:** Using Placket-Burmann optimization, it was found that the only parameters with significant difference between the analyzed data (considering $p < 0.05$) were the extraction time and the sample agitation speed. Therefore, a RCCD was conducted for both parameters, achieving 2 min and 500 rpm as the best values, respectively. Consequently, the optimized procedure was determined and it is aforementioned in methods section. The method are under validation following the guideline AAFS Standard Practices for Method Validation in Forensic Toxicology, for parameters as limit of detection, lower limit of quantification, linearity, precision, accuracy, carryover, selectivity, interference studies, extraction efficiency, dilution integrity, matrix effect, and stability. Further steps involves the analysis of authentic samples from indivudals clinically monitored at UNICAMP Hospital. **Conclusion:** A green analytical toxicology method was sucessfully developed using SPME-Tips and LC-MS/MS for the analysis of gefinitib and O-desmethylgefitinib in plasma, currently undergoing validation. **Acknowledgements:** This work was developed with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) through the granting of a master's scholarship, process number: 88887.711761/2022-00.

Use of surface response methodology in the optimization of the experimental conditions of imatinib extraction from capillary dried plasma spots in LC-MS/MS analysis

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Background/Introduction: Imatinib mesylate (IM), a selective tyrosine kinase inhibitor, is considered first-line therapy for Chronic Myeloid Leukemia (CML). However, patients in treatment with IM may show different responses due to interindividual pharmacokinetic variability. Plasma IM levels above 1.000 ng/ml are associated with major and complete molecular response, thus therapeutic drug monitoring is recommended. The capillary dried plasma spots (DPS) is an innovative less invasive sampling strategy that has been used for TDM of different drugs. Considering the influence of multiple factors into the extraction yield, the experimental conditions of the extraction of IM from the DPS must be carefully assessed. The response surface methodology is a set of statistics techniques employed to model and analyze problems in which a response variable is influenced by diverse operational variables. **Objective:** To optimize the experimental conditions for IM extraction from capillary dried plasma spots, generated from the Health ID® sampling device. **Methods:** Three 6mm DPS discs of quality control DPS sample at 3000 ng/mL were extracted with 500 µL of methanol and homogenized in a thermomixer system, followed by ultrasound extraction. The Box-Behnken experimental design was utilized, involving the variables of incubation temperature (20, 30, 40°C), incubation time (20, 40, 60 minutes), and ultrasonication duration (10, 20, 30 minutes) in a total of 17 runs. The obtained solutions were dehydrated at 60°C for 40 minutes. The extract was dried at 60 °C under vacuum, recovered with 100 µL of 0.1% formic acid in water and acetonitrile (50:50) and IM analyzed by LC-MS/MS with electrospray ionization probe. **Results:** The chromatograms of IM exhibited areas ranging from 26,540.678 to 31,053.502. The response was effectively modeled using a linear equation that incorporated various factors and their interactions, demonstrating a significant fit ($P<0.05$). Notably, temperature emerged as a significant variable, exhibiting an inverse relationship with the area. The model was fine-tuned to achieve the optimal response within the shortest incubation time. The equation, Area= 2,97x107 -102,8* incubation time -20,0005 * incubation temperature + 242 * ultrasound time. Consequently, the optimum reaction condition was set as: incubation time of 20 minutes at 20°C, followed by ultrasonication time of 10 minutes. **Conclusion:** It was possible to define ideal conditions for the detection reaction within a short interval, which proved applicable in the development of a method to quantify imatinib by LC-MS/MS. **Financial support:** CNPq, Capes.

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TOXICOLOGIA CLÍNICA

A platform for predicting pharmacokinetic natural product–drug interactions with clinical relevance

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The study of interactions between drugs and natural products (NDPI) is important due to the significant clinical implications that these interactions can have on the safety and efficacy of drug therapy. For example, herbal supplements are widely consumed in conjunction with conventional medications, often without proper medical guidance. However, the mechanism and clinical implication of NDPI are poorly studied given the complexity of in vitro and in vivo studies, which requires special attention due to the phytochemical complexity of NP, inconsistencies in formulations, differences in botanical taxonomy and nomenclature, and the scarcity of human pharmacokinetic data for most commercially available NPs. Therefore, this work aims to develop a platform for predicting pharmacokinetic NPDI with clinical relevance. The NPs selected for the initial screening were gallic acid, kaempferol, quercetin, isorhamnetin, epigallocatechin gallate, and rosmarinic acid. Initially, potentially clinically relevant interactions, considering the NP as an inhibitor of enzymes and membrane transporters, were mapped following the FDA guideline for conventional drug-drug interactions [In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry, 2020]. The ratio (R1) of intrinsic clearance values of a probe substrate for an enzymatic pathway (isoenzymes of Cytochrome P450 or UDP- glucuronosyltransferases families) in the absence and presence of the interacting natural product for reversible inhibition was calculated according to the following Equation: $R1 = 1 + (I_{max,u}/K_{i,u})$; where $I_{max,u}$ is the maximal unbound plasma concentration of the interacting NP, and $K_{i,u}$ is the unbound inhibition constant determined in vitro. If $R1 \geq 1.02$, a clinical interaction is possible and will be further investigated. Membrane transporters were investigated analogously considering the FDA guidance. Six NPs were evaluated as potential inhibitors of 13 membrane transporters and 16 drug metabolism enzymes. Twenty-four and 18 potential clinical NPDI on membrane transporters and drug metabolism enzymes, respectively, were detected. Forty interactions were not considered potentially clinically relevant and 92 in vitro Ki values were missing. The next step is mechanistically evaluating these interactions by applying Physiologically-based pharmacokinetic modeling and simulation. In the future, it is expected that this platform will serve as a guide for the rational prescription of NPs.

Accidental cannabis oil intoxication in a pediatric patient: a case report.

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Introduction: The use of medicinal cannabis is increasingly common among the population due to recent therapeutic applications. However, under specific exposure conditions, there is limited data regarding its safety. **Objective:** To report a case of accidental exposure to cannabis oil in a pediatric patient, who was undergoing treatment for Autism Spectrum Disorder (ASD), assisted by the Campinas Poison Control Center. **Case report:** A nine-year-old female patient, 40 kg, diagnosed with ASD, accidentally ingested 30 mL of commercial cannabis oil composed of CBD and THC (1:1 m/m, 10 mg/mL), which she had been using therapeutically. The product was prescribed and imported in accordance with ANVISA regulations. After ingestion, the patient developed drowsiness and was taken to a referenced emergency unit. Upon admission, the patient was unresponsive, developing hypotension and lethargy, which persisted for 12 hours. The treatment was supportive. The child remained hospitalized in the intensive care unit for 24 hours, until complete remission of symptoms. **Methods:** Blood and urine samples were collected as a laboratory diagnostic aid, as well as an aliquot of the oil for subsequent analysis of the actual composition and its respective content. Blood and urine analyzes were performed by LC-MS/MS, while the oil was analyzed by HPLC-DAD. After hospital discharge, the case was reviewed by toxicologists and classified according to the severity of the intoxication, using the Poisoning Severity Score. **Results:** From the data reported upon patient admission, the ingestible dose (ID) of CBD and THC was 7.5 mg/kg. However, with the analysis of the oil, CBD and THC quantification was obtained at 8 and 7 mg/mL, respectively. With the analytical data, the real ID was 6 and 5.25 mg/kg. In the analysis of whole blood by LC-MS/MS, the concentrations determined for CBD, THC and other metabolites were elevated (> 10 ng/mL). Urine analyzes confirmed the exposure of only CBD and THC, with no other psychoactive substances detected. The clinical picture of intoxication, considering the manifestations presented, was classified as mild. **Discussion:** This case highlights how accidental exposure to a new prescription medication can have significant implications for a child's health. The accidental overdose of cannabis oil ingested caused relevant symptoms of intoxication. Throughout clinical care, the patient responded positively to the support measures implemented. As the cannabinoids CBD and THC act on the endocannabinoid receptors CB1 and CB2, ingestion in high doses potentiated the effects generated on the cardiovascular system and the sleep cycle, which explains the symptoms presented by the child. Cannabinoid intoxication has no specific antidote, so treatment is supportive. It is certainly clear that the effects presented were exclusively related to CBD and THC, since no other psychoactive substance was detected. In the case presented, confirmation of the use of cannabis oil, both through laboratory analysis and parental reports, contributed to accurate and targeted therapy. However, in situations where there is no information or laboratory analysis available, clinical investigation may take longer, as the symptoms presented could be attributed to various causes. **Conclusion:** With the increase in the number of prescriptions for cannabis oil, healthcare professionals must be qualified to guide patients about the potential risks of erroneous manipulation that can lead to overdose. Furthermore, given the advancement in the use of medicinal cannabis in the country, the need for rigorous quality control over these products is expressed, with the application of validated analytical techniques to attest to the composition and quality of products originating from Cannabis sativa sp. and, thus, ensure that the amount of active ingredients present in the product is in accordance with the information reported on the packaging. **Acknowledgements:** INSPEQT and BACO projects. **Keywords:** Medical cannabis, intoxications, emergency services, clinical toxicology.

Accidental exposure to Cannabis sativa in a child: a case report

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Introduction: Accidental exposure to Cannabis sativa, commonly known as marijuana, in children is an emerging public health problem. Accidental ingestion of edible products containing marijuana is a common cause of such exposures. Several countries have already reported an increase in unintentional marijuana poisonings in children. In the United States, for example, a study indicated a 1375% increase in the number of children who inadvertently ingest cannabis-based products between 2017 and 2021. In Brazil, there are few studies related to unintentional intoxication in children, and it is important to report cases of this type. These products, such as gummies, chocolates and cookies, can easily be mistaken by a child as a common snack. Marijuana exposure in children can cause a variety of symptoms, including changes in mental state (confusion, hallucinations, agitation, lethargy) behavior (aggressiveness, hyperactivity, lethargy), nervous system (seizures, coma), cardiovascular (tachycardia, hypotension), respiratory (dyspnea, apnea) and gastrointestinal (vomiting, diarrhea). The diagnosis of accidental exposure to marijuana in children is made on the basis of clinical history, physical examination and laboratory test results. **Objective:** The aim of this case report is to highlight the importance of toxicological testing in the diagnosis and treatment of cases of exposure to Cannabis sativa in children. **Materials and Methods:** This report describes a case of "Cannabis" intoxication in a pediatric patient, using data from DATATOX/CIATOX/SUS at the José Frota Institute Municipal Hospital - IJF in Fortaleza-CE and toxicological testing was carried out by gas chromatography coupled with mass spectrometry (CG-MS/MS Agilent®) at the Forensic Toxicology Center of the Forensic Expertise of Ceará - PEFOCE. **Results:** The diagnosis of Cannabis sativa intoxication was made on the basis of the clinical history, physical examination and laboratory test results: Patient's history, clinical data, laboratory test results and clinical evolution. Fortaleza-CE, 2024. *Initial clinical evaluation:* Unconscious, but responsive to painful stimuli; Glasgow Coma Scale score of 8; bilateral miosis; normochromic skin and mucous membranes; eupnea; heart rate of 108 bpm; blood pressure of 81/45 mmHg; oxygen saturation of 99%. *Severity classification*: Moderate. *Management:* Intravenous hydration; monitoring of vital signs; general laboratory assessment (renal, hepatic, and electrolyte function); collection of samples for toxicological screening at Pefoce. *Laboratory results:* Full blood count indicating anemia (Hb: 9.0 g/dL, Ht 32%) without infection; serum electrolytes, renal and hepatic function: normal. Toxicology blood test: "positive" for delta-9-THC (Cannabis sativa). *Evolution - CIATOX:* At 8:45pm; woke up, fed, interacted well; no other symptoms of intoxication (discharged). *Pediatric Clinical Evaluation:* At 11:21pm, severity classification: mild, discharged Medical outcome: Cure. **Discussion/Conclusion:** The toxicology test detected the presence of delta-9-THC, confirming the presumption of exposure to the Cannabis sp. The child showed mild symptoms of marijuana intoxication, including lethargy, ataxia and miosis. The heart rate was elevated, but the blood pressure was normal. The patient recovered completely without any sequelae. The importance of toxicological testing in the diagnosis of exposure to Cannabis sativa is particularly important, as it can guide treatment and differentiate marijuana exposure from other medical conditions that may present similar symptoms. Early recognition and appropriate treatment are crucial for a favorable outcome. This case also underlines the need for greater awareness about the safe storage of edible cannabis products to prevent such exposures, especially in children.

Assessment of the effectiveness of the classification protocol for mild scorpion accidents recommended by the Ministry of Health

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Introduction: scorpion accidents have been a public health challenge with high severity potential, mostly in children, malnourished and patients with known comorbidities. The management of these accidents is guided by the Ministry of Health's guidelines, which orient about severity criteria and anti-venom therapy indications. However, some authors are in favor of the use of anti-venom therapy for all symptomatic children with less than 3 years old and less than 2 hours from the accident. **Objective:** analyze the effectiveness of the severity classification protocol for scorpion stings recommended by the Ministry of Health for mild/grade I accidents, which do not indicate anti-venom therapy for infants under 3 years old. **Methods:** descriptive and retrospective study based on electronic medical records from database DATATOX, 2.0 version, from Centro de Informação e Assistência Toxicológica do Espírito Santo (CIATox-ES) and from database e-SUS VS (Sistema Capixaba de Informação em Saúde) between 2021 and 2023. Variables analyzed include: age, sex, severity classification, time between sting and management at the hospital. **Results:** the sample registered 324 accidents, of which mostly were aged 1 year old (49,38%) and male (61,11%). Regarding the gravity stratification, 56,79% were mild/grade I, 29,01% were severe/grade III and IV and 14,2% were moderate/grade II. About the time elapsed between the accident, categorization and treatment, 59,46% were classified as mild within the first hour and 50,98% within the second hour, with no changes in severity during the observation period. **Discussion/ Conclusion:** the Ministry of Health's protocol proved to be effective even in age groups at higher risk of gravity. Not recommending anti-venom therapy in mild cases, even in children under 3 years old, avoids the exposure to heterologous and immunobiologicals and unnecessary hospitalizations. Also, contributes to the rational use of anti-venom serotherapy.

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Case report: 10-day-old neonate cocaine poisoning from human breast milk exposure

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Introduction: WHO determines recommendation of exclusive breastfeeding in first six months of life. Substances used by nursing mother can be excreted in breast milk and absorbed in infant's gastrointestinal tract. In this sense, mother's exposure to illicit drugs such as cocaine (COC) may pose a risk to child's health. **Objective:** Describe a case of COC poisoning of a newborn in addition to evaluating newborn's blood and urine samples and mother's breast milk to determine the presence of COC and its origin, in addition to determining a possible mechanism of action. **Methodology:** Case report: a 10-day-old newborn girl was admitted to municipal emergency room and showed cyanosis, abundant sialorrhea and without clinical response even after being stimulated. Mother offered the baby complementary nutritional formula at 4 a.m. and breast milk at 5:30 a.m. Mother admitted COC dependence and inhaled six lines of COC hydrochloride powder between 6 p.m. and 5 a.m. Toxicological Information Center (CIT) was called and following the CIT guidelines, it was requested a methemoglobin (MtHb) test and toxicological immunoassay screening in patient's blood and urine samples. In addition, a sample of breast milk (100 µL) from mother was collected for toxicological analysis. Samples of urine, blood and breast milk from patient and mother were stored at -80ºC and used for investigation of COC by immunochromatography. **Results:** It was found 2.14% of total MtHb in EDTA-whole blood sample while immunochromatography test detected COC and metabolites (cut off > 300 ng/mL) in urine and EDTA-plasma samples from baby. Mother's breast milk also detected COC and metabolites in toxicological immunoassay. In addition, aPTT (52.5 seconds) and INR (1.36) values were obtained. **Discussion and Conclusion:** COC exposure associated with poor swallowing of complementary foods may have caused sialorrhea through partial obstruction of the airways leading to peripheral cyanosis. MtHb assay was performed due to baby's cyanotic state and unawareness of exposure to any MtHb- promoting agent. The immunochromatographic assay detected COC in all biological samples tested, indicating lactational exposure. However, it wasn't possible to confirm it by mass spectrometry, which is limitation of technique. In animal experiments, it was found seven to eight times higher COC concentrations in breast milk when compared to maternal serum levels. The combined effect of very high blood concentrations during COC abuse and a large breast milk-blood partition can lead to toxic infant blood concentrations. However, COC breast milk partition coefficient in blood hasn't been established in humans. Breastfeeding is one of main causes of acute and chronic exposure in newborns and babies who still have breast milk in their diet. In cases where breastfeeding and COC consumption are maintained, it's advisable to interrupt breastfeeding for a period sufficient to eliminate the drug. Results of aPTT and INR tests were out of specification. Nevertheless, the patient's young age along with the COC intoxication affected the aPTT and INR values. APTT parameter is considered a more sensitive version of PPT. Exposure to COC in newborns, who still have some liver immaturity, could promote tissue damage that caused an increase in kallikrein (KAL) via factor XIIa. KAL has a proteolytic action and could increase in aPTT time. In turn, KAL promotes feedback from factor XIIa, which maintains the continuation of the normal process of the coagulation cascade and, therefore, the normal level of prothrombin time (PT). Since PT value did not change PT/INR rate that defines liver damage, it was expected that other traditional liver biomarkers would not be altered as we found in this case. Thus, immunochromatographic tests can be applied to biological fluids and are best screening option in toxicological emergencies, showing importance of these exams. **Acknowledgments:** CAPES

Chemical submission: analysis of 7 clinical cases

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Introduction: Chemical submission is defined as the ingestion of one or several psychoactive substances by a person without their consent, with criminal purposes of an economic or sexual nature. We do not have epidemiological data of this type of crime in Uruguay. **Objective:** to analyze clinical cases of chemical submission to approximate the epidemiological profile in our country. **Methods:** descriptive observational and retrospective study of 7 clinical cases received at the Toxicological Information and Advice Center (CIAT) in Uruguay between 2019 and 2022. **Results:** 7 clinical cases were analyzed, of which 6 were female. All were under 25 years old. Regarding origin, 6 cases were from Montevideo and 5 cases were treated at the Hospital de Clínicas. The latency of the consultations was variable and ranged between 5 and 36 hours. All cases corresponded to drug-facilitated sexual assaults. In 2 cases they were sex workers. The scenario was night parties in 4 cases, 1 on a public street, 1 at work (sex worker), 1 in a restaurant. In all cases there was voluntary consumption of ethanol. Some associated consumption of other substances: 1 case LSD, 1 case cocaine, 1 case energy drink. No patient reported use of psychotropic drugs. All cases presented subsequent amnesia and in 4 cases lesions were evident on physical examination. Alcohol testing was requested in 3 cases and was positive in 2. Urine drug screening was requested in all cases and was negative in only one case. Gas cromatography was requested in all cases, we have the results of 5 cases, all with findings of drugs of abuse and benzodiazepines. The police report was made in 5 cases and the prophylaxis of sexually transmitted infections in 4 cases. **Discussion:** Chemical submission is described in women under 30 years of age with a known aggressor who acts alone. Voluntary consumption of ethanol and other drugs of abuse is frequently associated. Amnesia is the most common symptom and genital or defensive lesions are not always evident. All of these features were observed in our series. Late latencies predominate in consultations, which makes it difficult to analyze toxicological results, since they are often toxics with a short half-life. Ethanol and benzodiazepines are the most frequent substances involved, alone or associated with other drugs of abuse, although a wide list of possible substances is known. This coincides with our case series, being the two the most common. These substances enhance central nervous system depression, generate disinhibition, amnesia and the inability to resist an assault. Toxicological results do not distinguish between voluntary or involuntary consumption, so care must be taken when interpreting them, considering that the most frequently detected substances are commonly used by the population. The negative toxicological analyzes and absence of injuries on physical examination do not invalidate the diagnose of chemical submission. The correct anamnesis of the scenario, the clinical symptoms, the consumption reported by the patient and the time since the intake is important. Most cases of chemical submission are carried out for sexual criminal purposes; once detected, the protocol aimed at avoiding unwanted pregnancies and sexually transmitted diseases must be initiated. **Conclusions:** Although we have a small series of cases, the data that emerges are similar to those reported in other countries. This is a public health problem that both health teams and the population must be aware of, since early consultation and high suspicion are essential for a correct diagnosis and collection of samples in a timely manner. It is necessary to establish preventive measures aimed at the most vulnerable population.

Clinical Toxicology of Medicinal Cannabis: risk assessment and impacts on clinical practice

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The therapeutic use of Cannabis has garnered increasing interest, necessitating a rigorous toxicological approach to assess risks and impacts on clinical practice. This work stems from the experience gained in two supervised internships during the undergraduate biomedical course, each lasting 374 hours, totaling 748 hours. These internships were conducted at the University Estácio de Sá and at the Instituto Medicina Canabinoide, with the purpose of exploring clinical toxicology related to the use of cannabinoids. **Introduction:** Medicinal Cannabis, grounded in the Endocannabinoid System and involving various active compounds such as THC and CBD, has gained acceptance in the therapy of various medical conditions. In this context, it is necessary to understand the associated risks and impacts on clinical practice to prevail in safe and effective administration. **Objective:** The main objective of this study was to evaluate the clinical toxicology of Medicinal Cannabis, focusing on optimizing pharmacological therapy and preventing intoxications. The activities provided an opportunity to explore practices in Medicinal Cannabis, specifically regarding risk assessment and implementing preventive measures in the administration of exogenous cannabinoids. **Methods:** Similar methodologies were adopted in both internships, integrating the prevention of adverse effects and toxicological control into clinical practice. Care, focused on clinical toxicology, was developed in two stages: before and after the medical consultation. The experience in the first internship contributed to the development of a standard medical record for patients undergoing cannabinoid therapy. In the second internship, it was possible to apply the standard record, facilitating the organization of essential information obtained in the dose titration process. This record assists in toxicological control and enables a more precise evaluation of each patient's response to the use of Cannabis-derived products. **Results:** The results highlighted the effectiveness of therapeutic monitoring in optimizing cannabinoid therapy, preventing intoxications, and minimizing undesirable side effects. The individuality of each patient emerged as a determining factor in the search for the ideal dose, emphasizing the complexity of cannabinoid therapy. The development of a standard medical record proved to be an important tool for organizing and analyzing relevant data for dosage adjustment. **Discussion:** Patients assisted during the internships expressed satisfaction with the care provided, reflecting the efficacy of the adopted practices. The implementation of the standard medical record in the second phase of the internship strengthened the ability to make more informed decisions and enhanced the monitoring of patients' clinical responses. In conclusion, the clinical toxicology approach to Medicinal Cannabis requires careful consideration of patient individuality and the implementation of effective tools to monitor and adjust therapies. These clinical practices are valuable, emphasizing the importance of therapeutic monitoring in risk minimization and promoting a positive therapeutic experience for patients. Additionally, the indispensability of more scientific research related to the topic is noted.

Consumption of antidotes flumazenil and naloxone in a University Hospital in Paraná

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Introduction: Flumazenil and naloxone are drugs used as chemical reversals in benzodiazepine (BZD) and opioid intoxications, which are psychotropic drugs applied in sedation and analgesia in hospital settings and require high vigilance due to their imminent risk of adverse effects such as respiratory depression, lowered levels of consciousness and significant overdose, which can be aggraveted in cases of synergism with other psychotropic drugs. **Objective:** To characterize the use of naloxone and flumazenil in patients in a university hospital in the state of Paraná and to define a profile of patients who may have contributed to BZD and opioid intoxication. **Methods:** A descriptive cross-sectional study was carried out in a regional hospital using data from 77 patients admitted between April 2022 and April 2023 through the DEDALUS HEALTHCARE SYSTEMS GROUP in its prescription and inventory control modalities (Esthos), interconnected to institution's electronic medical record system (MedView). **Results:** Of all patients, 51,9% used Flumazenil and 76,6% used Naloxone, and Pediatrics was responsible for most of the prescriptions. The results of albumin and liver enzymes tests were altered, and 37,6% had psychotropic drugs polypharmacy. Finally, 62,3% progressed to improved discharge and 37,6% progressed to death. **Discussion/Conclusion:** Age, polypharmacy and laboratory tests monitoring can help to understand and prevent poisoning, considering the drugs pharmacokinetic and pharmacodynamic profiles in diferente groups of patients. Analysis of prescriptions, laboratory tests and the patient's profile are essential for assessing risk and drug-related problems and the pharmacist, conjointly to the healthcare team, should engage in an active role in patients pharmacotherapeutic monitoring and colaborate to judge the need for such antidotes.
Acknowledgments: To HU-UEL Clinical and Hospital Pharmacy team, who together enabled this work to be carried out.

Crotalid snakebites death: the clinical epidemiological profile of the patients treated at the Toxicology Unit of João XXIII Hospital between 2011 and 2022

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Introduction: The amount of snakebite accidents in Brazil is recorded annually about thirty thousand cases. Crotalus durissus are responsible for 9% of the ophidisms in the country. The casuistry of Minas Gerais in its only toxicological reference unit differs from the national one and stimulates a detailed study due to the complexity of the care and its mortality. **Objective:** To outline the clinical, epidemiological and laboratory profile of fatal victims of crotalic accidents treated at the toxicological reference center of Minas Gerais, and the incidence of clinical complications. **Methods:** Retrospective cohort, by collecting data from consecutive patients, victims of crotalic accidents treated at the Toxicology Unit of Hospital João XXIII of the Hospital Foundation of the State of Minas Gerais, from January/2011 to December/2022. Data collection was based solely on data from medical records and the de-identification of the patients under analysis, and the free and informed consent form was waived. **Results:** Data from 411 visits in this period (median age [interquartile range 23-56], ranging from 1 to 86 years; 79.8% men; 70.0% mulatto. Seasonality was observed, with a period of higher number of accidents between December and March (63.3%), occurring in rural areas (76.8%). The accident occurred mainly in the lower limbs (77.8%), 67.6% of the consultations took place within 6 hours of the occurrence and the main local manifestation reported was pain (81.3%). Among the 304 patients who had systemic manifestations, 37.8% had myotoxic/hemolytic manifestations, 20.4% vagal manifestations, 17.3% renal manifestations, and 6.4% had hemorrhagic manifestations. Regarding the tests, 78.0% of the patients presented alterations in the coagulogram. Regarding severity, 22.7% were classified as mild, 34.0% moderate and 43.3% severe. A total of 4 deaths were identified: local and systemic manifestation occur in all these patients, 3 were men, 50% mullato, mean 71,5 years and median 79,5 years. **Conclusion:** The expertise of care based on good practices, considering the clinical manifestations and the timely management in a reference unit observed in this cohort, foster reflection on the importance of scientific dissemination and investment in the training of health professionals in the care of ophidism victims and prevention of these accidents. **Acknowledgments:** Special recognition to professionals from the Toxicology Unit at Hospital João XXIII - FHEMIG at the Toxicological Information and Assistance Center of Minas Gerais – CIATox/MG whose medical records made this study and excellent patient care possible and the institutions of which the authors belong.

Epidemiological profile of acute intoxications in Brazil from 2007 to 2019

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Background/Introduction: Poisonings are a significant global public health problem and common causes of demand for emergency care in health services around the world. Considering the relevance of the topic, the availability of publicly accessible data and the scarcity of published information on the subject, this work set out to characterize individual demographic variables, exposure variables and health indicators that outline the Brazilian national profile in relation to acute poisonings. **Objective:** Define the epidemiological profile of intoxications in Brazil, from 2007 to 2019. **Methods:** This is an ecological time- series study on acute intoxications in Brazil, from 2007 to 2019. Data were extracted through the Sistema de Informação de Agravos de Notificação (SINAN) using the TABNET, software developed by DATASUS that gives access to the public domain database of the Ministry of Health. Through it, the results were analyzed using descriptive statistics, and the health indicators were calculated: incidence, lethality, general and specific mortality. Furthermore, data were tabulated for analysis by macro-regions (Southeast, North, Northeast, South, Midwest) according to individual demographic variables (gender and age) and exposure variables (circumstance and toxic agents). The Prais Winsten model was used to analyze the trend of the time-series. **Results:** There was an increase of 684,35% in the number of notifications from 2007 to 2019. Considering the distribution of notifications by sex, most poisonings occurred in female patients (55,6%; n=648.390), while the majority of deaths (59,6%; n=7.076) were in males. The most notified age group in the period was adults (20 to 59 years old), and the toxic agent medication and the circumstance suicide attempt were the main ones related to the number of new cases and deaths. Regarding health indicators, the highest incidence and mortality occurred in the South region (59,1 cases per 100.000 inhabitants and 6,91 deaths per 1.000.000 inhabitants, respectively). Similar to notifications according to sex, in all major regions and in Brazil, the highest incidence occurred in females (48,9 cases per 100.000 inhabitants) and the highest mortality in males (5,56 deaths per 1.000.000 inhabitants), however, although notifications were more frequent in adults, the incidence was higher in children (≤ 9 years). Regarding toxic agents, almost half of the incident cases in the analyzed period were due to the use of medication (43,7%; 19,61 cases per 100.000 inhabitants), followed by the drug of abuse (11,9%; 5,33 cases per 100.000 inhabitants) and food and drink (8,2%; 3,69 cases per 100.000 inhabitants). With regard to the circumstances involved, the suicide attempt (38,1%; 17,09 cases per 100.000 inhabitants) is the main one, followed by accidental use (18,6%; 8,3 cases per 100.000 inhabitants) and abuse (12,4%; 5,57 cases per 100.000 inhabitants). Finally, there was an upward trend in incidence and general mortality in all macro- regions and in the country. **Discussion/ Conclusion:** The epidemiological results found in the Brazilian population allow us to conclude that acute intoxications occur more frequently and are more incident in females, due to the use of medication and in the context of suicide attempt, mainly in the South and Southeast macro-regions. Adults are the age group mostly notified, however the incidence is higher in children, mainly due to accidental use. Deaths, lethality and general mortality were higher in males and the is an upward trend in general mortality and incidence in the coming years. The results, therefore, may guide health promotion and prevention actions in the country. **Acknowledgments:** My sincere thanks to all my teachers in the Programa de Pós Graduação em Saúde Baseada em Evidências of the EPM/Unifesp, especially Professor Lucas Leite, Professor Thais Konstantyner and Professor Aécio Góis. Also, my gratefulness to Professor Marlene Zannin.

Epidemiological profile of cases of suicide attempts using diquat registered at the toxicological information and assistance center of Espírito Santo

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Introduction: Diquate (DQ) is a bipyridyl class herbicide and it́ s use is increasing in agriculture since the prohibition of Paraquat sales in 2020. Its mechanism of toxicity is through the production of highly reactive oxygen free radicals, and cellular damage due to oxidative stress, mainly affecting the gastrointestinal tract, kidneys, liver, heart, neurological system and lungs, and consequently, resulting in multiple organ failure and death in the vast majority of cases. The severity of poisoning revolves around the non- existence of an antidote, of more effective decontamination measures and of information among health agents regarding DQ poisoning. **Objectives:** To analyze the epidemiological profile of poisonings with Diquat registered by the Toxicological Information and Assistance Center of Espírito Santo (CIATox-ES). **Methods:** This is an epidemiological study with data collected in the Brazilian Poisoning Data System (DATATOX) and organized in Excel. The population considered in the study consisted of poisoning cases treated by CIATox-ES between 2020 and 2023, in which at least one of the agents involved was Diquat. **Results:** During the period analyzed, 16 cases of DQ poisoning were recorded in Espírito Santo, all of them related to suicide attempts, with death occurring in 9 cases (56.25%). There was a predominance of males (62.5%) compared to females (37.5%). Regarding age group, the most involved was 20-29 years old, with 5 cases (31.25%). The Alkali-Dithionite Test was performed in all cases, being positive in 13 cases (81.25%) and negative in 3 of them. Among the patients with a negative test, none died. The most prevalent clinical manifestations were vomiting (87.5%), renal failure (43.75%), coma - induced or not (56.25%) and reduced level of consciousness (37.5%). Hemodynamic changes (31.25%), odynophagia (31.25%), dysphagia (25%) and dyspnea (25%) were also significant symptoms. At par to laboratory tests, changes were noticed mainly in CPK (43.75%), creatinine (37.5%) and urea (37.5%), there were also reports of caustic lesions in EDA (25%). The treatment used, for the most part, involved Fuller's earth (50%), cathartic (68.75%), ventilatory support (56.25%), IV NAC (68.75%) and hemodialysis (43.75%) . In total, 9 patients required mechanical ventilation, with 8 of these dying (88.8%). Only 3 patients did not receive post-exposure decontamination measures. Furthermore, among the 4 eligible patients who underwent EDA, 2 did not receive a nasoenteric tube and 2 did not undergo prophylaxis with antibiotics. The average time from exposure to treatment was 219.68 minutes. **Discussion/Conclusion:** It can be concluded that DQ poisoning is serious and mostly fatal, affecting several organs within a few hours of ingestion. The clinical mainly involves symptoms of the gastrointestinal tract, neurological changes and renal failure. Furthermore, when analyzing the cases, the need to formulate an antidote for the agent and a single treatment protocol is evident, in addition to disseminating this knowledge among health services so that professionals can work on the situation; consequently reducing exposure time and offering the best possible treatment to the patient. Finally, more studies are needed to better understand the effects and consequent advances in the management of patients poisoned by this herbicide.

Epidemiological profile of patients with N-acetylcisteine indication after intoxication with Acetaminophen: data analysis between May and September of 2023 in a reference center in Southern Brazil

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Introduction: Acetaminophen, discovered in the XIX century and commercialized since 1955, is an analgesic and antipyretic largely consumed. Its toxic potential has been recognized in the 60's, occurring after gluthation depletion and accumulation of the metabolite N-Acetyl-p- benzo-quinone imine, that binds to hepatocytes membrane leadind to cellular death. As suicide rates rise in Brazil, specially in the youth, acetaminophen was responsible for mean 15% of the annual intoxications caused by medication at the Santa Catarina Toxicological Information and Assistance Center (CIATox/SC). **Goal:** To analyze the epidemiological profile of patients poisoned by Acetaminophen with indication of treatment with the antidote N-Acetylcysteine (NAC) at CIATox/SC from May to September of 2023. **Method:** Data collection using DATATOX-BI (Business Intelligence) - Computerized online access system developed based on an Open Source System that allows the extraction of data recorded in DATATOX - Platform for recording and documenting poisoning cases handled by brazilians CIATox. **Results:** During the period analyzed, 104 cases of acetaminophen poisoning received an antidote recommendation because they had ingested more than 10g, more than 200 mg/kg or an unknown dose of the medication. The most affected age group was 15 to 29 years old, responsible for 61% of cases. There was a predominance of females (64%) and the most common circumstance was attempted suicide (94%). The time lapsed between ingestion and our care was less than 2 hours in 42% of cases, but a significant portion still arrive late, with 26% of these cases showing up for care after more than 12 hours. During the period analyzed, there were no fatal cases, but 6 were classified as severe (6%). Therefore, the majority of poisonings were considered mild (81% of cases). **Discussion/Conclusion:** The toxicity of Acetaminophenl is dose dependent, there is a greater risk in chronic alcoholics, malnourished people and chronic users of cytochrome P450 inducers such as anticonvulsants and tuberculostatics. Regarding treatment, the only therapy approved for clinical use is the NAC, which has better results when started within the first 8 hours after ingestion, contributing to reducing mortality to less than 1% of cases. In May 2023, after a literature review and discussion at a scientific meeting, the CIATOx/SC team modified the toxic dose of acetaminophen and started to indicate NAC as an antidote only for ingestions of more than 10g of acetaminophen in adults, or more than 200mg/kg in children, or even in cases where the reported amount was uncertain. The evolution of tools such as DATATOX-BI and the services provided by specialized centers such as CIATox are extremely important as they will allow adequate documentation of poisoning cases, providing better epidemiological information and the outcomes of these patients.

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Epilepsy as a clinical manifestation of poisonings treated by the Toxicological Information and Assistance Center of Santa Catarina (CIATox/SC)

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Introduction: Epilepsy is a neurological disorder resulting from inadequate inhibitory influences or excessive excitatory stimulation. In intoxications, it is considered a potentially serious consequence. It is estimated that around 6% of new epilepsy episodes are induced by intoxications and, although most cases are self-limiting, up to 9% of status epilepticus cases may result from drug toxicity. Despite various substances having properties favoring epileptic activity, little is known about the main agents involved and the severity of intoxications. **Objectives:** The purpose of this study is to determine the main substanves involved and the severity in intoxications presenting with epilepsy treated by the Toxicological Information and Assistance Center of Santa Catarina (CIATox/SC) from 2014 to 2022. **Methods:** A retrospective analysis of cases attended by CIATox/SC between 2014 and 2022, presenting with epilepsy, was conducted. Analyzed data included the total number of cases, the agents involved, the distribution between isolated intoxications (involving a single substance) and mixed intoxications (involving two or more substances), and the number of fatal cases associated with each substance. **Results:** During the analyzed period, 789 cases presented with epilepsy. Of these, 453 (57.4%) were isolated intoxications, and 336 (42.6%) were mixed. The most frequently involved substances were: cocaine in powder and crack forms (153 cases: 80 isolated and 73 mixed); alcoholic beverages (118 cases: 18 isolated and 100 mixed); bupropion (76 cases: 41 isolated and 35 mixed); pesticides (62 cases: 50 isolated and 12 mixed), including cholinesterase inhibitors, coumarin-based rodenticides, pyrethroids, and other pesticides; phenytoin (43 cases: 19 isolated and 24 mixed); and amitriptyline (36 cases: 9 isolated and 27 mixed). Of the 789 intoxications presenting with epilepsy, death occurred in 61 cases (7.7%). Pesticides were the most frequently associated agent with deaths, accounting for 17 cases, followed by cocaine in 12 cases, and bupropion in 7 cases. **Discussion/Conclusion:** The results reveal a significant incidence of epilepsy in intoxications involving cocaine, alcoholic beverages, bupropion, and pesticides attended by CIATox/SC. The presence of this complication may be attributed to the direct neurotoxic effects of these substances on the central nervous system. Bupropion, with its dopamine and noradrenaline reuptake inhibition property, may trigger epileptiform activity. Similarly, pesticides and cocaine have the capacity to alter neuronal excitability. The high rate of fatal cases highlights the importance of early recognition and proper treatment of these intoxications to avoid severe complications. Epilepsy is a significant clinical manifestation in intoxications involving medications, pesticides, and substance abuse. It is crucial for emergency healthcare professionals to be aware of this association, enabling rapid diagnosis and appropriate management in this specific clinical context.

Evaluation of cases of exposure to substances that cause disorder, before and after the confinement of COVID-19

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Background/Introduction: The number of users of psychoactive drugs has increased in the world, and in Brazil, there are an estimated total of 3.5 million users. Social toxicology studies the effects of the non-therapeutic use of psychoactive drugs (stimulants, depressants, and psychedelics, or those disrupting the Central Nervous System), causing harm to the individual and society. Some medications have the potential for abuse and dependence, mostly used for non-medical purposes and not prescribed. Currently, the terms "substance abuse" and "dependence" have been replaced by a single terminology: "substance use disorder". In this scenario, the COVID-19 pandemic has brought historic problems for humanity in several areas in the whole world, such as in the health domain, including with regard to the pattern of use of psychoactive drugs. **Main aim:** The present study aimed to evaluate the socio-demographic profile of cases of exposure to substances that cause disorders, before and after the COVID-19 confinement, in order to estimate whether there was any influence of the COVID-19 pandemic on cases of psychoactive drugs intoxication. **Methods:** A survey was carried out at the Toxicological Information and Assistance Centre (CIATOx-CG), located at the "Dom Luiz Gonzaga Fernandes" Emergency and Trauma Hospital (HETDLGF). This was an epidemiological and retrospective study. The data were found in the Brazilian System of Data Intoxication (DATATOX) of the Toxicological Information and Assistance Centres. **Results/Discussion:** In the time frame evaluated, 289 cases were reported, with a predominance of male people (66.0%) at a young age, between 20 and 29 years old (35.9%), with complete secondary education (13.4%), mixed race (62.3%), and from urban area (90.5%). The occurrences were predominantly oral (72.2%), mild (65.6%), with the main outcome being the cure (83.8%). When related to the reports of "suicide attempt" and "abuse" with the time frames of "before confinement" (2018/2019) and "after confinement" (2021/2022), it was observed that there was a significant increase ($p<0.001$) of cases for both situations. There was also an increase, with statistical meaning ($p<0.001$), in the prevalence of almost all age groups after confinement, in comparison to the period before social distancing, except the ones between 10-14 years old ($p=0.9999$). Alcohol was the most consumed psychoactive substance (54.5%), followed by cocaine in powder form (19.0%) and marijuana (7.2%). **Conclusion:** Therefore, according to the data obtained regarding the use of substances that cause disorders in the pandemic scenario, there was an increase in the consumption pattern for the group of psychoactive substances in the city of Campina Grande (PB). **Acknowledgments:** The authors would like to thank CIATox of Campina Grande (PB) and the Brazilian Association of Toxicological Information Centres (ABRACIT) for the access to the DATATOX database.

First clinical case report of 25B-NBOH poisoning in Rio Grande do Sul state, Brazil

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Background/Introduction: Synthetic phenethylamines are an important class of new psychoactive substances (NPS). Initially, they were disseminated as NBOMe derivatives, but since the end of the 2010s, the reports of NBOH have increased in Brazil and around the world. Among the main clinical manifestations already described are hallucinations, delirium, psychotic episodes, and cardiovascular disorders. Diagnosing the use of these substances, as well as reporting clinical cases, is extremely important for fast and more appropriate management of new cases. **Objective:** This work aims to describe a clinical case of poisoning involving the synthetic phenethylamine 25B-NBOH. **Methods:** Retrospective case report of a clinical case of confirmed suspicion of synthetic phenethylamine exposure, attended by the Toxicological Information Center of Rio Grande do Sul (CIT/RS). **Results:** A 17-year-old male teenager is admitted to the hospital emergency room with intense agitation. During the examination, he stated the use of a drug called "N-bomb" in the form of a blotter paper, indicative of synthetic phenethylamines. The patient had a previous history of marijuana use. Antipsychotics and benzodiazepines were administered for agitation. Blood and urine samples were collected for laboratory analysis, including toxicological analysis. Laboratory tests showed leukocytosis (14,100 leukocytes/m3), glucose of 104 mg/dL, hyperbilirubinemia of 2.52 mg/dL, with normal levels of direct bilirubin of 0.34 mg/dL and high levels of indirect bilirubin of 2.18 mg/dL, and elevated levels of creatine phosphokinase 328 U/L. Other tests such as kidney function (creatinine, urea), liver function (AST, ALT), amylase, and electrolytes (sodium, potassium) showed normal levels. The qualitative urine test showed a positive result for red blood cells. Urine screening analysis using immunoassay did not indicate a positive result for any group. Screening toxicological analysis using gas chromatography coupled to mass spectrometry (GC-MS) only indicated a positive result for lidocaine, used in medical care. Subsequently, plasma analysis by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for classical drugs and pharmaceuticals indicated a positive result for chlorpromazine, diazepam, lidocaine, promethazine, and midazolam and the urine showed positive results for lidocaine, haloperidol, and promethazine. All of these substances detected were used in the management and treatment of the patient. Finally, due to suspected exposure to synthetic phenethylamine, an analysis for new psychoactive substances was performed using LC- MS/MS, where 25B-NBOH was detected in the urine sample. The patient was kept under observation for three days and referred for psychiatric evaluation. **Discussion/Conclusion:** A mild to moderate case of 25B-NBOH poisoning has been described. Intense agitation is compatible with synthetic phenethylamine poisoning but requires additional analysis to confirm exposure. The elevated CK levels demonstrate possible muscle damage due to agitation, as well as hemolysis due to the considerable increase in indirect bilirubin. Toxicological analysis allowed the correct diagnosis of the substance involved in the poisoning. This type of analysis is essential in cases with non- specific clinical manifestations. The identification of 25B-NBOH in the patient sample, as well as the case history, corroborates the presence and circulation of this NPS in Rio Grande do Sul state and Brazil, and should serve as a warning to health authorities. **Acknowledgments:** The authors would like to acknowledge the financial support from the Coordination of Improvement of Personal Higher Education, Brazil.

Illicit drug poisoning in children: toxicological data obtained from cases attended by the Toxicological Information Center of Rio Grande do Sul

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Background/Introduction: In recent years, there has been a discernible escalation in the prevalence of illicit drug exposure in Brazil, emerging as a substantial and pressing public health concern. Children emerge as an important vulnerable group in this scenario due to their susceptibility, curiosity, and exposure induced by other individuals. Consequently, it is essential to evaluate and describe the data from emergency toxicological cases to trace an epidemiological profile of illicit drug exposure in this specific population. **Objective:** This work aims to describe the profile of illicit drug exposure in children aged 0 to 12 years old from emergency cases attended by the Toxicological Information Center of Rio Grande do Sul (CIT/RS). **Methods:** Data were collected from poisoning cases attended by the Toxicological Information Center of Rio Grande do Sul (CIT/RS), wherein a urine sample was available for subsequent toxicological analysis. The cases were registered between 2018 and 2023. Specifically, only data on cases involving patients aged 12 years or younger were included in the analysis. Urine samples were analyzed to verify the presence of cocaine and its metabolites (benzoylecgonine, cocaethylene, EME, and AEME), 11-nor-9-COOH-Δ9- tetrahydrocannabinol (THC-COOH) and amphetamine-type stimulants (amphetamine, methamphetamine, MDA, MDEA, and MDMA). The analyses were performed on an LC-MS/MS (Shimadzu, Japan), employing a dilute-and-shoot protocol for sample preparation. The variables considered for statistical analysis were circumstance, gender, age, place of exposure, and patient evolution. The statistical analysis of the cases was carried out using Excel software. **Results:** The LC-MS/MS method used for the analyses showed a limit of quantification of 20 ng/mL for all analytes, except for MDA and THC-COOH which was 50 ng/mL. A total of 286 cases were included in this study. In 32.9% of the analyzed samples, at least one illicit drug was detected (n=94). The most identified substances were cocaine and its metabolites (71 cases; 24.8%), THC-COOH (27 cases; 9.4%), and MDA and/or MDMA (6 cases; 2.1%). Of positive cases, 56.4% were male (n=53) and 43.6% were female (n=41). The mean age was 3.4 ± 3.5 years old, and the most prevalent age group was 1-4 years old with 45 cases (47.9%), followed by 5-9 years old with 19 cases (20.2%), lower than 1-year-old with 17 cases (18.1%) and 10-12 years old with 13 cases (13.8%). Regarding the circumstances of exposure, 35 cases were individual accidents, eight mistreatments, three suicide attempts, two situations of abuse, and 46 had their circumstances ignored. Cases of suicide attempts and abuse occurred in children over ten years old only. The most common place for these exposures was in the home, with 58.5% (n=55), followed by public space (n=4; 4.3%) and school (n=1; 1,1%). In 36.2%, the exposure place was ignored. About 78.7% of patients were cured (n=74); only one had sequelae after exposure and 19 cases had their evolution ignored (20.2%). **Discussion/Conclusion:** The toxicological outcomes revealed a pronounced incidence of positive results for illicit drugs in samples collected from children. The occurrence of poisoning in children can be construed as a direct manifestation of the substances consumed by the adolescent and adult demographic. Cocaine was the most commonly detected drug in the urine samples. The major age group was 0-4 years old, which shows that such exposures are directly correlated with the conviviality of parents or guardians. This inference gains additional emphasis, particularly considering the predominant setting for these incidents, namely, within the confines of the children's residences. The analytical data obtained highlights the importance of toxicological analysis, to confirm drug exposure, thus facilitating decision-making by the medical and social assistance teams.

Intoxication due to indiscriminate use of phytotherapy in Brazil: literature review

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Introduction: According to the World Health Organization (WHO), 80% of the population in developing countries use traditional herbal practices for health, and 85% use plants or derivatives. Currently, there is a report of diversified clinical-traditional use of medicinal plants. Phytotherapy in general can connect human beings with the environment, normalizing physiological functions based on this access power of nature, restoring weakened immunity, promoting detoxification and rejuvenation (CAVALCANTE, 2019). In Brazil, the use of medicinal plants as therapeutics is mediated by social, economic, and cultural factors. Therefore, it is necessary to adopt standards that ensure its use (DE FRANÇA et al, 2021). There is a popular belief that plants, as they are natural products, do not present toxicity, especially if their toxic effects do not occur immediately after their use (DE FRANÇA et al, 2021). Thus, although it is believed that herbal medicines are natural substances, scientific studies state that their irrational use can cause health problems such as poisoning by intoxication; therefore, in order to avoid possible collateral damage, their management must be rationalized. **Objectives:** The objective of this study was to investigate the intoxication due to indiscriminate use of phytotherapy in Brazil. **Methods:** The work was prepared based on exploratory research in the PUBMED, LILACS, and Google Scholar databases, selected in Portuguese, involving the reading and study of these, using scientific journals related to the incorrect use of herbal medicines. To choose them, the keywords used were: phytotherapy, medicinal plants, risk, health, toxic, and intoxication. The oldest article used for the research is dated 2019 and the most recent article is from 2022. **Results:** Although the use of medicinal plants for the treatment, cure, and prevention of certain diseases is one of the oldest forms of medicinal practice in humanity, studies indicate that popular and even traditional use are not sufficient to validate medicinal plants as effective and safe medicines (DOS SANTOS et al, 2022). Regarding natural medicines, based on historical evolution, they are understood as products that are easy to acquire and handle. Most plants are used based on ancient cultural knowledge and family traditions, which impoverishes theoretical-scientific knowledge about their toxicological properties. Generally, the use of plants for therapeutic purposes is practiced without medical supervision, representing a potential danger to the population, as there is the possibility of interaction between these "natural" products and medicines, in addition to their interference with the results of laboratory tests (ÁVILA, 2023). According to Alexandre et al. (DE FRANÇA et al, 2021), medicinal plants and herbal medicines are made up of complex mixtures of bioactive substances, called secondary metabolites, which may be responsible for multipurpose actions. Therefore, misuse of medication can generate symptoms of intoxication, which can vary depending on the intensity, type of toxic material ingested, quantity, and physiological state of the individual who ingested it. **Conclusion:** As a result of the poor administration of herbal medicines, it is possible to conclude that accidental poisonings during treatment are due to the idea that treatment with plants does not contain chemically active substances. Research has shown evidence that medicinal plants can trigger adverse reactions depending on their chemical constituents. Furthermore, errors in the identification of plant species and use other than the traditional way can be dangerous, leading to overdoses, therapeutic ineffectiveness, and adverse reactions (DE FRANÇA et al, 2021). **Acknowledgments:** The students who carried out this research are grateful to the wisdom proposed by science.

Ophidian accidents by bothrops erythromelas and their association with the pandemic state of COVID-19

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Introduction: in Brazil, ophidian accidents are considered a public health problem, especially in poorer and rural regions of the country, despite the existence of the National Program for the Control of Accidents by Venomous Animals (PNCAAP), which aims to reduce the number of cases, through health education, and the lethality of accidents caused by this group of animals, through the appropriate use of serum therapy. Among the dozens of species of medically important snakes registered in Brazil, Bothrops erythromelas, predominant in the Northeast region, causes serious health risks and can even cause death. It is known that the COVID-19 pandemic caused changes in the population's daily lives and activities, for example: people started to avoid health services, for fear of contamination, besides confinement and social isolation may have impacted the notification of ophidism. **Main Aim:** the present study sought to evaluate accidents caused by Bothrops erythromelas, reported to CIATox-CG, in the period between 2018 and 2022, to verify a possible influence of the COVID-19 pandemic on these occurrences. **Method:** cross-sectional, quantitative, retrospective, and documentary study. Epidemiological data on ophidian accidents caused by Bothrops erythromelas were collected from January 2018 to December 2022, based on the Individual Notification Form of Accidents by Venomous Animals of the Notifiable Diseases Information System, in the city of Campina Grande/PB. Epidemiological, clinical and laboratory variables were analysed. Descriptive statistics were used to analyse the data and, for verifying possible associations between the research variables in the year of the beginning and peak of the pandemic (2020) with the previous (2018-2019) and subsequent (2021-2022) periods, the Chi-square test and Fisher's Exact test ($p < 0.05$) were applied. **Results and Discussion:** in the time frame evaluated, 669 accidents caused by Bothrops erythromelas were recorded. There was a predominance of cases in male people (75.3%) at a young age (20 to 49 years old) (45.4%), with low education (incomplete primary education) (24%), farmers (43 %), and in rural areas (85%). For the variables of "occurrence zone" and "age group", there was a statistically significant association with the pandemic ($p < 0.05$). Regarding the final classification of cases, moderate ones prevailed (48.7%). In the variable "time elapsed", between the accident and care, in the years assessed, there was a predominance of the interval of 1 to 3 hours (50.5%). For the variable "anatomical region" affected, the "foot" was predominant (52%). These two variables demonstrated a statistically significant association ($p < 0.05$) with the peak year of the pandemic (2020). The most frequent local events were observed over the years evaluated: pain (42% - 43%), followed by edema (31% - 41%). As for systemic manifestations, vagal ones predominated (29% - 41%) in all years of the time frame evaluated, except in 2018, when hemorrhagic changes stood out (18%). Such systemic manifestations also showed a statistically significant association ($p < 0.05$) with the pandemic event. For the "serum therapy" variable, it was observed that most patients (40%) required the administration of four to seven ampoules of Antibotropic Serum to reestablish hemostasis, thus manifesting a significant statistical association ($p < 0.05$) in relation to the year 2020. **Conclusion:** it was found that in the initial and peak year of the pandemic (2020), the occurrences presented an expressive record for the city, reaching almost double the number of cases when compared to the previous years, once applied to the Association Tests. Therefore, it can be stated that the COVID-19 pandemic had an impact on the number of ophidian accidents recorded in the city of Campina Grande/PB and that, despite the pandemic scenario, the PNCAAP managed to achieve its aims.

Overdose due to cocaine abuse: case report

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Introduction: Cocaine is an illicit psychoactive substance (PAS) with high analgesic and stimulating potential for the central nervous system (CNS). These properties make cocaine one of the most widely used substances in Brazil and around the world. Cocaine causes severe chemical dependence in the body, which is often associated with withdrawal syndrome and psychosis. Intoxication by this particular substance can occur in either a chronic or acute manner, commonly referred to as an overdose. An overdose can happen when a significant amount of the substance is used, either through injection (less common), smoking, or inhalation. Its complications can result in rhabdomyolysis, cardiac arrest, stroke, coagulation disorders, acute kidney failure, seizures, high blood pressure, hyperthermia, and other complications that can be fatal. **Objective:** The objective of this study is to report a case involving a fatal outcome attributed to cocaine abuse. **Methodology:** This study was developed based on the initiatives of the extension project titled "Poison Control Center" – registration number 1105/89 in the DEX. The data were obtained from the evolution of the epidemiological record registered in the Brazilian Poison Data System of the Toxicological Information and Assistance Centers (DATATOX). **Results:** A 34-year-old female was referred to the hospital in her city by SAMU after being found agitated and aggressive in a ditch. She had tachycardia, elevated body temperature (CT), heart rate (HR), and blood pressure (BP). A subsequent toxicological examination confirmed that the substance in question was cocaine. Benzodiazepines were administered for support, and laboratory tests and observations were performed. In less than 24 hours, she was transferred to the Intensive Care Unit where she continued with hemodialysis, an indwelling bladder catheter (SVD), and supratherapeutic doses of vasoactive drugs. The case presents a progressive evolution leading to a diagnosis of rhabdomyolysis, characterized by creatine kinase levels exceeding one hundred times the acceptable level. The patient also experienced acute myocardial injury, liver failure with very high levels of oxaloacetic transaminase and pyruvic transaminase, and severe coagulopathies as indicated by laboratory tests showing platelet counts below 35,000. Additionally, the patient exhibited respiratory depression, alterations in consciousness levels, and the presence of multiple hematomas throughout the body. On the fifth day, the patient exhibited no photomotor reflexes and had cyanotic extremities when she experienced bleeding from the dialysis catheter, which was suspended due to the effects of gravity. After the suspension of dialysis, the patient experienced a progressive decrease in blood pressure and bradycardia, ultimately leading to their death. **Discussion:** Cocaine is widely used as a drug of abuse, capable of causing strong physical and emotional dependence. Several studies have identified it as a major contributor to cardiovascular complications, ranking as the third most prevalent among individuals aged 12 to 65 years who report using illicit substances. Due to its vasoconstrictive activity, it results in an overload of the myocardium, leading to an inability to maintain sufficient cardiac output. This, in turn, can cause organ failure, as mentioned earlier. Basic functions necessary for the homeostasis of the organism can be compromised by overload and insufficiency of vital organs, such as excretion, metabolism, blood pressure control, hemostasis, and even the individual's consciousness. Depending on the circumstances, acute intoxication with high doses of cocaine can become nearly irreversible, potentially resulting in the patient's death. **Acknowledgments:** We would like to thank the State University of Maringá (UEM), the University Without Borders Extension Program, the Ingá University Center - UNINGÁ, the Maringá Poison Control Center, and the UEM Toxicology Laboratory (LATOX).

Poisoning in patients assisted at Toxicological Information and Assistance Center in Federal District, Brazil, during the COVID-19 pandemic

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Poisoning is defined as an adverse event resulting from the action of chemical substances that cause pathology in the individual. The WHO reported that three million hospital admissions occur annually due to toxic exposure worldwide. With the progression of cases of SARS-CoV-2 infection in 2020, calls to Poison Centers due to exposure to cleaning products, hand sanitizers and disinfectants increased rapidly in early March 2020 in the USA. Given this observation, it was hypothed that the pattern of exogenous intoxication changed for other toxic agents. Therefore, this work aimed to analyze the epidemiological profile of exogenous poisonings recorded by CIATOX-DF, during the months of March 2020 to February 2021, the period in which the COVID-19 pandemic was in force, and compared to the period between 2004 and 2019. During the COVID-19 pandemic, an increase in accidental and environmental cases of venomous (increased from 5,65% to 10,36% for scorpio and from 2,70% to 3,12% for snake) and non-venomous animals was observed (increased from 1,51% to 3,71%), especially in babies and pre-adolescents and adolescents (11 to 15 years old - increased from 2,15% to 3,11%) and in women (increased from 51,05% to 53,66%), due this range age group and gender remained more in the rural environment. The majority of poisonings are accidental, which intensified. It was possible to observe a decrease in suicide cases (decreased from 17.64% to 16.59%), but within suicide cases there was a tendency for an increase in cases of poisoning by pre-adolescents and adolescents (increased from 2,15% to 3,11% for 11 to 15 years old) in relation to other ages, being explained by the increase in anxiety within this range. age. There continued to be a high frequency of cases involving medicines (40,20%), pesticides (5,37%) and household cleaning products (12,11%), with a significant increase in cases involving 70% alcohol (increased from 0,01% to 3,01%). It is observed that patients living in cities further away from the plan suffer accidents with venomous animals. Accidental and attempted suicide cases occur more in cities closer to the pilot plan. It is concluded that these changes are explained by the new pattern of social behavior during the pandemic.

Poisonings involving bupropion: cases treated at the Toxicological Information and Assistance Center of Santa Catarina (CIATox/SC) between 2014 and 2022.

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Introduction: Bupropion is an aminoketone antidepressant commonly used to treat depression, smoking cessation and weight loss. Although its mechanism of action is not fully understood, it is known that it acts by inhibiting the reuptake of dopamine and norepinephrine. Bupropion intoxication can lead to cardiologic and neurologic manifestations, mainly related to serotonergic toxicity due to increased neuronal firing, with seizures being a major concern. It is estimated to occur as late as 24 hours after ingestion in 8 to 40% of poisonings. **Objectives:** The purpose of this study is to determine the clinical and epidemiological profile of poisonings involving bupropion treated by the Toxicological Information and Assistance Center of Santa Catarina (CIATox/SC) from 2014 to 2022. **Methods:** A retrospective analysis of bupropion poisonings treated by CIATox/SC between 2014 and 2022 was conducted. Analyzed data included the total number of cases, sex distribution, age groups, circumstances of exposure, distribution between isolated (only bupropion) and mixed poisonings (involving two or more agents), the average dose of bupropion ingested, the frequency of seizures occurrence, severity and final outcome of the cases. **Results:** From 2014 to 2022, CIATox/SC attended 738 cases of bupropion poisoning. Females accounted for a majority with 538 cases (72.9%), and the age group of 20 to 39 years was predominant with 389 cases (52.7%). Regarding the circumstance, attempted suicide (ST) was the main one, being attributed to 662 (89.7%) cases, followed by accidental ingestion in 36 cases (4.9%). Out of 738 poisonings, 237 (32.1%) were isolated bupropion exposures, while 501 (67.9%) one or more substances were associated. The ingested dose of bupropion was reported in 585 cases, ranging from 2.14 to 375 mg/Kg, with an average of 42.0 mg/Kg. Isolated poisoning cases had an average reported dose of 40.0 mg/Kg, while mixed cases had an average dose of 44.1 mg/Kg. Among clinical manifestations, one or more seizure episodes were reported in 78 cases (10.6%). Notably, of the 78 cases with seizures, 37 occurred in isolated poisonings (15.6% of 237 cases), and 41 occurred in mixed poisonings (8.2% of 501 cases). Regarding severity classification, 585 cases (79.3%) developed mild or no symptoms, followed by moderate cases in 66 (8.9%) instances. Severe poisonings, with intense clinical manifestations, life-threatening risks or sequelae, were attributed to 59 patients (8.0%), with 9 cases (1.2%) resulting in death and 19 (2.6%) are unknown. **Discussion/Conclusion:** The predominance of females and age group of young adults reflects potential demographic characteristics and substance prescription patterns. The high frequency of SA aligns with the previous studies, indicating an increase in cases and deaths from SA in recent years, emphasizing the need for preventive strategies. Although the average ingested doses of bupropion do not differ significantly between isolated and mixed cases, the association of bupropion with other substances highlights the importance of considering synergistic actions. The prevalence of seizures as a manifestation is in line with the literature, however, surprisingly, they point to a greater occurrence of seizures in isolated ingestions. Bupropion poisonings pose a significant risk, especially due to the frequency of seizures. It is crucial for emergency service professionals to be aware of this association for rapid and appropriate management of seizures in this clinical context.

Possible collateral and toxicological risks associated with the use of herbal remedies during pregnancy

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Introduction: It is widely known in history of humankind that the use of herbal medicine for the goal of curing certain diseases is widespread, specially in socioeconomically vulnerable communities. In addition to that, in the last two decades, there has been a return of popularity of herbal medicines, not only because of their easy access, but also for a preference for simple or complementary therapies. However, studies indicate that herbal therapy might represent health risks to its users, especially pregnant women, as the gestational period is marked by significant physiological changes connected to fetal development and potential clinical symptoms for the woman. The reason for using herbal remedies during pregnancy may vary, ranging from beliefs that such medications help in gestation, facilitate childbirth, assist in breastfeeding, and many of these beliefs are rooted in ancient cultural practices. **Objectives:** This literature review aims to investigate the possible side and toxicological effects of ingesting herbal medicines related to maternal health. **Methodology:** For the literature review, the subject headings "Phytotherapy" and "Pregnancy" were defined, using the Boolean operator AND, through the PUBMED database. After reviewing the studies, cross-sectional observational studies and systematic reviews were included, while other study models and publications unrelated to the proposed topic were excluded. Reviews in both English and Portuguese were included. **Results:** In total, 93 articles from the last six years were found, and six articles were selected to be included in this review. The studies report that Brazil ranks second in the world for pregnant women using herbal remedies. This data also indicates that the use of these resources is culturally influenced and varies according to the biome of each region. In light of this, it is remarkable that herbal therapy is classified as "healthy medication" without sufficient scientific basis and evidence regarding its use, which can lead to fetal malformation and negative repercussions for pregnant women. Therefore, self-medication with any herbal substance should be avoided by pregnant women, and the intention of administer such substances must be communicated to the healthcare professional to prevent adverse reactions like constipation, nausea and vomiting and toxic effects. **Conclusion and Discussion:** Based on the data studied that women from all regions wish to use or use herbal medicines, the studies state that there is little guidance regarding their use for pregnant women and the entire community, as the majority believe it is possible to use these substances without a medical prescription. and adequate monitoring by a health professional. It is concluded that there are few studies that confirm the safe use of medicinal herbs. Furthermore, it must be taken into account that the harm caused by inappropriate use of herbal medicine can cause damage to the infant's intra- and extra-uterine life, in addition to possible toxicology during lactation. **Acknowledgments:** We thank science and the event, the opportunity to study issues relevant to the development of the community.

Predicting fetus exposure to nifedipine employing a physiologically based pharmacokinetic (PBPK) modeling approach

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Nifedipine is used for treating mild to severe hypertension and preventing preterm labor in pregnant women. Nevertheless, concerns about nifedipine fetal exposure and safety are always raised. Nifedipine administration during pregnancy is thought to be associated with different levels of teratogenic risks ranging from atrial septal defect and hernia umbilicalis if administered in the first trimester of pregnancy in animal models and humans to minor birth defects and slight dysplasia of the hip. Furthermore, fetal exposure to nifedipine during the third trimester of pregnancy is associated with an increased risk of the neonate presenting jaundice and seizures. The aim of this study was to develop and validate a maternal-placental-fetal nifedipine physiologically based pharmacokinetic (PBPK) model and apply it to predict maternal, placental, and fetal exposure to nifedipine at different pregnancy stages. A nifedipine PBPK model was verified with non-pregnant data and extended to the pregnant population after including the fetoplacental multi-compartment model that accounts for the placental tissue and different fetal organs within the Simcyp Simulator version 22. Model parametrization involved scaling nifedipine transplacental clearance based on Caco-2 permeability, and fetal hepatic clearance was obtained from in vitro to in vivo extrapolation encompassing CYP3A7, CYP3A5 and CYP3A4 activities. Predicted concentration profiles were compared with in vivo observations and the transplacental transfer results were evaluated using two-fold criteria. The PBPK model predicted a mean (range) cord-to-maternal plasma ratio of 0.98 (0.86 – 1.06) at term, which agrees with experimental observations ranging 0.78 (0.59 -0.93). The PBPK model developed was used to predict fetus exposure in earlier phases of gestation. The Area Under the Curve (AUC) of amniotic fluid and umbilical vein concentrations versus time after 20 mg twice daily oral doses of nifedipine during first, second and third trimester of gestation were 13.24; 10.24; 8.41 ng.h/mL (amniotic fluid) and 105.75; 166.92; 155.76 ng.h/mL (umbilical vein), respectively. The reduced mother CYP3A4 activity and the increased, but small, metabolism and excretion of nifedipine by the fetus during the gestation development respectively explain these results. In conclusion, this innovative PBPK model can be applied to support maternal and fetal safety assessment for nifedipine at various stages of pregnancy. Marya Antônya Werdan thanks Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for the scholarship provided (process number E-26/204.526/2022).

Prediction of dapaconazole clinical drug–drug interactions using physiologically based pharmacokinetic (PBPK) modeling

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Background/Introduction: Dapaconazole is a new antifungal drug from the azoles class that has demonstrated good efficacy both in its systemic effect and in local fungal infections. Drugs from the azoles class have been shown to be inhibitors of cytochrome P450 (CYP450), an important family of enzymes involved in drug metabolism, which can generate many drug-drug interactions (DDIs). **Objective:** To investigate the potential interaction of dapaconazole on CYP450 isoenzymes using static and dynamic approaches. **Methods:** The in vitro inhibition of main CYP450 isoenzymes (CYP1A2, CYP1A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5) by dapaconazole in a human liver microsome incubation medium was evaluated. A dapaconazole PBPK model (Simcyp version 20) in dogs was developed and qualified using observed data and was scaled up for humans. Static and dynamic models to predict DDIs following current FDA guidelines were applied. **Results:** The in vitro dapaconazole inhibition was observed for all isoforms investigated, including CYP1A2 (IC50 of 3.68 µM), CYP2A6 (20.7 µM), 2C8 (104.1 µM), 2C9 (0.22 µM), 2C19 (0.05 µM), 2D6 (0.87 µM), and 3A4 (0.008–0.03 µM). The dynamic (PBPK) and static DDI mechanistic model-based analyses suggest that dapaconazole is a weak inhibitor (AUCR > 1.25 and <2) of CYP1A2 and CYP2C9, a moderate inhibitor (AUCR > 2 and <5) of CYP2C8 and CYP2D6, and a strong inhibitor (AUCR ≥ 5) of CYP2C19 and CYP3A (Table 1), considering a clinical scenario. Table 1. Evaluation of the potential of dapaconazole as CYP inhibitor through predicted area under the curve ratios using static and dynamic model. CYP Substrate [I] (µM) Ki (µM) R1 fm Static AUCR Dynamic AUCR FDA Classification 1A2 Phenacetin 9.5 1.84 1.20 0.71 1.86 1.17 Weak 2C8 Paclitaxel 9.5 52.05 1.01 0.5 3.00 1.461 Moderate 2C9 Diclofenac 9.5 0.11 4.43 0.87 1.95 1.382 Weak 2C19 S-Mephenytoin 9.5 0.03 15.14 0.89 3.86 5.36 Strong 2D6 Bufuralol 9.5 0.43 1.86 0.66 2.31 1.51 Moderate 3A4 Midazolam 9.5 0.004 98.26 0.88 19.45 5.14 Strong 3A4 Nifedipine 9.5 0.02 25.70 0.96 5.31 4.05 Strong 1 AUCR dynamic study was performed in Simcyp using repaglinide as CYP2C8 substrate. 2 AUCR dynamic study was performed in Simcyp using tolbutamide as CYP2C9 substrate. Abbreviations: R1, ratio of intrinsic clearance values of a probe substrate for an enzymatic pathway in the absence and in the presence of dapaconazole; CYP, cytochrome P450; fm, fraction metabolized; [I], inhibitor concentration that is the total plasma maximum concentration (Cmax); Ki, inhibition constant; AUCR, area under the curve ratio between AUC with inhibitor and AUC without inhibitor. **Discussion/Conclusion:** Knowing that the static model predicts the "worst clinical scenario", the results were consistent, demonstrating, in general, a greater impact of dapaconazole against CYP450 isoforms, when compared to the dynamic model, using the PBPK approach. Considering these results together, it is possible to determine that dapaconazole is a potential weak clinical inhibitor of CYP2C9, a weak inhibitor of CYP2D6 and CYP2C8 and a strong inhibitor of CYP2C19 and CYP3A4. The developed dapaconazole PBPK model could be used as a useful tool to guide future in vivo and clinical studies with this molecule.

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Prevalence and clinical-epidemiological analysis of intoxication cases in the intensive care units at a University Hospital of Campos Gerais

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Intoxication is defined as chemical and physical substances exposure where the interaction with the biological system generates imbalance, manifesting clinical signs and symptoms or abnormal laboratory results. They can be classified as intentional, accidental, self-inflicted or criminal. They vary in severity, but generally require medical attention and quick interventions. The exposure to toxic agents, usually exogenous can be acute or chronic and has different etiologies. An example of an acute consequence of a chronic exposure is upper gastrointestinal bleeding (UGH), which can manifest in cases of chronic exposure to alcohol. The present work investigated cases related to exogenous intoxication, hematemesis, melena and UGH at the four Intensive Care Units's (ICUs) physical records of the Regional University Hospital of Campos Gerais (HURCG) from January 2019 to April 2022. The medical records selected whose cause of intoxication was a consequence of substance abuse were studied regarding the variables: sex, age, prompter agent and outcome. A total of 112 cases of UGH were found, 94 (83.9%) caused by alcoholism, 15 (13.4%) by drug use only and three (2.7%) by alcoholism and concomitant drug use. Most cases of UGH occurred in men as a result of alcohol consumption, with the most affected age group being 55-64 years, and of the total number of cases studied 49.1% were discharged and 43.8% were to death. Also, 20 cases of hospitalization due to exogenous poisoning were recorded, two of which resulted in death and 18 were discharged from hospital. In 70% of cases the patient was female (n=14), aged between 15 and 75 years old, with 40% (n=8) of them being between 20-40 years old and 40% (n=8) between 41-59 years old. In 85% of poisonings (n=17), the causative agent was the use of medication. In other cases, the agents related to intoxication were alcohol, rodenticide, oil paint, illicit drugs and pesticides. The majority are administered orally and only one case is administered intravenously. Among the medications, amitriptyline and carbamazepine were used in 50% of cases, with 5 cases (25%) recorded with each of them. Other medications, such as chlorpromazine, diazepam, olanzapine, phenobarbital, methadone, warfarin, among others, were also related to the poisonings evaluated. It is interesting to note that in four cases (20%) more than 2 different medications were taken. Alcohol was the responsible cause in one of the studied cases, but it was present in 5 (n=25) other cases as an association. The circumstances observed in the registered cases were intentional and self-inflicted (60%) and the others were divided into accidental, criminal and unreported. The severity of drug poisoning depends mainly on the dose of the causative agent, but also on the characteristics of the individuals, the toxicodynamics and toxicocokinetics of the compounds involved, pharmaceutical properties, drug interactions and mode of use. Knowledge of the causes of the most frequent exogenous poisonings treated at HURCG is of great relevance, as it can help in more assertive preparation of teams to provide the best care available.

Rodenticide poisoning: a case study

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Introduction: Aldicarb, popularly known as "chumbinho", is a pesticide from the carbamate class, with anticholinesterase action. According to the National Toxic-Pharmacological Information System, from 2013 to 2022, 3003 deaths occurred due to pesticide poisoning in Brazil. **Objective:** Present the clinical manifestations and treatment used in a patient due to intentional carbamate poisoning using digital medical record data. **Methods:** This is a case study that took place in November 2023 related to the intentional poisoning by "chumbinho" of a teenager admitted to the Emergency Department of a University Hospital and notified to the Toxicological Assistance Center of Maringá. **Results:** Adolescent, 14 years old, male, with suspected carbamate poisoning, popularly known as "chumbinho". Initially treated by the Mobile Emergency Care Service, with exposure time to the toxicant of approximately seven hours. He was in support of defenses through orotracheal intubation. The patient had blood pressure: 160x90 mmHg, heart rate 131 BPM, temperature 35.5 °C, cholinergic syndrome characterized by sialorrhea, vomiting, fasciculations, sweating, reduced level of consciousness with a decrease in the Glasgow scale. The diagnosis of carbamate poisoning was confirmed by clinical manifestation and results of laboratory tests such as cholinesterase measurement. The treatment was guided according to the protocol for assistance in carbamate poisoning, including the use of the antidote, atropine, gastric decontamination with the use of activated charcoal in multiple doses, multiparametric monitoring , electrocardiogram to evaluate the effects on the cardiovascular system characterized by prolongation of the QT interval , supportive and symptomatic treatment. According to the assessment of the severity classification, the patient was transferred to the Intensive Care Unit of the same health service, using sedation with Fentanyl and Midazolam and vasoactive drugs. After 72 hours of hospitalization, although in a serious condition, the responses to clinical manifestations improved. On the fourth day of hospitalization, he was successfully extubated, maintaining respiratory autonomy and oxygen saturation at 100%, but still using atropine, justified by the clinical manifestations that were gradually reduced. The teenager was discharged from hospital without evidence of neurological damage on the eighth day of treatment at the health service.The results of the laboratory tests performed during hospital admission were as follows: plasma cholinesterase 0.2 U/mL (VR: 5.9 - 12.2 U/mL); partial thromboplastin time (APTT) 36.1 seconds (VR: 31.0 - 40.0 seconds); arterial blood gas analysis 7.071 (VR: 7.350 - 7.450); magnesium (Mg) 2.2 mg/dL (VR: 7 - 2.3 mg/dL); alkaline phosphatase 244 U/L (VR: 130 - 525 U/L); lipase 43 U/L (VR: 23 - 300 U/L); amylase 214 U/L (VR: 30 - 110 U/L). **Discussion:** Carbamates are compounds that despite the risks are widely used in agriculture and clandestinely, as rodenticides in the domestic environment. Easy access to these compounds results in an increasing risk of accidental or intentional poisoning. Poisoning by anticholinesterase agents presents as its main manifestations muscarinic, nicotinic and central nervous system cholinergic symptoms. As a severe clinical manifestation of cholinesterase inhibition, poisonings are characterized by severity and risk to life, which is why the main measure is atropinization. It is considered necessary to monitor clinical and laboratory manifestations, so that the appropriate and assisted dose is used, as well as gradual withdrawal to avoid early suspension of the antidote and consequently the "premature syndrome". **Conclusion:** Chumbinho poisoning is a serious public health problem with a risk of death for individuals. It is widely used in intentional poisoning and cases of serious toxicological incidents involving children. Rapid diagnosis and adequate guidance for assistance in carbamate poisoning are of enormous value in minimizing sequelae and avoiding unfavorable complications for patients.

Serious scorpion accidents caused by stings from scorpions of the genus Tityus that resulted in cardiac changes occurring at the Toxicological Information and Assistance Center of Espirito Santo

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Introduction: Scorpionism is recognized as a public health challenge due to its large frequency and severity potential, mostly in children and patients with known comorbidities. The poison pathophysiology is complex and may cause cardiotoxic effects threatening to life. **Objective:** describe the epidemiologic profile of severe scorpion accidents caused by genus Tityus that progressed with cardiac damage identified by laboratory tests and/or image. **Methods:** Retrospective longitudinal study from electronic medical record database, DATATOX system, 2.0 version, from Centro de Informação e Assistência Toxicológica do Espírito Santo (CIATox-ES), between 2021 and 2023. Analyzed variables include: age, gender, clinical manifestations, laboratory test, echocardiogram, use of mechanical ventilation (MV) or vasoactive drugs (VD) and hospitalization duration. Patients with anti- scorpion venom serum dose incompatible with severity classification were excluded. **Results:** Of the 317 severe scorpion stings records, 45 presented with cardiac troponin alterations, of which the majority were aged between 1 and 4 years (53,33%), male (62,22%). The most common manifestations were vomiting (88,89%), tachycardia (62,22%), tachypnea (60,00%), sweating (60,00%), hyperglycemia (62,22%), elevated lactate (60,00%) and hypokalemia (51,11%). Of the sample, 30 underwent echocardiogram (13 with reduced ejection fraction and 6 with other changes), 13 needed VD and 6 MV. Regarding the time of the accident and administration of anti-venom therapy, most happened between 1 and 2 hours (48,89%). The late treatment was directly related to the need of VD (12h: 50%). Death was observed in 2 cases, aged from 1 and 4 years old, female, anti-scorpion venom serum < 1h for one patient and 5h for the other. **Discussion/ Conclusion:** The majority of severe scorpionism with cardiac troponin evaluation occurred in the age group of 1 and 4 years old and male gender and progressed with vomits, sweating, tachycardia, tachypnea, hyperglycemia, hypokalemia and elevated lactate. Evaluation of echocardiograms in patients with positive troponin was relevant. The late treatment with anti-venom therapy was directly related with the severity and the need of VD. **Acknowledgments:** we are thankful for all the doctors at CIATox for all the help and for the constant improvement in our service. We thank, specially, doctors Rinara Angélica de Andrade Machado and Nixon Sesse.

Services provided by the Toxicological Analys Center (NAT) during the period 2021 - 2023

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Introduction: The Toxicological Analysis Center (NAT) is a service that assists the Santa Maria University Hospital (HUSM) when carrying out toxicological analysis of patients suspected or proven to be poisoning. **Objectives:** To present the services carried out by NAT in toxicological incidents that occurred in the municipality of Santa Maria-RS and the region between the years 2021 and 2023. **Methods:** NAT advises health professionals through telephone contact. Analyzes of biological matrices were carried out using immunoassays, spectrophotometry, CCD, LC-UV/Vis or DAD and/or GC-MS or FID, depending on the case. **Results:** During the last two years, from November 2021 to November 2023, NAT carried out 50 toxicological analyzes. In the last two months of 2021, 5 analyzes were carried out, in 2022, 24 analyzes and, from January to November 2023, 21 toxicological analyzes. Among the substances most detected in the analyzes are benzodiazepines (found in 20 patients), barbiturates (9) and cocaine (7). In 2021, four immunochromatographic assays for drugs of abuse and medications and one paraquat analysis were performed. In 2022, 19 immunoassays for drugs of abuse and medications. In 2023, in addition to 16 immunoassays for drugs of abuse and medications, two quantification analyzes of paracetamol and one of copper were carried out. In addition, a strychnine and paraquat determination analysis was carried out in the period 2022. Regarding the pesticide analyzes that were carried out, paraquat, the colorimetric method was carried out with sodium dithionite. In 2021, gas (GC) and liquid chromatography (LC) analyzes began, aiming at the detection and quantification of substances, given the increasing complexity of cases. Since the NAT does not have GC equipment and the LC equipment has limited performance, the techniques were developed in another laboratory, which has a high demand for work, a fact that restricts the execution of NAT analyses. **Discussion/ Conclusion:** Cases of poisoning generally represent a great challenge for the healthcare team, as they are difficult to define in diagnosis, complex treatment, there are often several agents involved and a potential risk of fatal complications. Although the NAT is not a Toxicological Information Center (CIT) and has a limited technological apparatus, it has contributed significantly to providing professional advice and elucidating toxicological occurrences attended to at HUSM. In addition to improving the patient's health status, NAT contributes to saving public spending. With the correct identification of the agent causing the damage and thus adequate treatment and monitoring, there is a faster hospital discharge and there is often no need to use an ICU bed, generating savings of R$ 1,600.00 / patient / day, given that this is the amount that a SUS patient at HUSM costs the public coffers per day, according to the AuditaSUS expense table in 2020. In this way, the important role that NAT plays for Santa Maria stands out. and region, both for health and the economy through its advice and analysis. **Acknowledgments:** UFSM.

Substance use disorder patients in rehabilitation process: difficulties faced, adherence to treatment, relapses causes and motivation

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Chemical dependency is a multifactorial and incurable chronic disease, which presents physical, psychological and behavioral symptoms, where the individual can no longer have control over that habit/substance, bringing various harm to them and the people around them. Therefore, this research aimed to understand the profile of drug addicts in the rehabilitation process. The aim of this work is to profile the patients, to point the difficulties, relapses causes and the motivations for the treatment, of people with substance use disorder undergoing rehabilitation in a therapeutic community. To this end, data collection was carried out through the application of a self-administered questionnaire to drug addicts hosted at the Copiosa Redenção Therapeutic Community, located in the district of Uvaia in the municipality of Ponta Grossa, Paraná. At the time of the research, the community hosted 21 male patients, aged between 18 and 60 years, who answered the questionnaires. According to the results obtained, the majority of those welcomed, 66% (n=14) are aged between 36 and 55 years. Regarding the socioeconomic profile, 61.9% (n=13) of the respondents declared that their family income did not exceed R$:4,000.00/month and 33.3% (n=7) reported having no income. Regarding education, 33.3% (n=7) did not finish Elementary School, 23.8% (n=5) did not finish High School, 4.7% (n=1) finished Higher Education and another 4 .7% (n=1) did not complete Higher Education. The vast majority declare themselves single or divorced, totaling 66.0% (n=16), with 76% (n=16) of respondents having children. The majority started using drugs with marijuana, followed by alcohol and, to a lesser extent, cocaine, cigarettes and cola were mentioned. Among the drugs mentioned responsible for the reception, the main ones were: crack and alcohol. When asked about the age at which drug use began, the majority responded that it was during adolescence, especially at the age of 16 in a greater proportion. Some even reported that they started using it during childhood, from 9 to 12 years old. Regarding the time of drug use, it varied between 6 and 45 years. The main reason given for why they used the drug for the first time was the influence of friends/relatives, followed by the situation they were in (party, bar, etc.) and finally curiosity. Among the motivations for treatment, the family's recovery and the desire for a new life stand out. Abstinence, socializing with colleagues and missing the family proved to be the greatest difficulties in rehabilitation. Among the causes of relapses, love problems, the use of cigarettes and alcohol and not recognizing impotence in the face of drugs were cited. Rehabilitation on drug use disorders become indispensable for the recovery of drug addicts. Thus, it is needed to know the addicts profile to assist better preparing and training the health professionals about the knowledge of the challenges in treating chemical addiction, besides better knowing the most vulnerable population to developing drug use disorder. Chemical dependency is a complex disease, about which very little is still known. However, knowledge of the profile of individuals most likely to develop it can help to strengthen more effective measures to increase treatment success.

Synthetic cannabinoids intoxications: relationship between case history and laboratory findings

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Introduction: Synthetic cannabinoid receptor agonists (SCRAs) are one of the most relevant New Psychoactive Substances groups, with hundreds of compounds, and frequently elicit severe negative health effects due to their unpredictable nature. These compounds are a global public health problem, but it was only in 2018 that they began to be detected in Brazil. Since 2021, the number of poisonings related to SCRAs has increased significantly. **Objective:** Thus, the objective of this work was to describe the relationship between case history and laboratorial findings of intoxications related to SCRAs assisted by Poison Control Centers in Brazil. **Methods:** This is a descriptive study carried out between April 2021 and April 2023 as part of the INSPEQT project (Brazilian NPS monitoring program) and resulting from the prospective evaluation of reports of SCRA intoxication cases i.e., clinical, toxicological, and laboratorial findings, among patients treated at the Poison Control Centers of Campinas and São Paulo. **Results and Discussion:** The analytical toxicology laboratory confirmed 13 cases of SCRAs poisoning by analysis of blood and urine through a sensitive liquid-chromatography tandem mass spectrometry (LC-MS/MS) method. The main SCRAs detected were MDMB-4en- PINACA, ADB-BINACA, ADB-BUTINACA, and 5F-MDMB-PINACA. SCRAs blood levels ranged from 0.18 to 13.59 ng/mL. Concurrently, other psychoactive substances were detected, on average of two per case, mainly delta-9-THC. 46% of the patients presented with severe systemic manifestations (PSS 3), mostly central nervous system depression; 8% progressed with moderate manifestations (PSS 2), presenting high levels of creatine phosphokinase (CPK); 46% manifested mild clinical effects (PSS 1), such as agitation. The most prevalent origin among the cases was home exposure, 46%. The treatment for SCRAs intoxication was supportive and the average hospitalization, for severe cases, was 7 days. Throughout the study period, two suspected samples were not sent for analysis, and three did not detect any SCRAs, probably due to the long interval between exposure and the material collection. **Conclusion:** The use of extremely potent SCRAs is increasing among the population in Brazil. Thus, it becomes evident that more research is needed to inform users and health professionals about the toxicity of this NPS group. The implementation of an early warning system in Brazil is a measure that could expand information on the SCRAs abuse in the country. **Acknowledgements:** INSPEQT project - Investigation of New Psychoactive Substances in Forensic Chemistry and Toxicology (process number 8888.808346/2023-00) and BACO project - Toxicology and Toxicological Analyzes as Sources of Information for Public Policies on Drugs (process number 08129.007064/2019-15).

The importance of the information and toxicological care center of Espirito Santo in pre-hospital care

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Introduction: The Center for Information and Toxicological Assistance (CIATox) is dedicated to providing toxicological information to healthcare professionals and institutions, as well as offering assistance to individuals exposed and/or intoxicated, to reduce morbidity and mortality. Teleconsultation in pre-hospital cases evaluates and recommends the best course of action, often on-site, without the need for referral to a healthcare facility. **Objective:** to assess the importance of teleconsultation provided by CIATox-ES in cases of pre-hospital intoxication or exposure (I/E), aiming to reduce the demand for in-person healthcare services. **Methods:** a descriptive and retrospective study of I/E cases recorded by CIATox-ES between 2020 and 2023 was conducted, identifying the causative agent, age group, location, time of year, and the necessity of seeking healthcare services. **Results/Discussion:** A total of 3649 cases were recorded, with 97,36% originating from residential areas. The age group of 1-4 years was the most prevalent (41,90%). The main agent groups were medications (46,55%) and household products (16,77%). Among medications, benzodiazepines were the most prevalent (14,59%). The majority of cases evaluated did not require referral to healthcare services (75,47%). When assessed by age group, in the under-15 age group, 80,81% did not need healthcare services. **Conclusion:** It was observed that the majority of requests were residential, involving the age group of 1-4 years, and related to medications. The study demonstrated that CIATox plays a significant role in the healthcare system, reducing the burden on services and the cost of public-private healthcare through appropriate screening. Therefore, there is a need to increase investment for greater visibility of the services provided by CIATox to the population.

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Use of dexamethasone in a hospital in Aracaju-SE from 2019 to 2022: before, during and after the covid-19 pandemic

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Introduction: Glucocorticoids are potent anti-inflammatory medications that have been widely used in severe cases of COVID-19, reducing the risk of death by a third among those hospitalized on mechanical ventilators. However, the use of corticosteroids can worsen the condition in milder cases of the disease, in addition to their indiscriminate use being related to several toxic effects of corticosteroid therapy. The great demand for the medicine was a challenge for hospital management, as the acquisition processes were made difficult due to the increase in demand. Given the importance of monitoring and competencies within healthcare organizations, the present work sought to analyze the changes that occurred, quantifying and establishing a direct relationship under the panorama of the total amount that was used of the drug dexamethasone in a reference hospital for hospitalizations with respiratory syndromes. **Objective:** to identify changes in dexamethasone prescriptions before, during and after the COVID-19 pandemic in a Aracaju hospital. **Methods:** quantitative and exploratory documentary research, based on data collected in the Hospital Municipal Zona Sul Desembargador Fernando Franco' pharmacy through reports, data entered into the pharmacy's internal management system, dispensing and prescription tables, in the period between 2019 and 2021. **Results:** In 2019, in the mentioned hospital, therefore in the pre-pandemic period, 8,154 ampoules of dexamethasone were used. The use of this medication increasing exponentially by 216.5% to 17,657 ampoules in 2020, decreasing to 13,874 in 2021 (reduction of 21.4%). The most commonly used dose was 10 mg. In 2020 there was a significant increase in the months of May and June (months with the highest number of deaths in the state of Sergipe resulting from COVID-19) followed by a reduction in subsequent months, with an increase in December, when cases rose again. In mid-2021, with the advancement of vaccination, cases decreased and the use of dexamethasone was reduced. However, with the resumption of elective surgeries and routine treatments, there was a high use of dexamethasone in 2022, reaching values of 18,796 ampoules, an increase above 50% of what was used of the drug in the pre-pandemic period. **Conclusion:** there were changes, both in the amount of dexamethasone used and in the routine of the health unit studied, which resulted in changes in pharmaceutical management, and in the need to monitor adverse and toxic reactions related to dexamethasone large use.

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TOXICOLOGIA COMPUTACIONAL

Empowering bioanalytical methods: development of a validation software using Python and SQL

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Background/Introduction: Statistical analysis plays a crucial role in the validation of bioanalytical methods, enabling precise interpretation and extraction of valuable insights from complex data. Python, known for its simple syntax and readability, has become a popular choice among data scientists and biochemical analysts. Besides, SQL (Structured Query Language) plays a fundamental role in efficiently manipulating databases. The combination of Python and SQL programming languages offers a powerful and versatile approach to conducting the validation of an analytical method. **Aim:** The aim of this work is to develop a software to facilitate data analysis during the validation of analytical methods. **Methods:** The Python and SQL programming languages will be used to create the software. Data from a previously published paper from the first author was used to evaluate the applicability of the software. The validation criteria for the analytical method were based on the SWGTOX guideline. **Results:** Microsoft SQL Server was used to create the software and, in order to evaluate if the software works, values from a paper published by the first author at the Journal of Analytical Toxicology (DOI: 10.1093/jat/bkaa138) was used, but in this case the parameters were evaluated using Excel®. The recovery parameter was evaluated and is in accordance with the published paper. **Discussion/Conclusion:** The next step is to add all the validation parameters in the software and compared to the results of the previously published method. The use of Python could provide robust tools for data manipulation, statistical calculations and visualization. By combining Python for data manipulation and SQL for querying databases, we will be able to perform a rapid and accurate statistical analysis during the validation of quantitative methods. This software could contribute for significant advancements in the development and validation of bioanalytical methods. **Acknowledgments:** We thank our colleagues Bruno Barraca Souza Lima and João Milton Butarelli Filho for their support during the development of the software.

Toxicological assessment of Tamoxifen computational - designed analogues to predict and avoid the Blood - Brain Barrier permeability

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Background/Introduction: Tamoxifen (TAM) is utilized as a hormonal treatment for patients who show estrogen receptor (ER)-positive breast cancer due to the antagonistic activity in this type of cell's ERs. Despite the benefits of TAM for breast cancer patients, losing adherence is highly prevalent. Around 70% of the patients rashly halt the treatment before the conclusion of the suggested 5-year period. TAM adverse effects (AE) are the most common reason for the treatment interruption. Primary AE's related to the lack of adherence are hot flashes, joint pains, fatigue/loss of energy, mood issues, sexual dysfunction, night sweats, and sleep issues. TAM permeability through the blood-brain barrier (BBB) into the Central Nervous System (CNS) and its action in numerous targets, such as Protein Kinase C (PKC), Calmodulin, and Voltage-dependent Ca2+ channels, is considered the main reason. **Objective:** This work aimed to generate a series of TAM analogues computationally planned to avoid BBB permeability and maintain estrogenic affinity by employing a 2D-QSPR (Two Dimensions Quantitative Structure-Property Relationships) prediction methodology. **Methods:** TAM derivatives were modeled using a Genetic Algorithm (logP≥4.0; TPSA(NO)>35Å^2; H-bond$_{acc}$≥10; SAScore≤3.0) provided by AlvaBuilder software v.1.0.10. An already validated 2D-QSPR method (sensitivity=0.915; accuracy=0.820; specificity=0.649) using AlvaRunner v.2.0.8. Multiple Linear Classification Model using the kNN method was employed to evaluate BBB permeation. All molecules were also analyzed using the SwissADME server to assess BBB crossing. The selected molecules were then evaluated in terms of ER (PDB: 1ERR), Calmodulin (1 and 2; PDB: 1CTR and 6XXF), α-PKC (PBD: 3IW4), and Voltage-Dependent Ca^{2+} Channel (CaV 2.1 and 2.2; PDB: 8X91 and 7VFW) affinities using CCDC GOLD Docking (ChemPLP.Fitness score) v.2023.3.1. The results were compared (One-Way ANOVA, Dunnett *post hoc* test) to those of the same analysis performed with TAM (or 4-OH TAM to ER). **Results:** 150 TAM derivatives were obtained, and after BBB permeation analysis using two methods (Alva Runner QSPR and SwissADME), 10 were selected and evaluated regarding their affinities for the pharmacological ER target. Seven compounds presented a statistically significant higher ($p<0.0001$) ChemPLP.Fitness score than the control 4-OH TAM. When comparing the interaction of these derivatives with TAM for the CNS targets, it was possible to verify that in silico ER affinity is directly correlated to the affinity with the other targets. The molecules that obtained the lowest ER affinity scores were the same that presented lower affinities to the CNS targets. **Discussion/Conclusion:** TAM is a widely used breast cancer treatment and prophylaxis drug. However, some of the main reasons for the treatment abandonment are the CNS- related adverse effects. Several TAM derivatives have already been developed, with different pharmacokinetic profiles, but avoiding crossing the BBB is difficult. For example, Raloxifene, a TAM derivative, has very low permeation to the CNS, and so a desirable adverse effects profile, but has a much lower (when compared to TAM) efficacy to prevent breast cancer. We present here seven new TAM derivatives, designed using computational tools, focusing on the ER high affinity and lower BBB permeation. We also infer that developing TAM derivatives for treating breast cancer with low CNS adverse effects patterns and high ER interaction must focus on the BBB low permeation with the same importance as the ligand's selectivity. The next step of this research involves the synthesis and in vitro testing of the selected molecules.

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TOXICOLOGIA DE MICRO E NANOPARTÍCULAS

Characterisation, quantification and toxicological assessment in zebrafish embryous of silver nanoparticles for antimicrobial purposes in textile substrates

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Introduction: Nanotechnology is growing exponentially, with nanomaterials being applied in various sectors to benefit society. Fabrics impregnated with silver nanoparticles (AgNPs) stand out due to their biocidal properties, being used in masks and protective clothing against the SARS-CoV-2 virus. Despite their benefits, AgNPs can be toxic, with cytotoxic and genotoxic impacts on human cells, influenced by particles. In animals, AgNPs crossed biological barriers, induced neurotoxicity, and accumulated in vital organs. Regarding the toxicological potential, it is crucial to optimise products and understand the environmental and health impacts associated with using these nanoparticles. **Objectives:** The study aimed to assess the toxicity of AgNPs synthesised with controlled dimensions and concentration used to impregnate textile substrates. **Methods:** The AgNPs were synthesised for this study, following the methodology described by AGNIHOTRI et al. (2014). Single particle inductively coupled plasma mass spectrometry (spICP-MS), model NeXion 300D was used for detecting, characterizing, and quantifying nanoparticles and total silver was quantified using inductively coupled plasma optical emission spectrometer (ICP OES). The fish embryo toxicity test (FET test) OECD TG 236 (2013) was performed to assess the toxicity of AgNPs using zebrafish (Danio rerio) as animal model. Possible sublethal and lethal effects were observed in addition to eye, body and heart rate. **Results:** The synthesis of the nanoparticles was confirmed using the UV-visible technique with an absorbance spectrum with peaks at 400 nm. The average hydrodynamic diameter was 31.68 nm in distilled water. MET showed that all the nanoparticles synthesised had a spherical morphology. The spICP-MS technique confirms a 38 ± 1 nm. It provides important information for toxicological evaluation such as number of particles/mL of 2.4 x 107, the distribution range with 45% das nanoparticle between 21-40 nm and the total silver concentration of 12.6 mg.L-1 obtained by ICP OES. The concentrations used for the FET test were 2.4 x 106, 1.2 x 105, and 4.8 x 104 AgNP/mL and no morphological changes or sublethal effects were observed. No significant changes were observed in body length, eye, and heart rate. **Discussion/Conclusion:** This study highlights the role of particles in the developmental toxicity of AgNPs in fish embryos. AgNPs of specific s (30-72 nm) entered zebrafish embryos, resulting in dose- and -dependent toxic effects. Deformed zebrafish exhibited higher concentrations of larger AgNPs, indicating -dependent nanotoxicity. Larger AgNPs (41.6 ± 9.1 nm) were found to be more toxic than smaller ones (11.6 ± 3.5 nm). Another study with 4 nm AgNPs showed significant impacts on zebrafish embryos by 72 hpf, while no significant differences were observed with 10 nm AgNPs. Those studies underscore the need for further research to understand key factors responsible for specific toxic effects and the association between AgNP uptake and toxicity in fish embryos. **Acknowledgements:** This project has received funding from Fundação Carlos Chagas Filho de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ), Finance Code 001, (Grant: Rede NanoSaúde-E-26/2010.139/2019; E-26/2010.255/2020) and Project LabVisa, Instituto Nacional de Controle de Qualidade em Saúde (INCQS/Fiocruz).

Toxicity comparison of polymeric nanoparticles containing amphotericin B and the free drug by the zebrafish model

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Introduction: Amphotericin B (AmB) is a drug primarily employed to treat fungal infections. It is recognized as a drug with limited oral bioavailability, and it poses a notable nephrotoxicity associated with dosage and duration of use. Polymeric nanoparticles (NPPs) refer to those composed of either synthetic or natural polymers, garnering attention in the pharmaceutical industry for their attributes like constructing controlled release models, safeguarding sensitive drugs, enhancing bioavailability, and diminishing adverse effects. The utilization of zebrafish (Danio rerio) as an animal model in toxicological studies has witnessed a surge in recent times. The OECD fish embryo toxicity test (FET), utilizing zebrafish embryos, has gained widespread popularity due to its rapid response and the ability to estimate the dosage range for future tests in mammals. Since its creation in 2014, the ISO 16197 standard has already highlighted the FET test as a pertinent in vivo model for examining the toxicity of nanomaterials. **Objective:** To compare the toxicity of polycaprolactone (PCL) and poly(lactic acid) (PLA) NPPs containing amphotericin B against the free drug, using zebrafish (Danio rerio) embryos. **Methods:** The Fish Embryo Toxicity (FET) test was performed following the guidelines set out in TG 236 of the OECD. Samples were diluted to the desired concentrations with E3 medium solution (5 mM NaCl; 0.17 mM KCl; 0.33 mM CaCl2; 0.33 mM Mg2SO4). 20 fertilized embryos were used for each tested concentration and also for the negative control. Concentrations tested were 0,05; 0,1; 0,2; 0,5 and 1,0 µg/mL. At the end of 120 hours, the live animals were contained in a drop of carboxy-methyl-cellulose (CMC) at 6%, on a slide for microscopy, with the purpose of being photographed and filmed for 10 seconds. Additionally, measurements of body , the diameter of the eyes of the larvae and heart beats per minute (bpm) were also performed. **Results:** Toxic Concentration 50% (TC50) and Lethal Concentration 50% (LC50) were calculated as it follows: free AmB TC50 1.034 µg/mL, LC50 < 1.5 µg/mL; PLA-NPs+AmB TC50 0.1536 µg/mL, LC50 0.3982 µg/mL; PLC-NPs+AmB TC50 0.3204 µg/mL, LC50 0.5575 µg/mL. In the bpm analysis all groups tested with free AmB showed elevated heartbeats when compared to negative control. In body and eye diameter analyses, the larvae tested with PLA-NPs+AmB at the concentration of 0.5 µg/mL showed reduced growth in relation to the negative control. **Discussion and Conclusion:** With the data obtained, it can be suggested that PCL+AmB nanoparticles proved to be safer than PLA+AmB nanoparticles in the tested model. Although AmB, which was not nanoencapsulated, presented CT50 and LC50 values that could mean that it is less toxic or lethal than nanoparticles, it is suggested that this effect may have occurred due to the drug's tendency to aggregate and form precipitates when is in an aqueous medium, reducing its absorption by embryos. However, a study on the penetrability of AmB in embryos is necessary to obtain certainty about this hypothesis. If proven, it could lead us to conclude that, by nanoencapsulating the drug, we increase its penetrability in the embryo, and consequently its bioavailability.

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TOXICOLOGIA EXPERIMENTAL

CYP450 metabolism of a semisynthetic naphthoquinone, an anticancer drug candidate, by human liver microsomes

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Background/Introduction: CNFD (6b,7-dihydro-5H-cyclopenta[b]naphtho[2,1- d]furan-5,6(9AH)-dione) is a semisynthetic naphthoquinone derived from Lausone that has cytotoxic action in different tumor lines and anticancer activity in vivo. Therefore, this molecule is a relevant candidate for drug development, but there is still no information on its human metabolism and systemic elimination. **Objective:** This study aimed to investigate the in vitro metabolism of this naphthoquinone by human liver microsomes. **Methods:** Initially, in order to determine the in vitro enzymatic kinetic parameters, an HPLC method to quantify the CNFD was developed and validated. In addition, the enzymatic kinetic data, the predicted pharmacokinetic in vivo parameters and the phenotyping study were presented. The main metabolism sites and metabolites have been suggested in silico. **Results:** The developed HPLC method was linear, reproducible, selective, accurate, and stable. The enzymatic kinetic parameters revealed a sigmoidal profile. In vitro to in vivo extrapolation hepatic metabolic clearance was 10.39 mL/min/kg protein and the liver extraction rate was 51%. **Discussion/Conclusion:** In summary, the metabolism of the CNFD drug candidate was characterized for the first time. The data established in the kinetic study were used to predict important pharmacokinetic parameters, determining liver clearance. CYP450 is involved in CNFD metabolism, with all enzymes showing important roles, especially CYP3A4 and CYP2C9. Metabolism sites were predicted. These results may be useful for future in vitro studies, as well as for clinical studies. **Acknowledgments:** The authors are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Evaluation of oxidative parameters in rats after repeated daily exposure to the herbicide clomazone

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Introduction: Brazil is one of the largest consumers of pesticides in the world and the exposure to these agents has been studied, observing the interaction with several non-target species. Therefore, they are harmful to human health and interfere with ecosystems. Clomazone is a herbicide that can be used on various crops and, in spite of limited data, its toxicity has been reported in fish, amphibians, rats and humans. **Objective:** The objective of this work was to evaluate oxidative parameters in adult male rats treated with clomazone. **Materials and Methods:** The animals were treated orally for 28 days with doses of 15, 30 and 60 mg/kg of clomazone. Control group was treated with distilled water (n=6 animals/group). After euthanasia, organs such as liver, kidney, spleen and heart were collected. Blood was collected from the vena cava in tubes with EDTA. All the samples were used for quantification of thiobarbituric acid reactive substances to (TBARS), as measure of lipoperoxidation, and total non-protein thiols (GSH), as a measure of antioxidant defenses. **Results:** No significant differences were observed in TBARS levels in spleen, heart, kidney, liver and plasma. Total non-protein thiols showed no significant difference in the spleen, heart, liver and erythrocytes, however an increase was observed in the kidney in the group treated with 15 mg/kg clomazone. **Conclusion:** Although oxidative stress does not seem to be one of the toxicity mechanisms of the herbicide clomazone, an increase in total thiols in renal tissue was observed, which may be due to an increase in antioxidant defenses or herbicide conjugation reactions. **Acknowledgments:** Capes. Approval number: CEUA-UFRGS 43478.

Gut microbiota contribute to abdominal aorta and PVAT dysfunction induced by chronic plus binge ethanol exposure

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Background: Gut microbiota affects health and disease, including cardiovascular disease (CVD) and ethanol-induced injuries. Ethanol effects in the cardiovascular system involves many mechanisms, but the impact of gut dysbiosis in ethanol-induced vascular dysfunction is not understood. **Objective:** The aim of this study is to determine whether the gut microbiota plays a role on abdominal aorta (AA) and perivascular adipose tissue (PVAT) dysfunction induced by chronic plus binge ethanol exposure. **Methods:** Male C57BL/6J and germ-free (GF) mice were randomized in two groups: 1) Control (C): mice that received water and food ad libitum; 2) Ethanol (E): mice that received a 5% (v/v) ethanol solution for 1 week, 10% (v/v) ethanol solution for 10 days, with an additional higher dose of ethanol (5 g/kg, oral gavage) on day 11. Mice were sacrificed 9 hours after the oral gavage. Gut microbiota was evaluated by sequencing of the 16S rRNA gene through next-generation DNA sequencing. Leaky gut was evaluated by FITC-dextran, colony forming units (CFU) and LPS. Pro-inflammatory cytokines were measured by ELISA. Nitric oxide (NO) and reactive oxygen species (ROS) were determined on a Sievers NO analyzer and with the dehydroethidine (DHE) probe, respectively. Immune cells were evaluated by flow cytometry, and mitochondrial function with an oxygraph-2k respirometer (Oroboros). Vascular function (AA) was evaluated in the presence of vehicle, MitoQ (mitochondria-targeted antioxidant), L-NAME [non-specific NO synthase (NOS) inhibitor] or CsH (FPR-1 antagonist). Student's t test and one-way ANOVA were used to compare the data among groups ($p<0.05$) (CEUA: 1007/2021; IACUC: 2648-101791-060723). **Results:** Chronic plus binge ethanol induced gut dysbiosis in male mice, which was accompanied by leaky gut, characterized by high levels of FITC-dextran [μg/mL, C: 0.9±0.1; E: 1.6±0.2 (n=9-10)], increased CFU and serum LPS levels [EU/mL, C: 0.02±0.003; E: 0.1±0.03 (n=7-9)]. Ethanol-treated mice showed increased TNFα levels in the colon [pg/mg prot., C: 7.4±0.7; E: 14.6±2.8 (n=6-7)]; high plasma levels (pg/mL) of IL-1β [C: 15.4±1.8; E: 69.7±17 (n=9-10)], IL-6 [C: 19.8±0.3; E: 26.2±2.7 (n=10)] and IL-22 [C: 5.2±0.6; E: 19.2±4.6 (n=4-5)]; and increased IL-17a in PVAT [pg/mg prot., C: 1478±212; E: 4283±958 (n=4-5)], which was accompanied by augmented M1 macrophage and Th17 cells. NO levels were increased in plasma [μM, C: 98.7±9.9; E: 152±12 (n=7-8)] and decreased in the PVAT [μM/mg prot., C: 15.4±2.9; E: 7.5±1.5 (n=5-6)] of ethanol-treated mice. Ethanol increased ROS generation in AA and PVAT and promoted mitochondrial dysfunction in AA. In addition to decreased PE-induced contractions in AA-PVAT(-) [Emax, C: 2.3±0.3; E: 0.99±0.2 (n=5-8)], ethanol induced pro-contractile effect of PVAT [Emax, C: 2.9±0.5; E: 1.86±0.3 (n=5-6)]. L-NAME increased vascular responses in AA from C and E groups. Interestingly, in the absence of gut microbiota, i.e. in GF mice, the anti-contractile effect of PVAT in C group [Emax, PVAT(-): 2.3±0.6; PVAT(+): 1.6±0.3 (n=4)] was observed, and the ethanol-induced pro-contractile effect was abolished [Emax, PVAT(-): 1.6±0.4; PVAT(+): E: 1.7±0.6 (n=4)]. The hyporesponsiveness of AA-PVAT(-) was partially reversed in GF mice and after FPR-1 inhibition [Emax, C: 2.7±0.6; E: 1.5±0.1 (n=4-6)]; and restored by MitoQ incubation [Emax, C: 2.4±0.3; E: 2.2±0.5 (n=3-4)]. **Discussion/Conclusion:** Excessive ethanol exposure induces gut dysbiosis, leaky gut, bacterial translocation into the systemic circulation and, consequently, immune system activation, systemic and PVAT inflammation. Gut microbiota-derived metabolites, such as fMLP, can activate FPR1, contributing to oxidative stress and mitochondrial dysfunction, leading to AA dysfunction. The pro-contractile effect of PVAT may be related to the activation of LPS/TLR4 and/or IL-17a/IL-17R pathways, as well as uncoupled NOS. Altogether, our data suggest that the gut microbiota is critical to the ethanol effects on vascular tone of AA and PVAT function. **Acknowledgments:** FAPESP (2020/11339-2; 2022/12426-1).

Mechanisms of cytotoxicity of diuron and its metabolites DCA and DCPMU in hepatic cells

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Background: The use of pesticides raises concern due to their potential toxic effects on human health. Diuron, a herbicide derived from urea, ranks fourth in pesticide sales in Brazil, despite being labeled as "probable carcinogen for humans" by the North American Environmental Protection Agency (USEPA). Our research team, TOXICAM, has consistently published studies in prestigious international journals investigating the potential mode of action (MoA) of Diuron in rat urothelium. However, it remains unknown what is the mechanism of cytotoxic action of this herbicide, and which of its forms is responsible for cytotoxicity, whether the parental Diuron or any of its metabolites. **Objective:** Considering that the initial biotransformation of Diuron occurs in the liver, the present study aims to evaluate the mechanism of in vitro damage induced in HepG2 cells after exposure to Diuron and its metabolites, DCA and DCPMU. **Methods and Results:** The study involves the evaluation of compounds in cultured HepG2 liver cells obtained from the Rio de Janeiro Cell Bank (BCRJ). Pre-incubation with Diuron, DCA and DCPMU will be carried out together with the experimental cell culture model. Cell proliferation was assessed using the Sulforadamine B probe, which binds stoichiometrically to the basic amino acids of cellular proteins and our results show that both Diuron, DCA and DCPMU alter cell proliferation at the highest concentrations tested (100 and 200 µM), and cell viability was determined by the MTT assay, which measures cellular metabolic activity and our findings demonstrate that the substances tested alter cell viability also at the highest concentrations (100 and 200 µM) and, additionally, the mitochondrial membrane potential of HepG2 cells was analyzed to assess mitochondrial depolarization, which is indicative of cell death. This was done by monitoring the fluorescence of tetramethylrhodamine methyl ester (TMRM) and it could be observed that Diuron, DCA and DCPMU altered the cell membrane potential at the highest concentrations tested (100 and 200 µM). **Discussion/Conclusion:** Our findings suggest that Diuron and its metabolites may have cytotoxic effects, altering cell proliferation, cell viability and dissipation of mitochondrial membrane potential when evaluated using HepG2 cells and additional studies are being carried out to better understand these effects. **Acknowledgments:** Supported by São Paulo Research Foundation (FAPESP) [Grant No. 2022/03045-4] and National Council for Scientific and Technological Development (CNPq) [Grant No. 141101/2024-5].

Myrcia splendens (Sw) DC crude hydrocoholic extract exhibit anti-inflammatory activity in vivo and in vitro

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Some species of the genus Myrcia have described anti-inflammatory activity. As for the species Myrcia splendens, data in the literature are scarce, and this is the first study demonstrating the anti-inflammatory activity of the crude hydroalcoholic extract. This study aimed to evaluate the in vivo and in vitro anti-inflammatory activity of the Crude Hydroalcoholic Extract of M. splendens leaves (EBH-MS). After obtaining the EBH-MS by macerating the dry leaves in 70% alcohol, in vivo and in vitro tests were performed. The in vivo anti-inflammatory activity was evaluated using the air pouch method, with total and differential leukocyte counts, nitric oxide dosage in the bag toilet, and histological analysis of the tissue lining the bag. The in vitro inflammatory activity of EBH-MS and isolated compounds (gallic acid and salicylic acid) was evaluated using cell viability (macrophages (RAW 264.7) and neutrophils), dosage of nitric oxide and cytokines (IL-1β, IL- 6 and tumor necrosis factor (TNF)) in macrophage supernatant (RAW 264.7), chemotaxis (neutrophils) and efferocytosis. EBH-MS reduced polymorphonuclear migration at all doses (3, 30, 100, 300 mg/kg), corroborating histological analysis and reduction of nitric oxide secreation. Treatment with EBH-MS (1, 10, and 100 µg/mL) and the isolated compounds did not induce cytotoxicity at tested doses, however gallic acid at 100 µg/ mL induced cytotoxicity in neutrophils. There was a reduction at concentration of nitric oxide in neutrophils and macrophages (RAW 264.7). Regarding macrophages, the M. splendens extract reduced the secretion of cytokines IL-1β, IL-6, and TNF. Furthermore, there was increase in efferocytosis and IL-10 at all concentrations and TNF reduction in the supernatant of this assay. Thus, the results demonstrate that M. splendens has anti-inflammatory activity in vivo and in vitro, without showing cytotoxicity.

Pulmonary toxicity of DMT pyrolysis products: characterization of the effects in a new drug-addiction pathway

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Background: The production and trade of N,N-Dimethyltryptamine (DMT) are globally prohibited by the Convention on Psychotropic Substances. However, in Brazil, the ritualistic use of DMT in the form of ayahuasca is legally permitted for certain syncretic churches. Despite this, there is a growing illicit market focused on online sales of DMT extracted from lyophilized plants, such as Mimosa hostilis and Mimosa tenuiflora. This substance can be consumed via multiple routes and inhalation is of particular concern due to its rapid absorption, immediate effects, and the potential for repeated dosing, contributing to the risk of drug addiction. The widespread recreational use of ayahuasca products and the uncertain dangers linked to vaporized product delivery technologies may lead to an escalation in intoxication cases. **Objective:** Therefore, the purpose of this work was to develop and implement a pulmonary toxicity assessment model associated with toxicological tests to evaluate the effects of DMT and/or its pyrolysis products consumed by inhalation. **Methods:** The experimental groups consisted of 8 mice each, having previously fasted for 4 hours, and 4 groups were determined, 3 groups with doses of 500 mg, 1 g, and 2 g of lyophilized material respectively, and 1 control group. The exposure was carried out for 15 minutes in the exposure chamber with the pyrolysis of the material occurring after 5 minutes of heating. After the evaluations, it was determined that the 2 g dose would be used in experimental protocol 2. It was conducted by evaluating 4 euthanasia times after exposure, namely times 1, 5, 15, and 30 minutes. After the exposure time (15 minutes) and observation time, the animals were sacrificed, and biological materials were collected for analysis. The identification and quantification of DMT and its pyrolysis products in plasma, and the simultaneous quantification of neurotransmitters in the brain were carried out using UHPLC-MS/MS equipment. **Results:** According to the results, in the control group the concentration was zero, consistent with the group evaluated. In the 1-minute group, there was a peak plasma concentration (0.53 ng/mL); in the 5-minute group, there was a reduction in concentration to 0.28 ng/mL. After 15 minutes, the concentration was found to be 0.09 ng/mL, remaining stable after 30 minutes (0.08 ng/mL) and 60 minutes (0.06 ng/mL). **Discussion/Conclusion:** Based on the analysis of DMT levels in the blood, it can be inferred that there is a significant decrease in plasma concentrations of the drug after 5 minutes, aligning with the expected pattern for this mode of administration. Additionally, this reduction follows a similar profile observed in other pulmonary psychoactive substances. Examination of neurotransmitters in brain tissue revealed pronounced serotonergic activity of DMT, consistent with findings from published works and studies that have elucidated the mechanism of action of this drug.

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Allegation of suspected sexual misconduct facilitated by psychotropic drugs during hemorrhoidectomy: a case report

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The utilization of substances that modify an individual's behavior as facilitators of criminal activities. These substances are commonly employed in surgical procedures as sedatives (benzodiazepines, non-benzodiazepine hypnotics, opioids, phenothiazines, etc.). We present a case report of an accusation of an alleged sexual crime during a surgical procedure. A 44-year-old female patient reported in a statement that she underwent an ambulatory hemorrhoidectomy; on this occasion, she received local anesthesia and oral sedatives (paracetamol with codeine, midazolam, zolpidem, and Lisador®). At the end of the procedure, still in the medical office, she claimed to have been sexually abused before being discharged. According to the medical report, the procedure lasted 40 minutes, and the patient remained awake and alert. After the procedure, she stayed under observation for an additional 30 minutes, stood up, and dressed without difficulty, totaling 70 minutes inside the medical office. After discharge, the patient walked out unassisted. Toxicological examination of blood and urine samples collected seven days after the procedure detected the presence of codeine and promethazine (postoperative medications). According to scientific literature, zolpidem is a potent sleep inducer that induces virtual incapacitation within 30 minutes of ingestion (usually in 10-15 minutes), and its effect lasts no less than 3-4 hours (half-life of 2.4 hours, total elimination time of >12 hours). Its most common side effects include anterograde amnesia (the person will not remember what happens after the onset of the medication's effects), motor coordination impairment, fatigue, dizziness, hallucinations, decreased libido, and sleepwalking. Oral midazolam has similar effects to zolpidem when used for insomnia but has broader applications, such as seizures, anxiety, or panic attacks. With a half-life of about 2.5 hours, it shares with zolpidem the rapid onset, a duration of at least 3 hours, and total elimination in >12 hours. Its main adverse effects include respiratory depression (with a risk of death in case of overdose), memory impairment, and paradoxical agitation. It is important to note that the combination of zolpidem and midazolam has additive effects, adding to the sedation already produced by each of them, with their own risks and consequences. Regarding codeine and promethazine, their half-life varies between 2-4 hours and 10-15 hours, respectively. Therefore, the patient's narrative is incompatible with the sedation she claims to have received, as the effects of the enumerated medications would significantly incapacitate her – even for being able to leave the bed, dress, and walk on her own – for no less than 3-4 hours after oral administration. A proper investigation of these cases must take into account not only the isolated elements - the victim's account, the results of expert examinations and toxicological tests - but must consider them in an integrated perspective, verifying the greater or lesser plausibility of the allegations in light of the toxicokinetics and toxicodynamics of the drugs allegedly used.

Alternative screening method for qualitative identification of clobenzorex

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Introduction: Clobenzorex (N -(2- chlorobenzyl) amphetamine , N -(2- chlorobenzyl)-1- phenylpropan -2- amine) is a stimulant drug, banned in Brazil , belonging to the amphetamine class and used as an appetite suppressant. Due to its stimulating effect, it is used by drivers who want to prolong their state of alertness. Analysis of these materials also identified substances such as caffeine, lidocaine and other amphetamines (COMPOSITION OF CLOBENZOREX SEIZED BY POLICE IN BAHIA, CBTOX, 2019). The tablets were white, with the impression of a star symbol in low relief and the cards had the inscription " nobésio " or " nobésio forte". To identify this substance, forensic laboratories often use the gas chromatography coupled to mass spectrometry (GC-MS) technique. However, it is not always available at Criminalistics Institutes. In the present work, a study is presented using two screening techniques together to identify clobenzorex . The initial test is based on color development with the sodium nitroprusside - acetaldehyde reagent , indicated for aliphatic and alicyclic amines . The second technique is thin layer chromatography (TLC) with the T B elution system and platinum-iodine developer, both respond very well to alkaloids. **Objective:** Propose a screening methodology using chemical testing and thin layer chromatography (TLC) to identify clobenzorex in materials seized by the police. **Methods:** Fifteen materials were selected, previously analyzed by GC-MS, among which thirteen were positive for clobenzorex and two were negative. In the latter case, presenting other similar substances (caffeine, benzocaine and theophylline). The samples were initially subjected to chemical testing using 1/4a tablet pulverized on a touch plate and adding 2 drops of the reagent obtained by mixing equal volumes of 5% nitroprusside solution in 10% acetaldehyde solution in water and Na 2 CO 3 2 solution % in water. The samples were analyzed by TLC, using the methanol extract of 1/8the tablet, on silica gel 60, F 254 plates (MACHEREY- NAGEL), T B elution system (cyclohexane : toluene : diethylamine , 75:15:10 v/v/v) and developed with UV light (λ 254 nm) and platinum-iodine solution. All samples were white tablets , except the material identified with the number 15, which had a yellowish color, with the star symbol and was presented in cards with the imprint "nobésio". **Results/Discussion:** In the color test, a bluish-green color was observed for all positive samples (Samples 2 to 14) and another brown or white color was seen for negative materials (Samples 1 and 15). Caffeine, a substance also present in the materials analyzed, presented a negative result for the test. In CCD, caffeine remains very close to the application point of the samples, it is visible under UV light, but does not reveal itself with platinum-iodine. Clobenzorex has Rf = 0.80 and brown color after chemical development. A positive result was observed for samples 2 to 14 and a negative result for samples 1 and 15, confirming the expected result. Based on the results obtained it is suggested to use the gas chromatography coupled to mass spectrometry (GC-MS) technique to confirm the presence of Clobenzorex.

AmberNPS: a prototype QSAR model for predicting lethal blood concentrations of new psychoactive substances in forensic toxicology

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Background: Over the past decade, the emergence of more than 40 new psychoactive substances (NPS) annually has posed significant challenges to forensic scientists. The rapid introduction of novel NPS members demands continuous updates to quantitative analytical methods and dose-effect correlations. Interpretation of analytical results becomes particularly complicated due to the scarcity of toxicological data, especially when information on lethal blood concentrations (LBC) is limited or unavailable. **Objective:** This study addresses this challenge by describing the development of a predictive model for LBCs of NPSs within opioid, benzodiazepine, cathinone, synthetic cannabinoid, and phenylethylamine classes. The model relies on a Quantitative Structure-Activity Relationship (QSAR) concept, which utilizes structural information to make predictions. The model development targeted the provision of a robust framework for understanding the toxicological implications of acute NPS exposure. **Methods:** Compilation of LBC ranges from the literature, primarily sourced from *post-mortem* cases in forensic laboratories, formed the basis for our dataset. The negative logarithm of the range's median value, denoted as pLBC50, served as the model endpoint. To enhance the model's reliability, the dataset was categorized into high and low toxicological significance (TS) subsets based on the correlation between intoxication and the cause of death. Molecular descriptors for each substance were calculated using the Mordred tool, and pLBC50, along with these descriptors, formed the variables for training support vector machine (SVM), linear regression, and multilayer perceptron (MLP) regression algorithms in the Weka suite. The algorithms were evaluated using correlation coefficient (R) and mean absolute error (MAE) metrics. **Results:** Our comprehensive dataset, comprising 113 substances, was divided into high (70; 62%) and low (43; 38%) TS subsets. After training and testing, the MLP algorithm emerged as the optimal choice for building our model (R = 0.89; MAE = 0.44), outperforming SVM (R = 0.78; MAE = 0.73) and linear regression (R = 0.64; MAE = 0.78). Subsequent evaluations through 10-fold cross-validation (R = 0.73; MAE = 0.65) and external validation (R = 0.76; MAE = 0.73) confirmed the model's reliability when applied to unseen data. Utilizing our model, LBC predictions for 1200 substances listed on the NPS DataHub repository were conducted, and the results were visualized in a chemical map using the TMAP Python library. To facilitate broader accessibility, a Python-Streamlit script was developed to create a simple, user-friendly web app named AmberNPS. This app enables users to input chemical structures easily via SMILES notations, obtaining rapid predictions of potential lethal concentrations with minimal computational requirements. **Discussion/ Conclusion:** The developed model's versatility in predicting LBCs across different NPS classes emphas its robustness, overcoming the challenges posed by the heterogeneous nature of data sources and the complexities of *post-mortem* toxicology. In light of the largely unknown toxicity of NPS and the lack of quantitative data, our model, coupled with the interactive map and web app, represents a valuable tool for predicting lethal blood concentrations of NPS, contributing to advancements in forensic toxicology.
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Application of switchable hydrophilicity solvents to extract synthetic cathinones from urine samples

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Introduction: Synthetic cathinones (SC) are an important class of New Psychoactive Substances (NPS), which appeared in the illicit drug market in the mid-2000s as legal alternatives to traditional drugs of abuse. Hundreds of these stimulants have been reported so far and due to their toxicological relevance, different analytical methods have been proposed to analyze SC in biological samples. Similarly, the aim of this work was to develop a sample preparation technique to analyze these drugs in urine samples, however, the principles of Green Analytical Toxicology (GAT) were considered. To that end, switchable hydrophilicity solvents (SHS) were studied as extraction solvents in a miniaturized technique, named homogeneous liquid-liquid microextraction with switchable hydrophilicity solvent (SHS-HLLME). The use of SHS to extract drugs from biological fluids is a recent approach and makes use of their chemical feature of switching between the hydrophilic and hydrophobic forms depending on the pH. It is possible to add these chemicals while in their polar state to an aqueous sample, such as urine, and change the pH of the system to make them immiscible, thus allowing the collection of the organic layer. **Objective:** Test the application of SHS-HLLME for the analysis of the SC ethylpentedrone, ethylpentylone, 4-chloroethcathinone (4-CEC), methylone and pentylone in urine samples. **Methods:** Two SHS, dipropylamine (DPA) and N,N-dimethylcyclohexylamine (DMCHA), were initially considered and the best candidate was used in a subsequent optimization study with other relevant parameters for the technique. The volume of sample (200-500 µL), SHS:HCl 6 mol/L mixture (1:1) (200-500 µL), and NaOH 10 mol/L (200-500 µL) in combination with extraction time (0-6 min) were included in a Box-Behnken design. All analyses were performed using a UPLC-MS/MS and, once optimized, the method was validated according to the ANSI/ASB Standard 036 1st Edition 2019 validation guidelines. **Results:** DPA was the SHS yielding the best analyte recovery for the SC included in this study. Additionally, the combination of extraction conditions providing the best analyte recovery was: 350 µL of urine, 350 µL of SHS:HCl 6 mol/L, and 400 µL of NaOH 10 mol/L, while dismissing any extraction time ($p < 0.05$). Thus, after adding NaOH, the samples were directly centrifuged (8.000 rpm for 5 min) to achieve separation of the organic layer, which was evaporated under a gentle N2 stream. The extracts were then ressuspended with 50 µL of mobile phase A, of which 5 µL were injected into the instrument. The technique was successfully validated within accepted limits: linearity range of 10-100 ng/mL, bias and imprecision ≤ 20%, limit of quantitation of 10 ng/mL, and limit of detection of 1 ng/mL for all the analytes. **Discussion:** In this work, the applicability of SHS-HLLME to extract the SC ethylpentedrone, ethylpentylone, 4-CEC, methylone and pentylone from urine samples was studied. This sample preparation technique proved to be a simple, quick, and efficient procedure to analyze this class of NPS in urine samples making it a valuable tool for toxicological applications. In addition, the cost-effectiveness of this miniaturized technique in combination with the low toxicity of using SHS are fit to GAT guidelines, thus contributing to reducing the environmental impact of practices in analytical toxicology. **Acknowledgments:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - Projeto INSPEQT, Edital Nº 16/2020), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Processo 2021/09857-8), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Processos 142056/2002-0 and 121221/2022-9) are gratefully acknowledged for funding this work.

Comparison of 4-aminophenol and Duquenois-Levine colorimetric tests for preliminary detection of Cannabis sp.

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Cannabis is the most consumed drug in the world, with approximately 4% of the global adult population using it in 2021, according to the United Nations Office on Drugs and Crime, with a 21% increase in the number of users of the drug since the last decade. The hallucinogenic psychoactive effect that leads people to use Cannabis comes mainly from tetrahydrocannabinol (THC), including its isomers and stereochemical variations, with Cannabis psychoactivity being proportional to the concentration of THC present in the drug. On the other hand, cannabidiol (CBD), also found in marijuana, has therapeutic properties and has been widely studied for medicinal use. In the forensic context, the colorimetric methods commonly used as preliminary tests to identify Cannabis usually operate with toxic reagents, such as Duquenois-Levine test, making their use outside the laboratory difficult, in addition to not allowing the differentiation of drugs that have mainly THC or mostly CBD in their composition. Therefore, it is important to seek simple and efficient method that can be used on site by criminal experts for preliminary analysis of suspected Cannabis samples. Thus, this work aimed to evaluate the efficiency of the colorimetric reagent containing 4- aminophenol (4-AP) compared to the Duquenois-Levine test, using thin layer chromatography (TLC) or gas chromatography-mass spectrometry (GC-MS) as confirmatory method. To carry out the analysis, 0.5 mL of the acidic ethanolic solution of 4-AP 0.03% w/v (solution A) was added to a microtube and subsequently 3 drops of ethanolic sodium hydroxide solution 3% w/v (solution B) were added to the sample (approximately 20 mg), followed by vigorous shaking for 10 s and evaluation of the color after 2 min. The results were considered positive after the development of blue color (samples with high THC content) and pink/purple (samples with high CBD content). To check the applicability of the colorimetric test, 168 samples seized in different preparations (leaves, inflorescences, hashish, vape, dried and burnt herbs) and sent to the Criminalistics Institute of Minas Gerais, in the year of 2023 and 2024, were analyzed by both colorimetric tests. Moreover, 28 species of plants other than Cannabis purchased locally were also analyzed (negative samples). The rates of false-positives, false-negatives, sensitivity, selectivity, and reliability for the 4-AP test were 9.4%, 2.4%, 97.6%, 90.6%, 88.2% respectively, and for the Duquenois-Levine test were 6.3%, 3.0%, 97.0%, 93.8%, 90.7% respectively (n=198). The plant species Jaborandi (Pilocarpus jaborandi Holmes), Aroeira (Schinus molle L) and Carobinha (Jacaranda decurrens Cham) developed similar colors to the positive samples, generating false-positive or dubious results with the 4-AP test. Considering the positive samples, 97.5% had a higher THC content in relation to CBD and 2.5% contained more CBD than THC, according to the color developed in the 4-AP test. Hence, the 4-AP test proved to be suitable for analyzing seized Cannabis samples and can be used in the routine of forensics laboratories as a quick, simple, cheap, and effective method for detecting vegetables containing phytocannabinoids, and can even be used in the differentiation of samples with higher or lower THC content in relation to CBD.

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Design of experiments approach to determine organophate pesticides by gc-ms in larvae of *Lucilia cuprina* (calliphoridae) for entomotoxicological studies

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Introduction: When human body is in an advanced state of decomposition where tissue toxicological analysis is impracticable, insect larvae can be considered an alternative option for researching toxicants. Entomotoxicological studies with cholinesterase inhibitors (carbamates and organophosphates) are scarce using this alternative matrix. **Objective:** The objective of the present work was the optimization of an analytical methodology based on a Design of Experiments approach, with the development of a 24 full factorial design as a screening step, followed by a response surface methodology to optimize the cleanup step of the QuEChERS methodology, having as independent variables commercial sorbents commonly used in QuEChERS methodologies for the determination of organophosphate pesticides through GC-MS in Lucilia cuprina larvae matrix. **Methods:** For the development of the factorial screening planning, the sorbents used were and their levels were as follows: anhydrous MgSO4 (0.056 g and 0.112 g), PSA (0.025 g and 0.05 g), C18 (0.025 g and 0.05 g) and GCB (0.0025 g and 0.0078 g) with quadruplicate at the central point and as dependent variable (response) the sum of the areas of quantitative ions referring to each analyte under study (Dichlorvós – 109 m/z; Methyl chlorpyrifos – 286 m/z ; Fenitrothion – 260 m/z; Methyl Pyrimifos – 290 m/z; Ethyl Chlorpyrifos-D10 – 324 m/z; Ethyl Chlorpyrifos – 314 m/z; and Ethion – 231 m/z), totaling 20 isolated experiments. Afterwards, a response surface methodology was followed, using a Doehlert matrix for two variables – GCB (0.025 g and 0.05 g) and C18 (0.0025 g and 0.0078 g), which were the variables that best influenced factorial 24, with PSA and MgSO4 maintained at the central point. A GC-MS system (GCMSQP-2010 ultra; Shimadzu, Japan) with an Rtx®-5 MS column (30m x 0.25mm, 0.25µm film thickness) was used. Helium 5.0 (Air Liquide, Brazil) was used as carrier gas at constant linear velocity (39.8 cm/sec) and 2 µL was injected in splitless mode (1.5 min. sampling time). The injection temperature used was 175ºC. The interface and ion source temperatures were maintained at 300 °C. Data were obtained in SIM mode with an acquisition rate of 0.30 scans/sec. For the development of extractive tests, larvae free of analytes were contaminated with 100 µg/kg of the respective reference analytical standards. The method consisted of the following steps: 1) extraction (0.2 g of contaminated larvae+0.4 mL of ultrapure water+0.4 mL of acetonitrile+vortex for 30 seconds); 2) partition assisted by the salting-out effect (0.160 g of anhydrous MgSO4+0.04 g of NaCl+30 seconds of vortexing+14,000 RPM for 5 minutes); 3) Cleaning with test experiments (0.350 mL of extract+test sorbents+centrifugation 14,000 RPM for 5 minutes); 4) Injection into GC-MS. **Results and Discussion:** The statistical optimization of the data obtained showed that the variables that most positively influenced the effects were, for the complete factorial design 24, C18 and GCB, while PSA and MgSO4 generated better responses at their central point. Finally, the optimum points of the studied experimental region indicated by the response surface methodology used in the present study were as follows: 88.4 mg of MgSO4, 37.5 mg of PSA, 34.16 mg of C18 and 7.37 mg from GCB. **Conclusion:** The proposed methodology allowed the determination of all tested analytes. Furthermore, it was possible to optimize the methodology through the experiment design approach used. This work is part of doctoral thesis and the next steps of the study consist of validating the analytical methodology followed by application in real cases. **Acknowledgements:** We wish to thank PROBIC/FAPERGS-CNPq for providing financial support.

Detection of amphetamine derivative in electronic cigarettes

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Background/Introduction: Popularly known as vapers, pods, e-cigarettes, e-ciggy, e-pipes, and e-cigars, electronic smoking devices (ESDs) have become popular among both young people and adults in Brazil. Different flavors, designs resembling digital accessories, and the illusion of being less harmful than traditional cigarettes are among the attractive features. However, the solutions used (e-liquids), in addition to the solvent (e.g., glycerin, propylene glycol), may contain a variety of chemical compounds such as nicotine, flavorings, and even undeclared psychoactive substances in the product labels. Despite the prohibition of marketing, importation, and advertising by the regulatory agency ANVISA, the number of seizures of these devices has increased, reflecting their widespread availability and use in the national territory. **Objective:** This study aims to identify the chemical profile of seized ESDs sent for forensic examination at Scientific Police of Santa Catarina in Joinville region. **Methods:** Ten distinct samples from three ESDs brands were analyzed. Qualitative analysis was performed by Gas Chromatography coupled with Mass Spectrometry (Agilent 7890A/5975C, HP-5MS column 30 m x 250 µm x 0.25µm). The e-liquids extracted from the ESDs were diluted in methanol (1:10) and subjected to analysis under the following chromatographic conditions: 100°C for 2 min, then 15°C/min to 300 °C for 10 min, with the transfer line and ion source held at 280°, and the quadrupole temperature set at 150 °C. **Results:** Substances related to the vehicle (glycerin derivatives), flavorings, and nicotine were detected in all samples. Additionally, all samples showed the presence of octodrine (dimethylhexylamine - DMHA). Octodrine has a structure similar to amphetamine, serving as a Central Nervous System stimulant used in pre-workout and 'fat-burner' products, listed in the World Anti-Doping Agency's (WADA) List of Prohibited Substances . Its adverse effects are still under-researched, but it is known to have the potential for cardiovascular effects, and chronic use may lead to tolerance, withdrawal symptoms, and a risk of dependence. **Discussion/Conclusion:** The chemical composition of ESDs raises significant toxicological concerns, considering that the liquid vehicle and flavorings, when exposed to high temperatures, can produce harmful substances, especially with chronic use. Moreover, the presence of undisclosed substances such as octodrine intensifies the harm to users, increasing concerns about the addictive nature of the product. To better elucidate the chemical profile of ESDs, there is a plan to expand the research with a greater number and variety of samples. **Acknowledgments:** Scientific Police of Santa Catarina and Federal University of Santa Catarina (UFSC).

Development and validation of a quantitative analytical method for drug-facilitated sexual assault (DFSA) in urine samples

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Background/Introduction: The drug-facilitated sexual assault (DFSA) is a kind of drug- facilitated crime (DFC), in which the victim is incapacitated or unconscious due to the effects of psychoactive substances. The literature indicates a wide list of substances being associated with this type of crime, including licit and illicit drugs. Currently, in Brazil, there is no standardized scope for the detection of DFSA-related substances in biological samples. Furthermore, DFSA analysis requires relatively low limits of detection to expand the detection window. **Objective:** The aim of this study was to develop and validate a quantitative method for analysis of 56 psychoactive substances (antidepressants, benzodiazepines, antihistamines, opioids, stimulants, and miscellaneous) and its metabolites in urine samples, using liquid-liquid extraction (LLE) procedure followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). **Methods:** The list of substances selected to this study was based on the substances recommended by the ANSI/ASB Standard 121 guidelines 2021. Urine samples (400 µL) were submitted to enzymatic hydrolysis and extracted with methyl tert-butyl ether (MTBE). For benzoylecgonine extraction, an aliquot of the urine samples was extracted by protein precipitation with acetonitrile. After agitation, centrifugation, and evaporation of solvent, samples were resuspended with 100 µL of mobile phase and 2 µL was injected into LC-MS/MS system (LCMS8045, Shimadzu) with electrospray ionization. The chromatographic separation was performed with a Raptor™ biphenyl column (100x2.1 mm, 2.7 µm). The mobile phase consisted of ultrapure water (A) and methanol (B), both containing 0.1% formic acid (v/v) and 2 mmol/L of ammonium formate, eluted in gradient mode. The method was validated according to ANSI/ASB Standard 036 recommendations. **Results/Discussion:** The method attended the guideline acceptance criteria, with limits of detection and quantification as low as 0.5 ng/mL, and linearity from 0.5 to 750 ng/mL. Bias and imprecision values were better than 14.3% and 14.8%, respectively. The maximum value of matrix effects observed was -57.4% and recovery were better than 5.1%. Neither carryover nor interferences were observed. Autosampler stability study showed that all analytes were stable for 24 h at 10 °C. Forty-two authentic urines samples were analyzed; twenty-six samples were positive at least for 1 substance (61.90%) and sixteen different substances were detected in the samples. THC-COOH was the most common drug found (26.92%), followed by cocaine (23.08%), and benzoylecgonine (19.23%). **Conclusion:** A sensitive method based on simple LLE and LC-MS/MS analysis was developed and validated to quantify 56 psychoactive substances in urine, showing low limits of detection and quantification, adequate linearity, bias, and imprecision, being successfully applied to authentic urine samples analysis. **Acknowledgments:** The authors thanks the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- Brasil-CAPES, Superintendência da Polícia Técnico Científica do Estado de São Paulo, and the Hospital da Mulher Prof. Dr. José Aristodemo Pinotti (CAISM). **Keywords:** drug-facilitated sexual assault (DFSA); drug-facilitated crime (DFC); LC- MS/MS.

Development of a dispersive liquid-liquid microextraction for the detection of new psychoactive substances in oral fluid

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Background/Introduction: The growing emergence of new psychoactive substances (NPS) has been a challenge for responsible institutions by substance control and toxicologist, as new substances are constantly incorporated into the market, being marketed in ways that make it difficult to identify the inspections. NPS can be considering of high risk to public health, since there is an unknowing regarding toxicity, effects, damage and dissemination. In view of this situation, oral fluid (OF) is a good alternative sample for testing drug users on the street or in hospitals. Thus, OF has the advantage of non-invasive collection without the need for a trained professional, represents a recent use and be of less complexity. This project is based on the principles of Green Analytical Toxicology, and therefore, intends to use a dispersive liquid-liquid microextraction (DLLME). In this technique, an organic extractor and a dispersive solvent are quickly added to an aqueous sample, creating an emulsion solution. The extracting solvent is dispersed in the sample in the form of microdrops, depending on the presence of the dispersing solvent. **Objective:** This project aims to develop and validate a method for the determination of synthetic cannabinoids and synthetic cathinones in saliva through DLLME and liquid chromatography coupled to mass spectrometry (LC-MS). **Methods:** 20 µL of the pool of analytes and 20 µL of the internal standard were added to the 200 µL of OF collected by Quantisal™ and buffer (1:3, v/v) to obtain the desired concentrations. Initially, protein precipitation was performed by adding of 200 µL of acetonitrile to the sample. In addition, in the next step, this solvent provides dispersion of extractor solvent. Then, this solution was vortexed, centrifuged (5 min at 6000 rpm) and the supernatant was transferred to a thin glass vial. For extraction, 100 µL of chloroform was added to the samples, briefly vortexed and centrifuged at 6000 rpm for 5 min. The organic layer was collected, being dried under nitrogen flow. Finally, it was resuspended with 50 µL of ammonium formate (1 mM) and formic acid (999:1, v/v) and injected into the LC-MS. **Results:** To evaluate the application of the microextraction technique, a sample with analytes at 100 ng/mL in triplicate was analyzed using two different analytical methods. One method contained 31 synthetic cannabinoids and another method contained 15 synthetic cathinone. Of the total analytes, 30 synthetic cannabinoids and 9 synthetic cathinones were detected. **Discussion/Conclusion:** Based on these results, it can be concluded that DLLME is capable of extracting synthetic cannabinoids and synthetic cathinone in OF. For the next steps, it is intended to develop a single method that includes all analytes, in addition to adding new substances. After defining the method, extraction optimization will be carried out, followed by validation in accordance with the validation guidelines of Standard ANSI/ASB 036 1st Edition 2019. Finally, the developed method will be applied to real samples collected from NPS users.

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Development of an ultrasound-assisted liquid-liquid microextraction for benzodiazepines analysis in total blood

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Introduction: Benzodiazepines (BZDs) are part of a pharmacological group that is widely consumed due to their depressant effects on the central nervous system. In neuropsychiatric disorders such as insomnia, anxiety, and epilepsy, for example, BZDs are highly present in medical prescriptions. Despite the benefits that these BZDs, there is an increase in the misuse of these substances, such as abusive consumption, mixing with other drugs or even doping an individual to commit crimes (theft, rape), which can even lead to fatal cases. Considering this and the demand for toxicological and forensic analyzes, the ultrasound- assisted dispersive liquid-liquid microextraction (UA-DLLME) can greatly benefit many laboratories due to its practicality, effectiveness, less time consumption, reduced sample volume and low cost throughout the world. **Objective:** This work aimed to design a ultrasound-assisted dispersive liquid-liquid microextraction (UA-DLLME) method for the investigation of benzodiazepines in whole blood. **Methods:** Samples of 500 µL of whole blood were fortified with 10 BZD (oxazepam, lorazepam, chlordiazepoxide, temazepam, midazolam, flunitrazepam, bromazepam, clonazepam and alprazolam) and Diazepam-D5, achieving a final concentration of 3, 5, and 7 µg mL-1 for the analytes. The UA-DLLME was developed by a Full Factorial Design (FFD) investigating ACN and MeOH as precipitating and/or dispersing agents, and CH2CH2, CH2CH3, and AcOEt as the extracting solvent. The FFD also assessed the need for a salting-out step. During the UA-DLLME, the samples received 1 mL of precipitating solvent, 1 mL of dispersing solvent, and 300 µL of extracting solvent. Analysis of the obtained extracts was performed by DART-HRMSOrbitrap with a data acquisition time of 1 minute. **Results:** All the selected BZDs were detected by the UA- DLLME-DART-HRMSOrbitrap technique. The results obtained by the FFD's assays generated a Pareto Chart where, along with the desirability function results, the best result was the one involving the salting-out step, ACN as precipitating solvent, MeOH as dispersive agent, and CH2CH2 as the extracting agent. The recovery assays of the BZDs in the 3 concentrations ranged from 79.4 to 118.6% at 3 µg mL-1, 82.87 to 111.33 at 5 µg mL-1, and 78.51 to 99.07 at 7 µg mL-1. **Discussion/ Conclusions:** By this method, the recovery of BZDs was based on the National Institute of Metrology, Quality and Technology (INMETRO) standards in which results of 80 to 110% are considered optimal. Some results presented limits beyond the INMETRO's ideal range, however, in literature it was observed that de addition of ± 5 % in recovery are still acceptable. It can be said, therefore, that the recovery of BZDs in blood by UA-DLLME-DART-HRMSOrbitrap was effective, demonstrating, therefore, the analytical confidence of the developed method. This research proved to be viable for application in forensic laboratories, benefiting them with a high- recovery, fast and lower-cost extraction method. **Acknowledgments:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES); Instituto Estadual de Hematologia Arthur de Siqueira Cavalcanti (HEMORIO).

Ecstasy cutting agents over a decade: a case study at Rio de Janeiro – Brazil

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Introduction: Misuse of illegal drugs is currently one of the main problems faced by public health professionals. These illegal substances, such as MDMA, which is the major ecstasy compound in Brazil, can expose users to a variety of health hazards, including the possibility of developing serotonergic syndrome. In addition, cutting agents are frequently added to illicit drugs by drug dealers to boost revenues, improve their pharmacological effects, make them appear prettier, or make them easier to transport and consume. This practice exacerbates public health problems. However, the inclusion of such cutting agents can render drugs even more dangerous. Therefore, continuous assessment of the chemical profile of illicit drugs is essential. This helps determine their potential toxicity, elucidate trends in the illicit market, and enhance police intelligence. **Objective:** This study aimed to determine the cutting agent profile of ecstasy seized in Rio de Janeiro State (RJ, Brazil) between 2012 and 2021. **Methods:** Samples were extracted with methanol by ultrasound-assisted extraction and characterized using gas chromatography-mass spectrometry and high-resolution mass spectrometry. **Results:** Over the evaluated decade, 27 distinct combinations of adulterants were identified in 32,756 ecstasy tablets. The major adulterants were caffeine, clobenzorex, lidocaine, ephedrine, sildenafil, and ketamine. In 57.9% of the ecstasy tablets, caffeine was used as the sole cutting agent. Some of the cutting agent combinations found in the tablets correspond to over-the-counter licit drug composition, such as acetaminophen with caffeine (flu treatment), caffeine with orphenadrine (muscle relaxant), lidocaine with theophylline (respiratory disorder), and benzocaine with lidocaine (local anesthetic). In addition, the veterinary dewormer levamisole was found in the evaluated tablets. **Discussion/Conclusions:** Worldwide, caffeine is a prevalent cutting agent used in different types of illegal drugs, extending beyond ecstasy. The primary objective of adding caffeine is to increase the mass of tablets without using excessive amounts of the primary illicit substance, which is typically more costly and challenging to obtain. Caffeine's inclusion in ecstasy can reduce the user's experience of exhaustion, which increases the risk of overdosing. The occurrence of different combinations of cutting agents over time indicates changes in the supply chain. This highlights the importance of continuous monitoring of the chemical profiles of illicit drugs. For instance, clobenzorex frequently occurred as a cutting agent until 2021, the year it was outlawed in Brazil, whereas acetaminophen was first detected in ecstasy seized in Rio de Janeiro only in 2015. Since acetaminophen was a prevalent adulterant detected in ecstasy in neighbouring states prior to 2015, acetaminophen highlights the potential establishment of a new traffic route. Clobenzorex and sildenafil are adulterants typical of the South American region and are used to produce the popularly known "generic ecstasy" and "sextasy," respectively. Each of these variations was created to modify ecstasy effects. Thus, illicit marketing of generic ecstasy promises to increase user attention, whereas sextasy illicit marketing promises to enhance user sexual performance. Abuse of clobenzorex and sildenafil combined with ecstasy can increase the risk of cardiovascular events. Chemical fingerprints of drugs produce data that help police intelligence predict the activities of the illicit market, elucidate traffic routes, and assist in the development of public health measures. **Acknowledgments:** Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES).

Enhanced portable raman sensing: trace detection and analysis of priority drugs of abuse using handheld devices in mexico city

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The development of Surface-enhanced Raman Spectroscopy (SERS) has ushered in a new era for the rapid and highly sensitive detection of trace chemicals. Harnessing this cuttingedge approach, we aim to create a paradigm shift in on-site drug screening, making it a valuable tool for law enforcement and medical first responders. This study focuses on the application of a handheld SERS-based analyzer, tailored for the detection and quantification of street drugs in Mexico City. Methodological enhancements were made to establish concentration-dependent calibration curves, assess effects on SERS signal, and allow for accurate multi-component analysis of drug samples, including those adulterated with fentanyl. Our findings highlighted the robust quantitative potential of the device, revealed through limits of detection (LODs) and limits of quantification (LOQs) for drugs like cocaine and phenylcyclidine at impressively low concentrations—7.0 ng/ml and 3.57 ng/ml for cocaine, and 20.0 ng/ml and 0.92 ng/ml for phenylcyclidine, respectively. These results underscore the practical applications of portable SERS units in rapid, reliable, and fieldsensitive drug analysis.

Evaluation of the chemical profile of new psychoactive substances (NPS) through high-performance analytical techniques

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Introduction: The last decade has brought significant changes in the rise of drugs on the world stage. Since the 2000s, hundreds of new drugs have emerged from the alteration of the chemical structure of already known drugs of abuse, the so-called New Psychoactive Substances (NPS). The main objective of these drugs is to circumvent existing laws. Therefore, it becomes a great challenge to control, identify and characterize these drugs at a high speed. **Objective:** Application of analytical methods for characterization at the molecular level of new psychoactive substances (NSP) seized in the State of Rio de Janeiro using colorimetric tests, gas chromatography coupled to mass spectrometry (GC-MS) and high-resolution mass spectrometry (HRMS) with a Orbitrap mass analyzer. **Methods:** Colorimetric tests were performed with Ehrlich and Hoffman reagents. For each analysis, one drop of the reagent was used separately, and the process was monitored for 30 minutes. The analyzes carried out in GC-MS used He as carrier gas at 1 min mL-1, a HP-5MS (Agilent) column, and the mass range from 35 to 500 m/z. The Orbitrap-HRMS analyzes were performed only in positive mode by direct infusion (10 µl min-1). The capillary temperature used was 380°C and the mass range analyzed varied from 50 to 500 m/z. **Results:** A total of 11 samples were analyzed. From the samples tested, two presented a positive result for indole compounds using the colorimetric test. Only in two samples containing the Ehrlich's reagent a change in color was observed. With the GC-MS analysis, the phenomenon observed in the colorimetric tests was confirmed, with the presence of LSD in two samples. It was also possible to identify amphetamine, methamphetamine, 25I-NBOMe among the samples analyzed. Subsequently, with the analysis carried out on the Orbitrap-HRMS, minority compounds were identified in the samples. Ecstasy could be identified in samples that contained amphetamine compounds and 25I-NBOH in samples whose major compound was 25I-NBOMe. **Discussion/ Conclusion:** There is great importance in carrying out these studies both for greater knowledge of new drugs that emerge and in relation to the effects of these substances on users. Another factor that can be analyzed is the combination of effects that can be caused by these changes in the structures and/ or mixture of different drugs. Colorimetric tests showed good results for samples containing indoles when analyzed with Ehrlich's reagent, however, visual colorimetric changes could not be observed with Hoffman's reagent. The results obtained through GC-MS analysis allowed the characterization of the majority compounds present in the samples, as well as confirmation of the presence of indole compounds identified in the colorimetric tests. Due to its greater sensitivity, the Orbitrap made it possible to identify compounds in smaller quantities that may have been added to add volume to commercialized samples, such as ecstasy and 25I-NBOH. **Acknowledges:** CAPES, FAPERJ, and CNPq.

Exogenous intoxication by methomyl: a case report

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Introduction: Exogenous intoxication is characterized by clinical manifestations and biochemical changes resulting from exposure to chemicals found in the environment or isolated. Among the most commonly involved substances are pesticides, drugs, industrial chemicals, and household sanitizers. Methomyl, a systemic and contact insecticide, is classified as extremely toxic (CLASS I). It belongs to the chemical group of carbamates, which reversibly inhibit the enzyme acetylcholinesterase, resulting in the accumulation of acetylcholine at muscarinic receptors (effect on cholinergic cells), nicotinic receptors (skeletal neuromuscular junctions), and in the central nervous system. **Objective:** Report a case of exogenous intoxication caused by ingestion of Methomyl. **Methods:** Relevant data for this case report were obtained from the Regional Hospital North (Sobral/CE, Brazil) and the forensic management software Sistema Galileu (PEFOCE/CE). A review of the specialized literature on the subject was also conducted. **Case Report:** A 44-year-old male patient was admitted to the hospital after the probable accidental ingestion of methomyl. He presented with electrical activity without pulse and in asystole, undergoing cardiopulmonary resuscitation with four ampoules of adrenaline and ten ampoules of sodium bicarbonate. The patient also exhibited bilious gastric residue, large quantity diarrhea, reddish urine, secretion in the upper airways, hypothermia, sweating, hypotension, hyperglycemia, bradycardia, spasms, and choluria. Gastric decontamination was not reported. In laboratory tests, he showed elevated creatinine (3.51 mg/dL) along with hematuria (40 per field), as well as a leukocytosis (33,200/mm3) and increased platelets (500,000/mm3). The patient did not improve during hospitalization and died due to failure of bodily systems caused by cholinergic syndrome. The initial suspicion was cyanide poisoning, hence the treatment administered to the patient consisted of Cyanocobalamin and Thiamine, in addition to Atropine. The Forensic Expertise Agency of the State of Ceará (PEFOCE/CE) was requested to perform a toxicological examination for pesticides in gastric lavage, blood, and serum samples. An acidic organic extract was obtained, which was subjected to Gas Chromatography coupled with Tandem Mass Spectrometry. The result of the toxicological examinations in the three biological samples identified the carbamate Methomyl. **Discussion/ Conclusion:** The clinical picture presented by the patient is consistent, in some aspects, with exogenous intoxications by carbamates. These intoxications can cause irritation in the gastrointestinal tract, manifested by burning, abdominal pain, nausea, vomiting, and diarrhea. In large quantities, they can cause central nervous system (CNS) depression with sedation, drowsiness, dizziness, loss of concentration, ataxia, and, in severe cases, seizures, coma, and/ or death. In a complete blood count, reversible leukocytosis or leukopenia can be observed, and in such intoxications, transient hyperglycemia may occur. Regarding treatment, atropinization was useful as it is an effective therapeutic approach to combat the symptoms presented by patients victims of acute exogenous intoxication by carbamates. The collection performed while the patient was still alive was essential, as it increased the chance of obtaining a more accurate result in the toxicological examination, since carbamates are rapidly excreted by the body and may not be detectable after death.

Fractional factorial planning as a screening method to determine organophosphate pesticides by GC-MS in larvae of *Lucilia cuprina* (calliphoridae) for entomotoxicological studies

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Introduction: When the human body is in an advanced state of decomposition where tissue toxicological analysis is impracticable, insect larvae can be considered an alternative option for researching toxicants. Entomotoxicological studies with cholinesterase inhibitors (carbamates and organophosphates) are scarce using this alternative matrix. **Objective:** The objective of the present work was the optimization of an analytical methodology based on a Design of Experiments approach, with the development of a 26-2 fractional factorial design as a screening step, to optimize the cleanup step of the QuEChERS methodology in Lucilia cuprina larvae matrix in QuEChERS methodology for the determination of organophosphate pesticides through GC-MS using rice husk as cleanup step sorbent. **Methods:** For the development of the fractional factorial screening planning, the independent variables and the sorbents used and their levels were as follows: (0.04 to 0.08 g) (extraction/partition) anhydrous MgSO4 (0.0525 g and 0.150 g), Rice husk (0.075 g and 0.150 g), vortex (0.5 and 1 min) and centrifugation time (5 and 10 min.) throughout the process. The tested analytes were dichlorvos– 109 m/z; Methyl chlorpyrifos – 286 m/z ; Fenitrothion – 260 m/z; Methyl Pyrimifos – 290 m/z; Ethyl Chlorpyrifos-D10 – 324 m/z; Ethyl Chlorpyrifos – 314 m/z; and Ethion – 231 m/z), totalizing 20 isolated experiments. Afterwards. A GC-MS system (GCMSQP-2010 ultra; Shimadzu, Japan) with an Rtx®-5 MS column (30m x 0.25mm, 0.25µm film thickness) was used. Helium 5.0 (Air Liquide, Brazil) was used as carrier gas at constant linear velocity (39.8 cm/sec) and 2 µL was injected in splitless mode (1.5 min. sampling time). The injection temperature used was 175ºC. The interface and ion source temperatures were maintained at 300 °C. Data were obtained in SIM mode with an acquisition rate of 0.30 scans/sec. For the development of extractive tests, larvae free of analytes were contaminated with 100 µg/kg of the respective reference analytical standards. The method consisted of the following steps: 1) extraction (0.2 g of contaminated larvae+0.4 mL of ultrapure water+0.4 mL of acetonitrile+vortex for 30 seconds); 2) partition assisted by the salting-out effect (0.160 g of anhydrous MgSO4+0.04 g of NaCl+30 seconds of vortexing+14,000 RPM for 5 minutes); 3) Cleaning with test experiments (0.350 mL of extract+test sorbents+centrifugation 14,000 RPM for 5 minutes); 4) Injection into GC-MS. **Results and Discussion:** As the hypothesis of the existence of a quadratic behavior was evidenced, it was necessary to develop more optimization tests, aiming to understand with greater precision the global interaction behavior of the variables chosen for the respective planning before the analytical methodology validation stage. For this purpose, a response surface methodology based on Doehlert planning was chosen for the 3 variables that had the most prominent main effects in the initial testes (NaCl, Water/Sample Ratio and MgSO4). As the other 3 variables studied proved to be important when associated with each other in 2nd order effects, they will be maintained at their central levels for the development of the next planning. **Conclusion:** The proposed methodology allowed the determination of all tested analytes. Furthermore, it was possible to partially optimize the methodology through the experiment design approach used. The next steps are the development of response surface methodologies to finalize the optimization of the methodology to continue with its validation This work is part of the doctoral thesis and the next steps of the study consist of validating the analytical methodology followed by application in real cases. **Acknowledgments:** We wish to thank PROBIC/FAPERGS-CNPq for providing financial support.

Homicide caused by poisoning with diethylene glycol added to beer

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Introduction: At the end of 2019 and beginning of 2020, several cases of poisoning by diethylene glycol (DEG) occurred in Brazil resulting from the consumption of beer contaminated. It was widely publicized by the country's press, reaching the population's attention. Approximately one year after the incident, in April 2021, one husband buys DEG on the internet and adds this substance to his wife's beer, simulating the 2020 intoxication case. One of the biotransformation products of DEG, after the action of alcohol dehydrogenase and aldehyde dehydrogenase, is diglycolic acid (DA). Studies indicate that DA is responsible for the toxic action of DEG. **Objective:** The objective of this study was to search for DA in the blood of the victim to aid in the diagnosis of DEG poisoning. **Methods:** In a microtube, 20 µL of blood were added to 50 µL of internal standard solution of GHB-d6 diluted in methanol and 45 µL of acetonitrile. The tube was then vortexed for 15 seconds, sonicated for 5 minutes and centrifuged for 15 minutes at 5000 rpm. The supernatant was transferred to vial and dried with air flow at room temperature. Was added 50 µL of BSTFA/1% TMCS, the derivatization reaction occurred at 70°C for 20 minutes and the samples were injected into GC-MS/MS. **Results:** The victim's blood was received and analyzed for DA research while she was still alive in hospital, 4 days after the possible intoxication and 3 days after of the first symptoms. The value obtained in the quantification was 106 µg/mL which was close to the maximum value found in victims of intoxication in mass per DEG occurred in Brazil, which was 108 µg/mL and much higher than the maximum value found in the case of Panama, which was 75.2 µg/mL, it could be an indication that the dose of DEG consumed by the victim could be high. The DA in the victim's blood was measured after necropsy, 27 days after the supposed occurrence, 26 days after the first symptoms and 23 days after the first collection, obtaining a value of 40 µg/mL. **Conclusion:** DA, a metabolite of DEG, was found in the victim's body. High concentrations of this analyte were found a few days after consumption of DEG and this concentration decreased over time. **Acknowledgments:** This study was carried out with the support of the Civil Police of Minas Gerais.

Identification of counterfeit anabolic medicines: a case report

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Introduction: Forensics plays a crucial role in identifying and solving crimes, providing analysis and evidence that guide investigations and guide judicial systems. Among the various types of crime, the counterfeiting of pharmaceutical substances, especially anabolic steroids, has gained notoriety, due to the growing demand and consumption by the population in search of aesthetic improvements or sports performance. These counterfeit products not only violate intellectual property laws and health regulations (Portaria 344/98 ANVISA/MS), but also present significant risks to the health of users due to the lack of quality standards and possible contamination. This work describes a specific case report in which the falsification of anabolic steroids was central to the criminal investigation. The case analysis will illustrate the challenges faced by experts, the techniques used and the legal and public health implications that emerge from these situations. **Objective:** Report the expert analysis of anabolic medications that were seized and sent to the Forensic Expertise of the State of Ceará for identification. **Methods:** The chemical characterization of four analyzed samples followed the standardized procedures at the Forensic Toxicology Center (NUTOF) of Fortaleza for the analysis of medicines. The techniques used were: Vibrational Spectroscopy in the Infrared Region, Raman Spectroscopy and Gas Chromatography coupled to Mass Spectrometry. **Results:** The characterization and identification of the anabolic steroid samples analyzed made it possible to determine the presence of drugs from the anabolic steroid class in all products. In this case, the three Landerlan products, Methandrosterone 10 mg, Decaland Depot (Nandrolone Decanoate 200 mg/mL), Trenbolone Enanthate 200 mg/mL, had a composition compatible with the descriptions on the respective packaging and leaflets. **Discussion:** These medications appear in Federal Police reports among those most frequently described in expert reports.1 The product Wuanavar (Oxandrolone 20 mg) showed a different constitution from that indicated. In this formulation, the only active ingredient found was the hormone Testosterone. The drug Wuanavar is considered by the Ministry of Health to be a clandestine product,1 since the supposed manufacturer (WU Laboratory/ Germany) is not registered with any health agency. The results obtained allowed the correct characterization of the anabolic steroids examined and the identification of a false product among them.

In silico prediction and forensic detection of cinnamylpiperazines biomarkers of exposure

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Introduction: Opioid-related drug overdoses have been rising for the past thirty years in the U.S. Synthetic opioid-related deaths accounted for over 82% of opioid deaths in 2020. Many illicitly manufactured synthetic opioids show higher potency than morphine or heroin. AP-237 and its derivatives (2-methyl-AP-237, para-methyl-AP-237, and AP-238) have gained popularity due to their online easy availability. In 2019, 2-methyl-AP-237 and AP-238 appeared on the illicit market and were identified through drug seizures. AP-237 is considered a weak opioid receptor agonist. However, pre-clinical studies have shown that 2- methyl-AP-237 and AP-238 are also u-receptor agonists presenting the same or higher potency than morphine. A few data are available for the prevalence and potency of para- methyl-AP-237, but it was already observed in forensic cases. Forensic and clinical identification of these substances is challenging and usually relies on the identification of their metabolites and the parent drug in biological samples. The study of the metabolism of NPSs is extremely important for the proper forensic identification of these compounds in case of intoxication. **Objectives:** This study aimed to identify the metabolites of four cinnamylpiperazines using in silico predictions and experimental data using Liquid Chromatography – Quadrupole Time- of-Flight – Mass Spectrometry (LC-QTOF-MS). **Methods:** In silico prediction of the metabolites was applied by combining four different software. Way2Drug was used to predict the sites of metabolism (SOM), SwissADME and ADMETlab for pharmacokinetic information, and GLORYx to predict phase I and phase II metabolites for all 4 drugs. Different concentrations of the target compounds were incubated with microsomes from different species (human, rat, mouse, and rabbit), NADPH, NADPH regeneration system, and buffer at 37°C at different times with a maximum time of 90 minutes. After incubation, cold acetonitrile was added to stop the reaction. A centrifugation step was conducted and the supernatant was collected for LC-QTOF-MS analysis. **Results:** A total of 34, 43, 30, and 35 phase I and phase II metabolites were predicted for AP-237, 2-methyl-AP-237, AP-238, and para-methyl-AP-237, respectively. The in silico predictions suggest that the cinnamylpiperazines share the same SOM, a reactive carbon adjacent to the nitrogen-substituted ring, for most metabolism reactions catalyzed by the CYP3A4, CYP2D6, CYP2C19, CYP2C9, and CYP1A2 isoforms. The predicted pharmacokinetic data showed that the compounds analyzed are inhibitors of CYP2D6 and CYP2C19, and only AP-238 doesn't inhibit CYP2C19. All drugs are substrates of CYP1A2, CYP2C9, CYP3A4, but AP-238 is also substrate for CYP2C19. Experimental results showed that the main metabolites for all the compounds are the result of hydroxylation, reduction, desaturation, and oxidation, with important differences among species, showing the predominance of hydroxylation in rabbits, when compared to humans. **Discussion:** In silico predictions showed to be effective as an initial tool for the detection of NPS metabolites and for the comparative assessment of interspecies metabolism. From the metabolites predicted 67%, 72%, 56%, and 51% for AP-237, 2-methyl-AP-237, para-methyl- AP-237, and AP-238, respectively, were found experimentally. The in silico approach allowed the identification of the most reactive carbon in all compounds studied suggesting that the main site for metabolization is the carbon adjacent to the nitrogen-substituted ring due to its electronegativity an structural availability. In addition, metabolization differences were found among the species. The understanding of species differences in drug metabolism improves our abilities to predict the pharmacologic and toxicologic properties of a given compound in man from experimental data obtained in animals. Overall, this dual approach for the metabolites' identification enabled to propose relevant candidates as future biomarkers of consumption for the cinnamylpiperazines currently in the market. The addition of these biomarkers to mass spectrometry libraries will help diagnose intoxication cases. **Acknowledgments:** The authors acknowledge the Experimental Toxicology

In vivo toxicity evaluation of the russian homemade drug krokodil

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Introduction: "Krokodil" is the street name for the injectable mixture that has been used as a cheap substitute for heroin since 2002 in Russia, Ukraine, Georgia, and other European countries. The main psychoactive compound in krokodil is the opioid desomorphine. However, several other compounds were described to be found inside the krokodil mixture, mainly opioid derivatives and reaction by-products. Krokodil is usually prepared at home by the users themselves in a harsh uncontrolled reaction that starts with codeine tablets, bathroom cleaners, gasoline, car battery fluids, iodine tincture, and matchboxes, all of which are easily available in retail outlets, such as supermarkets and drugstores. The resulting product is a light brown liquid that is called krokodil. Krokodil users present several symptoms such as the formation of black ulcers in the injection site, necrosis, limb amputation, jaw osteonecrosis, speech impairment, and many others. **Objectives:** The goal of this study was to understand the toxic effects presented by krokodil users using Wistar male rats as the experimental model. **Methods:** Krokodil samples were synthed mimicking the homemade method applied by krokodil users. Samples were analyzed by GC-MS to confirm the presence of desomorphine. Animals were divided into seven groups and exposed subcutaneously to NaCl 0.9% (control group), krokodil mixture free of desomorphine (blank krokodil group), desomorphine 1 mg/Kg, and four different concentrations of the synthed krokodil (1, 0.5, 0.25 and 0.12 mg/Kg). Animals were treated once a day for five consecutive days. Animals were monitored daily and euthanized 24 hours after the last administration. The toxicity study was performed by the analysis of biochemical biomarkers using a clinical chemistry analyzer, histology assays, and toxicity assays. The toxicity assays were based on the formation of reactive oxidative species (ROS) and consisted of the analysis of reduced and oxidized glutathione (GSH/GSSG ratio) and lipid peroxidation. Krokodil's capacity to inhibit acetylcholinesterase was also evaluated. **Results:** The use of krokodil for a few days showed to cause injury at the injection area, with the formation of necrotic zones. The biochemical results evidenced alterations in cardiac and renal biomarkers of toxicity, namely creatine kinase, creatine kinase-MB, and uric acid. Significant alteration in levels of reduced and oxidized glutathione in the kidney and heart suggested that oxidative stress may be involved in krokodil-mediated toxicity. Although urinary biomarkers such as N- acetyl-β-glucosaminidase evidenced slight alterations, histological analysis revealed only mild alterations in kidney, liver, and lung tissue. Cardiac tissue necrosis was the most relevant finding of continuous krokodil administration. Krokodil has also been shown to inhibit acetylcholinesterase, explaining some of the krokodil effects that resemble the ones presented by patients intoxicated with organophosphate pesticides. **Discussion/Conclusion:** This study contributed to a better understanding of the toxicity of krokodil abuse, mainly the local and systemic toxicological impact of this complex drug mixture on major organs. As opioids are the main active compounds inside krokodil, when patients intoxicated with krokodil arrive in the ER, the first action is to revert the opioid effects. However, due to the formation of several by-products and the high pH and phosphorous concentration inside krokodil, patients need to be monitored for their renal functions and calcium and phosphorous levels in the blood. We hope these findings will be applied to develop an appropriate treatment strategy for the toxicological effects of krokodil.

Last Report of national database on toxicological criminal information (ToxCrim system)

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Introduction: There are three different databases in Brazil with toxicological information, the Information System of Grievances Notifications (SINAN) of Health Ministry, the National System of Toxic and Pharmacologic Information (SINITOX) of Oswaldo Cruz Foundation and the National Health Surveillance Agency and Brazilian System of Intoxication Data (DATATOX) of Brazilian Association of Information and Toxicological Assistance Centers. Unfortunately, there is no collection by any of them of criminal toxicological information because of the clinical approach to the toxicology of these systems. Such absence of criminal toxicological information and identified analyte data creates a gap of knowledge that is of vital importance for official laboratories to use in delineating criminal toxicological investigations. The ToxCrim system, as a pilot of a national database of criminal toxicological information, provides an important knowledge of the scope of intoxication cases in the Brazilian scenario, especially involved with crimes. **Objective:** To present the last data of a national database on toxicological information prepared by official forensic laboratories in the 2,022 year. **Methods:** The records in the ToxCrim system or by Excel® spreadsheet were based on postmortem information from forensic reports issued/signed and provided by the participants. The data were collected between January and December 2,022 from official forensic laboratories of the Brazilian states of Bahia, Espírito Santo, Goiás, Sergipe, Paraíba, Rio Grande do Sul, São Paulo and Rondônia, consolidated, processed automatically by a R-language and an environment for statistical computing and graphics developed. The cases were classified based on ANSI/ASB Standard 119 A, Standard 119 B and 120. **Results:** 9,548 postmortem cases were consolidated, with 22,834 reports of 224 different analytes detected in postmortem blood, urine, vitreous humor, gastric content, liver, brain, kidney, lung and hair samples. The cases were classified as a) a known anatomical cause of death 2,705 (28.3%), b) suspected toxicological cause of death determination 4,626 (48.4%) and c) impaired driving investigations 2,217 (23.2%). The top 10 analytes detected by known anatomical cause of death cases were ethanol (1,393), benzoylecgonine (873), cocaine (663), ecgonine methyl ester (587), ecgonine (580), 7-aminoclonazepam (197), carboxy-THC (185), diazepam (151), cocaethylene (119) and nordiazepam (113); by suspected toxicological cause of death determination cases were ethanol (1,776), benzoylecgonine (1,523), ecgonine (1,304), ecgonine methyl ester (1,293), cocaine (1,113), diazepam (431), midazolam (406), fentanyl (294), 7-aminoclonazepam (279) and nordiazepam (254); and by impaired driving investigations cases were ethanol (1,741), benzoylecgonine (275), ecgonine methyl ester (207), cocaine (200), ecgonine (182), midazolam (76), carboxy-THC (72), cocaethylene (47), fentanyl (43) and Δ9-THC (37). The statistics of all analytes detected were also obtained and assessed. **Conclusion:** Ethanol and cocaine/metabolites were still the main analytes detected for three different types of cases in 2,022 Brazilian scenario based on ToxCrim system.

Potential in the use of georeferencing tools to characterize seizures by the Scientific Police in the north of Santa Catarina

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Background/Introduction: In the current scenario of utilizing technological resources in criminal investigation, a promising methodology is geographic analysis using information systems. Considering the diversity of synthetic drugs seized and the increase in clandestine laboratories identified in the Santa Catarina territory, it is necessary to apply criminal intelligence based on the mapping and traceability of substance trafficking in the state. Santa Catarina Scientific Police is developing an automated tool to visualize data, such as the physical-chemical profile of synthetic drugs, in search of correspondence or similar patterns between different seizures. **Objective:** Test an automated distribution geolocation map tool for the of seizure chemical profiles of synthetic drugs analyzed by Santa Catarina Scientific Police. **Methods:** Geographic data and occurrence data related to synthetic drugs (municipality and address with latitude and longitude of seizure, macroscopic characteristics of the seized samples, and chemical substance detected) were obtained using the database of the Scientific Police of Santa Catarina. The data were tabulated in an Excel® spreadsheet, filtering the latitude and longitude of the item of interest and, through these two geographic points, grouping each line of the table, highlighting it on the map. The data obtained was exported to create maps on Google My Maps®. **Results:** In the period from 2016 to 2020, in the northern region of the State of Santa Catarina, 631 seizures of synthetic drugs in pills, blotters, powder, or crystals were recorded. 17 different substances were detected where only one compound was determined in the product and 35 different substances with a mixture of two compounds or more, including classic drugs (MDA and MDMA) and new psychoactive substances (cathinone, methamphetamine, and tryptamines). The most seized substances and their most seized formats were used to test the georeferencing tool. The application of georeferencing was carried out in the North region of Santa Catarina in the seizures with the substance MDA and design of “skull” and the substance MDMA and design of “Netflix”. A pattern was observed in seizures, demonstrating that most substances seized had a similar trafficking route when compared geographically. **Discussion/Conclusion:** The presence of different substances in the same seized drug reinforces the need for a continuous study to elucidate the clandestine laboratories and understand potential new substances introduced on the market. Considering the complexity of substances found applications of forensic intelligence are increasingly necessary to establish links between samples and strategies for identifying trends and patterns in a given illicit drug market. With georeferencing, it was possible to correlate the data from each seizure with the spatial aspects that can reveal a pattern, like the distribution of synthetic drugs along the coast. Thus, these data, in addition to promoting discussion about synthetic drugs seized and analyzed by the Scientific Police in Joinville, reinforce the need for continued surveys of this nature and the implementation of automated tools for data processing and issuing strategies. **Acknowledgments:** Scientific Police of Santa Catarina and Professional Master’s Program in Pharmacology of the Federal University of Santa Catarina (UFSC).

Prevention of false negative results of 11-nor-9-carboxy-Δ-9-tetrahydrocannabinol in urine by replacing HPTLC by GC-MS/MS

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Introduction: The most consumed illegal drug in Brazil is marijuana. It is important for criminal investigation to determine the use of this drug by victims of rape, car accident or violent death. In the legal medical Institute of Belo Horizonte (IML/BH), Minas Gerais, Brazil, a preliminary analysis of cannabinoids was carried out in the urine by immunoassay, if the result was positive it was confirmed by high performance thin layer chromatography (HPTLC). The limit of detection (LOD) reached by the HPTLC was 70 ng/ml for 11-nor-D9-tetrahydrocannabinol carboxylic acid (THCCOOH), the main metabolite of marijuana. This LOD is above that suggested by the UNODOC in the "Guidelines for the Forensic analysis of drugs facilitating sexual assault and other criminal acts" which is 10 ng/ml. It was possible to reach the LOD of 10 ng/ml with the use of gas chromatography coupled with mass spectroscopy with a triple-quadrupole mass filter (GC-MS/MS). **Objective:** The objective of this study was to reach the LOD of 10 ng/ml for THCCOOH and to reduce by five the volume of urine and solvents needed to carry out the extraction, which brings less generation of toxic and flammable materials. It was in accordance with the new concepts of green chemistry. **Methods:** A volume of 5 mL of urine was aliquoted into a 50 mL plastic tube, added 0.3 mL of 10M KOH and vortexed for 5 seconds. The solution was placed in an ultrasound bath at 55°C for 15 minutes and left to cool down for another 15 minutes. In the solution was added 0.4 mL of concentrated acetic acid and 3 mL of 50 mM phosphoric acid, was checked the pH, which was between 4 and 5. In the solution was added 5 mL of n-hexane, vortexed for 5 seconds, stirred for 30 minutes in the inversion homogenizer with around 70% of maximum speed, centrifuged at 5000 rpm for 15 minutes. After centrifugation, the organic phase was collected and evaporated to dryness using a sample concentrator at 45°C. The residue was resuspended with 150 µL of hexane and the extract was analyzed by HPTLC. The procedure described above was used for GC-MS/MS analyses, reducing the amount of urine and solvents by 5 times. After centrifugation, the organic phase was collected and evaporated to dryness and was derivatized with 50 µL of BSTFA-1%TMS at 70 °C for 20 minutes, the derivatized extract was injected into GC- MS/MS. **Results:** A total of 1189 urines were evaluated with the immunoassay from January 1 to October 29, 2021 and 181 urines were positive for cannabinoids. A total of 171 exams were confirmed in the analysis by HPTLC. The 10 cases that obtained negative or doubtful results were analyzed by GC-MS/MS and a positive result was obtained avoiding the production of false-negative results. **Conclusion:** It was possible to reach the LOD of 10 ng/ml for THCCOOH and to reduce by five the volume of urine and solvents used. After this result, the HPTLC was replaced by GC-MS/MS in the analysis carried out in IML/BH. **Acknowledgments:** This study was carried out with the support of the Civil Police of Minas Gerais.

Qualitative chromatographic comparison of analyte presence in multiple pharmaceutical forms of ayahuasca

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Introduction: Ayahuasca is a south american psychoactive beverage used in religious rituals, with its main active components being the β-carboline alkaloids, harmine (HRA), and harmaline (HRL), known for their monoamine oxidase inhibitory properties, in addition to the potent hallucinogen N,N-dimethyltryptamine (DMT). Despite its growing consumption, information regarding its clinical applications and toxicological effects remains limited. This study proposes a qualitative analysis, using GC-TSD, of the chemical composition of ayahuasca tea and its ointment, as well as the presence of these analytes through different administration routes of ayahuasca. **Objective:** Conduct a qualitative evaluation of the chromatograms generated after the extraction of ayahuasca tea and ointment, in addition to analyzing the extraction of biological samples. **Methods:** For this analysis, a method previously developed by the research group was employed, optimized for the use of a mini centrifuge and eppendorf tubes. With the eppendorf tubes loaded with samples, internal standard, and extraction solvents, vortex agitation was performed for 1 minute, followed by centrifugation in a mini centrifuge at 6000 rpm for 5 minutes. The eluate was evaporated under a nitrogen flow at 35°C. Separation was carried out on the fused silica capillary column SLB-5MS (15 m X 0.25 mm X 0.25 µm) in a gas chromatograph coupled to the thermionic specific detector (GC-TSD). **Results and Discussion:** Chromatograms revealed distinct absorption patterns between the ointment formulated from ayahuasca tea and the tea itself, indicating greater absorption in the topical administration of the ointment. Notably, DMT demonstrated more prominent absorption in the ointment, suggesting a potentially higher concentration of DMT in ointment form, influencing physiological responses to the compound. Additionally, the investigation of blood absorption through three different routes (dermal, oral, and inhalation) showed results below the detection and quantification limit of the employed method, making it impossible to determine the concentration of the analytes. Future results aim to enhance sample preparation and adapt chromatographic conditions to validate the qualitative method. **Conclusion:** These results highlight the need for methodological adjustments to improve the sensitivity of blood sample analysis, aiming to more comprehensively elucidate the effects and safety of different ayahuasca administration routes. Therefore, the discussion regarding the potential toxicity of each administration route is constrained by the detection limits of the analytical method. **Acknowledgments:** I express gratitude to laboratory colleagues for their support, to Professor Bruno Spinosa de Martinis for trust and encouragement, and to the Department of Chemistry at FFCLRP-USP.

Quantification of synthetic drugs of abuse in seized tablets by Direct Analysis in Real Time-High Resolution Mass Spectrometry

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Introduction: The growing popularization of a wide range of synthetic drugs known as New Psychoactive Substances (NPS) has been a serious threat to global health. Accompanying the increase in the diversity of structures on the market, there is an increase in the diversity of substances simultaneously present in each matrix (e.g., tablets), which inevitably leads to an expansion of the concentration dynamic range within a single sample. Consequently, combating NPS requires increasingly scientific efforts focused on toxicological studies. The pace of market changes in NPS is scarcely matched by the speed at which research laboratories can identify and quantify these substances. In this context, Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART-HRMS) can represent a faster alternative for the identification and quantification of drugs of abuse in forensic matrices. **Objective:** Quantify synthetic drugs of abuse in seized tablets in the state of Rio de Janeiro using the DART-HRMS technique. **Methods:** Ten seized tablets were provided by the Civil Police of the State of Rio de Janeiro. The tablets were extracted and subsequently analyzed using DART-HRMS (Orbitrap analyzer). Analyses were conducted by depositing 10 µL of the solution onto a QuickStrip™ sample card and positioning the card between the DART source and the mass spectrometer. Prior to analysis, the tablet extract was prepared by macerating the solid matrix, separating 150 mg, and extracting it with 1.0 mL of methanol by vortexing for 10 s. Subsequently, the mixture was centrifuged at 2,000 rpm for 4 min, and the supernatant was diluted by a factor of 500. **Results:** Five different synthetic drugs and one adulterant (caffeine) were quantified in the analyzed matrices: MDMA (in the concentration range of 0 – 347 milligrams per gram of tablet), MDA (53 – 254 mg g^{-1}), Amphetamine (133 – 160 mg g^{-1}), Methamphetamine (12 – 354 mg g^{-1}), 25I-NBOMe (60 – 80 mg g^{-1}), and Caffeine (60 – 140 mg g^{-1}). Calibration curves used for quantification were constructed (concentration dynamic range of 0 – 125 µg mL-1) using certified standards and exhibited the following parameters: MDMA (R2 = 0.9969, p-value = 0.00007), MDA (R2 = 0.9811, p-value = 0.0011), Amphetamine (R2 = 0.9800, p-value = 0.0012), Methamphetamine (R2 = 0.9946, p-value = 0.00017), 25I-NBOMe (R2 = 0.8748, p- value = 0.019), and Caffeine (R2 = 0.9562, p-value = 0.0039). **Discussion and Conclusion:** The concentrations found are within the expected ranges reported in the literature for each analyte individually. However, it is worth noting that the majority of analyzed tablets contained not one but a mixture of various substances, each at different concentration values and with distinct pharmacological effects (e.g., hallucinogens alongside stimulants), which can further increase the dangers associated with their consumption. This highlights the complexity of the challenge that needs to be addressed by toxicological studies in the face of the dynamic nature of the current synthetic drug market. In conclusion, DART-HRMS presented itself as an alternative technique with promising potential for combating NPS in the toxicological context, enabling the rapid detection and quantification of synthetic drugs of abuse in tablet extracts. **Acknowledgments:** FAPERJ, CNPq, CAPES.

Review of literature: the hair fiber as a biological matrix for the analysis of psychoactive substances in toxicology

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Introduction: The process for analyzing the use of psychoactive substances involves three stages: pre-analytical (preparation and collection), analytical (processing using specific equipment), and post-analytical (interpreting results for a comprehensive diagnosis). The choice of biological matrix for analysis should consider the peculiarities of each phase, and it is essential to strictly follow the laboratory instructions to ensure the quality of the samples. Matrices such as blood, serum, urine, gastric content, and hair can be used in toxicological investigations. Hair, due to its extended detection window, has become prominent in these investigations, offering non-invasive collection, ease of transportation, storage, and prolonged stability. The growth and segmentation of hair strands allow for the creation of a timeline to analyze the history of exposure to xenobiotics, with drugs gradually incorporated throughout the hair's lifecycle. **Objective:** The present work aims to describe why hair fibers are more efficient for toxicological analyzes with a wide detection window. It explores how psychoactive substances are retained in the chosen matrix and finally, it points out why toxicological tests have become mandatory to obtain a driver's license (CNH). **Methods:** This work is a literature review that resulted in a descriptive research using theoretical foundations, including academic articles, dissertations, and scientific journals available both online and in print, obtained in both Portuguese and English languages. The obtained data will be interpreted and applied to the context of forensic toxicology. **Results:** Drug tests are ordered for a variety of reasons, including legal issues, occupational safety, and driver's license renewal. Once the purpose of the exam has been determined, the necessary matrix can be chosen. Urine indicates recent consumption, while hair offers long-term information. Therefore, for examinations with a wide detection window, hair emerges as a more suitable choice, offering a variety of analytical techniques such as GC-MS, HPLC-MS and capillary electrophoresis. After reviewing more than sixteen articles, it was concluded that the individual physiological characteristics of each person have a great influence on the incorporation of toxic components, therefore, it is essential to take some special care and compare the data obtained with those in the "cut-off" table . "to ensure the accuracy of the results. In March 2016, an executive order was instituted requiring toxicological tests to be carried out for the renewal and classification of driver's licenses in categories C, D and E, as well as for the hiring and firing of professional drivers. This measure seeks to increase traffic safety and reduce accidents, given the frequent use of psychoactive substances by many drivers in these categories to deal with the demands of work. This need was evidenced by a study carried out in MG, where 61% of drivers used amphetamines during trips and 54% were under the influence of these substances near highways. **Conclusion:** The capillary expansion process occurs in three phases (anagen, catagen and telogen), and absorption of the medication generally occurs through the bloodstream, sweat and sebaceous secretions. To ensure the accuracy of results in hair toxicology tests, measures are taken to minimize failures, such as decontamination to remove external interferences. Cutoff values are established for each analyte, considering that chemicals can reduce drug concentration. Hair is widely recognized as an effective matrix due to its non-invasive collection, ease of transportation and storage, although it has low efficacy in short-term drug detection. **Acknowledgments:** We extend our gratitude to the Universidade Paulista (UNIP) and DB Toxicologico.

Soundwave substances: exploring drug consumption through hair analysis at electronic music festivals

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Background: Recently, there has been a concerning surge in the consumption of psychoactive substances, particularly at electronic music events, posing a substantial challenge to public health. In response, the Substance Consumption Information Research (SCIRe) project emerges as a guiding light, aiming to uncover patterns and trends in drug consumption through advanced toxicological analysis techniques. The selection of the hair matrix for analysis is deliberate, given its remarkable capacity to delineate a timeline of drug usage. Our mission is to dispel the opacity surrounding this consumption and advocate for the implementation of harm reduction policies, fostering a safer and more informed society. **Objective:** This study aims to investigate the prevalence and patterns of psychoactive substance consumption among attendees of electronic music festivals, utilizing hair analysis as a novel approach. **Methods:** Volunteers were enlisted at an Electronic Music Festival in Rio Grande do Sul, Brazil. Hair samples were obtained (Ethics Committee number CAAE: 64674122.0.0000.5345) by cutting a pencil-thickness section from the posterior vertex region of the head and securely wrapped in individually labeled foil pouches enclosed in paper envelopes. Each participant completed a questionnaire comprising 22 inquiries ranging from demographics to drug usage habits, accompanied by requisite consent forms. Hair samples underwent methanolic extraction for 16 hours, followed by a clean-up step and identification via liquid-chromatography tandem mass-spectrometry (LC- MS/MS). **Results:** Among all samples collected (n=30), 26 tested positive for at least one of the 14 substances examined. Nicotine (n=22), cocaine (n=19), and MDMA (n=10) emerged as the top three detected substances. Sociodemographic findings from the pilot study revealed an equal gender distribution (50% female, 43.3% male, and 6.3% undisclosed) with volunteers averaging 30.5 ± 4.8 years of age, 70% of whom had completed higher education. Moreover, 86.7% reported recreational use of psychoactive substances within 24 hours preceding collection. This is a pilot study, still undergoing research, to expand the drug panel to NPS as well. **Discussion/Conclusion:** A notably high positivity rate for one or more psychoactive substances (26 out of 30) was observed. Notably, nicotine, cocaine, and MDMA, stimulants of the central nervous system, exhibited the highest positivity rates. This underscores the prevalent occurrence of substance abuse at electronic music festivals and emphas the importance of formulating effective harm reduction measures and safe usage guidelines to benefit users and promote safer festival environments.

Synthetic cannabinoids (k-drugs) mixed in herbal fragments: a new strategy for purification and identification

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Introduction:Currently, the emergence of New Psychoactive Substances (NPS), including synthetic cannabinoids (SCs) – popularly known in Brazil as K-drugs, represents one of the main challenges for forensic professionals. **Objectives**:Due to the unavailability of standards for the NPS, there is great difficulty for the scientific police to identify them, especially in cases in which the drugs are incorporated in herbal fragments. So, the aim of the work is to develop a new strategy to purify synthetic cannabinoids present in herbal fragments to make possible their further identification by mass spectrometry and NMR. **Materials and Methods**: SCs impregnated in herbal fragments, previously seized by the STSP-IC of São Paulo, were purified using a developed extraction procedure followed by an optimized semi-preparative HPLC method, and confirmatory techniques (NMR, Q-TOF) to identify the substances purified. **Results**: The developed new strategy to purify SCs sprayed in herbal fragments was applied in actual drug samples seized by São Paulo Police. With this strategy, it was possible to purify and characterize MDMB-4en-PINACA, ADB-BUTINACA, MDMB-INACA, and 5F-ADB. Also, it was possible to identify, for the first time in Brazil, ADB-INACA, an SC not listed in any forensic library as well as it was not included in the Brazilian list of prohibited substances (Portaria 344). **Conclusions**: Proving to be effective and simple, the purification that enabled the correct identification of the substances contained in plant samples, the developed strategy can be used in the future by the scientific police to identify other SCs present in herbal fragments and even to produce homemade chemical standards for the easier identification of SCs in further apprehensions.
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Synthetic drugs and new psychoactive substances detected in the state of Ceará, Brazil

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Introduction: New psychoactive substances (NPS) are synthetic chemical substances that are designed to produce psychoactive effects, such as changes in perception, mood, cognition or behavior. NPS are a subset of designer drugs. They are new drugs that have not been regulated and are not well understood. They can be found online as well as in physical stores, and present a potential risk due to the unpredictability of their effects. On the other hand, synthetic drugs (SD) are substances designed to mimic the effects of classic drugs such as marijuana, cocaine and ecstasy. They are generally produced in clandestine laboratories and are considered a health risk, since their effects are not fully known. These two classes of substances can have unpredictable effects and can lead to overdose, addiction, withdrawal and interactions with other drugs or medications, which means they are a rising concern for public health authorities. It is therefore of the utmost importance to know the types of substances that are circulating in the various regions of the country in order to develop prevention and control strategies. **Objective:** To ascertain the prevalence of new psychoactive substances and synthetic drugs in the state of Ceará between 2020 and 2023 detected at the Forensic Expertise of Ceará (PEFOCE). **Methodology:** The data was obtained from the reports produced at the Forensic Toxicology Center - NUTOF of PEFOCE for the identification of synthetic drugs. All samples were analyzed using the gas chromatography technique coupled to a mass spectrometer (GC 7890A - MS 5975C Agilent®). **Results:** The detection of new psychoactive substances (NPS) and synthetic drugs in the state of Ceará showed a downward trend between 2020 and 2023. The most prevalent substance was MDA, accounting for 43.1% of all substances detected. MDMA was the second most prevalent, with 32.4%. LSD-25, Clobenzorex, 2C-E and Amphetamine were detected in significantly lower proportions, accounting for 5.3%, 4.3%, 2.7% and 2.7% respectively. The substances 25E-NBOMe, 2C-B, 25I-NBOMe, 2C-C, Ketamine and Methamphetamine were found in even smaller quantities, ranging from 1.1% to 2.1% of the total substances detected. The least prevalent substances were 25B-NBOMe, 25H-NBOMe and Eutilone, each representing just 0.5% of the total. The substances were categorized into three forms of presentation: Crystal/Granular Powder, Seal and Tablet (COMP). The most prevalent form of drug presentation was tablets (71.0%), followed by seals (16.0%) and crystal/powder (13.0%). **Discussion/Conclusion:** The most prevalent substances were MDMA and MDA, this data indicates that they are the most commonly used or distributed substances in the region during the study period. The significant prevalence of synthetic drugs in relation to NPS suggests the need for more sensitive and comprehensive detection methods. The presence of NPS, although fewer in number, indicates the eminence of new substances on the recreational drug scene. It is therefore essential to continue monitoring and studying these substances in order to better understand their effects and associated risks. The results of this study contribute to a better understanding of the Brazilian NPS and synthetic drug landscape, providing evidence for the development of effective control and prevention strategies and actions to protect the health of the population.

Toxicological analysis of synthetic opioids in bones: Introducing new animal models for blood-bone correlation

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Introduction: In 2021, 75.7% of overdose deaths were due to opioids in the U.S. Many of these deaths were attributed to novel synthetic opioids. Synthetic opioids are particularly dangerous considering that most of them are extremely potent requiring lower concentrations to be toxic. Their analysis in biological samples such as blood and urine is already very challenging. In the case of extreme body decomposition, the only matrix available for toxicological analyses is bone tissue. Due to the time-consuming extraction methods and the lack of correlation between drug concentration in bones and blood, bones are currently not considered a good forensic toxicology sample. **Objectives:** The aim of this study was to study the presence of fentanyl in bones using New Zealand White male rabbits as the experimental model, for further bones:blood correlation studies. To achieve this goal, a faster extraction method coupled with GC-MS analysis was developed and validated. **Methods:** A total of 12 New Zealand White male rabbits were used in this study. Animals were divided into two groups (n=6 per group) and exposed intravenously to NaCl 0.9% (control group), and fentanyl 200 mg/Kg. The injections were performed twice a week for 8 consecutive weeks. Animals were monitored daily and euthanized 24 hours after the last injection. Each animal was divided in half and bone samples from the scapula, ribs, and femur were collected fresh after death. The remaining bones were left to decompose for 4 weeks in an open field. After complete decomposition, the scapula, ribs, and femur were collected for fentanyl analysis. A previously validated method was used for the extraction of all the fresh and decomposed bone samples. For this extraction, 500 mg of bone samples were mixed with methanol and ISTD and homogenized using the Bead Ruptor. Samples were then centrifuged and the supernatant was subject to SPE for further clean-up. For quantitative analysis, an ANSI/ASB 036 validated GC-MS method was utilized. The quantitative analysis was performed in selected ion monitoring (SIM) mode and fentanyl was identified by its designated ions. **Results:** The results of the study showed the presence of norfentanyl in rabbit bones after 8 weeks of treatment. Due to the complexity of the bone matrix, a further LC-MSMS study will be performed for the complete evaluation of the possible compounds present in the bone after the treatment. **Discussion/Conclusion:** Bones are important forensic matrices especially when no soft tissue is available. Rabbits were chosen due to their bone metabolism similarity with humans, a characteristic not shown in rodents. The use of rodents as animal models can be the reason for several disparities in the literature data about blood-bone correlations using different drugs. The presence of the major fentanyl metabolite in bones confirms the capacity of bones to be a reservoir for opioids and consequently to be a good forensic sample in cases with massive body decomposition. In addition, the developed method showed to be fast and reliable and could be applied by forensic laboratories routinely. Given the increase in illicit synthetic opioid cases, it is of forensic interest to develop a method to detect these substances in alternative biological matrices, especially to be applied in cold cases. **Acknowledgments:** The authors thank Aesha Patel, Julia Perschbacher, and the members of the Experimental Toxicology Research Laboratory for their help with the collection of the samples.

Toxicological effects of MD-NBOH in zebrafish: assessing acute and chronic impact on different life phases

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The abuse of New Psychoactive Substances (NPS), synthetic or semisynthetic recreational drugs reported worldwide, poses a significant challenge to public health systems. Despite this, studies on their effects are lacking, especially on animal models. In the phenethylamines class, which includes NBOHs compounds, knowledge is particularly limited. To expand our understanding of the effects of these compounds, this study aimed to assess the toxicity of MD-NBOH, a phenethylamine, on both adult and larval stages of Zebrafish (Danio rerio) (ZF) following acute and chronic exposures lasting 96 hours. The assays were conducted following the standards described in NBR 15088:2016 for adult fishes and NBR 15499:2015 and OECD 236/2013 for newly hatched ZF larvae. In both assays, the test organisms were randomly subdivided into groups: (1) Control (dilution solution) (ADULT: 1000 mL/N=5; LARVAE - 250 mL/N=10); (2) MD-NBOH: (ADULT - 20 mg/L; LARVAE - 2 mg/L - both subdivided into subgroups corresponding to 6 serial dilutions of the solution - 100, 50, 25, 12.5, 6.25, and 3.12%, respectively) ADULT: total volume of 1000 mL/N=5; and LARVAE - final volume of 250 mL, N=10/subgroup. The adult assay recorded mortality and behavioral changes indicative of toxicity every 24 hours for 96 hours. The larval assay evaluated lethality and morphological changes indicative of toxicity daily, expressed as a percentage in each sample. The results were assessed with a confidence level of 95% in all experimental groups ($p<0.05$, ANOVA - Graph Pad Prism 8.0). The preliminary results for adult ZF showed no mortality in the control group; however, lethality was observed in all components of the system at the 100% MD-NBOH dilution at the end of the assay (TF = 1; $p<0.05$, ANOVA). During the experiment, in the test with adult fish, cases of total or partial loss of balance, aggressiveness, convulsions, and erratic swimming were observed in fish from 100% dilution (tf= 1; $p<0.05$, ANOVA compared to control) among 24 and 48 hours. Agitation was observed at the 50% dilution, and in the other dilutions, no behavioral changes indicative of toxicity were observed. These behavioral changes are very similar to the clinical signs of intoxication described in humans. In the larval test, there was no mortality in any group during the experimental period, with only a slight increase in larval locomotor activity being observed in the 100% group and, under optical microscopy, morphological changes such as tail deformation and scoliosis in different concentrations. In conclusion, MD-NBOH produced chronic lethality only at 100% dilution in adult ZF. Furthermore, in the adult ZF test, behavioral changes indicative of toxicity were observed mainly at higher concentrations of MD-NBOH. In the larval stage, MD-NBOH did not produce lethality, however, morphological changes indicative of toxicity were observed in all dilutions evaluated.
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Toxicological findings in a fatal multidrug intoxication involving cocaine: case report

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Cocaine (COC), considered the most potent natural stimulant, is extracted from the leaves of the coca plant (Erythroxylum coca), and remains one of the most common causes of acute drug-related emergency department visits in the United States. COC is well absorbed following contact with the oral, nasal, gastrointestinal, rectal, and vaginal mucosa, or through the pulmonary alveoli after inhalation. The vasoconstrictive properties of COC extend the absorption rate and delay its maximum effect when absorbed by mucous surfaces. The bioavailability of COC is approximately 90% when smoked and around 80% after intranasal use. We present a case report of suspected multiple drug intoxication associated with COC use in a 22-year-old female. History of COC and alcohol use throughout the night and early morning, she arrived at the hospital around 8:00 AM, brought in by firefighters in a cardiopulmonary arrest (CPA) state. Resuscitation procedures were performed for about 2 hours using medication and defibrillation; during intervals, the patient exhibited a heartbeat three times, but subsequently went into a new CPA, resulting in death. Post-mortem examination revealed a significant amount of free blood in the abdominal cavity, a large infrahepatic right paravertebral hematoma with vena cava injury and blood drainage. No external lesions or signs of aggression were found. Qualitative toxicological analysis of a whole blood sample detected the presence of COC, anhydrous methylecgonine (AEME), methylecgonine (EME), cocaethylene (CE), bupropion (antidepressant), and sertraline (antidepressant). In the urine sample, COC, AEME, EME, CE, bupropion, sertraline, and diphenhydramine (antihistamine) were found. Gastric content analysis revealed COC, AEME, EME, CE, and bupropion. COC was quantified in whole blood as above 50.0 µg/mL. According to medical reports, the individual arrived with dilated pupils and pale lower extremities, clear signs of acute intoxication. COC induces a dose- dependent increase in heart rate and blood pressure, accompanied by heightened excitement, improved performance in surveillance and alertness tasks, and a sense of self- confidence, euphoria, and well-being. Typically, its use is followed by a desire for more drug. COC produces target organ toxicity in almost all body organ systems, primarily through its hemodynamic effects. At elevated blood concentrations, COC's negative inotropic effects can cause acute depression of left ventricular function and heart failure. COC can induce supraventricular and ventricular arrhythmias through direct actions on myocardial receptors or as a complication of myocardial ischemia. Toxic effects resulting from COC consumption can be detected at blood concentrations between 250 ng/mL (0.25 µg/mL) and 500 ng/mL (0.5 µg/mL). Ethanol (ethyl alcohol) was not reported in the blood; however, the presence of CE was observed in all analyzed samples. CE is considered more toxic to the cardiovascular and hepatic systems when compared to COC, with a longer plasma elimination half-life (approximately 2 hours) than COC (around 1 hour). Additionally, the use of COC combined with other drugs, such as antidepressants and antihistamines, produces toxic effects at a lower dose. In this specific case, the presence of various substances was observed, indicating intoxication by multiple drugs, where the lethal concentration of COC in the blood could be below 1.0 mg/L or 1.0 µg/mL. The measured COC concentration in the sample in question was at least 50 times higher than the lethal dose in similar cases. The injuries found in the autopsy examination are consistent with injuries caused by CPR maneuvers present in up to 80% of cases, as extensively documented in the literature.

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TOXICOLOGIA OCUPACIONAL

Benzene and toluene exposure assessment: individual-related confounding factors

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Background: Benzene and toluene are organic solvents of great importance to public health, with a stronger presence in large urban centers and in the occupational environment. As they pose a risk to health, they have a control required by law for the concentration in the work environment and for the levels of exposure biomarkers. However, factors related to the individual can confuse the interpretation of the results of the exposure assessment. **Objectives:** In this study, these confounding factors will be addressed, describing the form of interaction, in order to better control these variables in the studies, obtaining more representative results of occupational exposure. **Methods:** This review study was carried out using the PubMed, Cochrane, Scielo and ScienceDirect databases. The selection criteria were articles published between 2013 and 2023, full articles, published in English and Portuguese, related to environmental and occupational exposure. The descriptors used were "benzene", "toluene", "exposure", "biomarkers", "confounding factors", "ortho-cresol", "phenylmercapturic acid", "trans, trans-Muconic acid". **Results:** It was possible to perceive an absence of a pattern of the factors taken into consideration in the exposure assessment studies: more frequently, smoking, consumption of alcoholic beverages, and of the food preservatives sorbic acid and benzoic acid were evaluated. Other studies took into consideration additional factors, such as genetic polymorphism, workplace/residence and work shift. **Conclusion:** The collection of information through a well-directed questionnaire is essential. The recognition of confounding factors in exposure assessments led to clearer and more representative results, as by taking into account the interference of confounding variables, there is greater assertiveness in estimating the internal dose and interpreting the data.

Combination of rural workers' exposure to pesticides and obesity as risk factors for cancer

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Background: Chronic occupational poisoning among family farm workers is a concerning and often overlooked issue. When combined with other risk factors, such as obesity, pesticide exposure can pose a significant threat to the health of both men and women. Pesticide use is necessary to control pests in crops, providing benefits in the socioeconomic sphere. However, some of these substances may have a high potential for causing mutations and cancer. When combined with chronic inflammation in the body, which promotes the excessive release of hormones and inducers of abnormal cell growth, as observed in obesity, the risk can be significantly increased and more immediate. **Objective:** This study aims to investigate the correlation between obesity and the reported exposure to pesticides among rural family farm workers in Marialva-PR. **Methods:** A cross-sectional descriptive study was conducted with 112 representatives of family farming to investigate the risk factors associated with cancer. These factors included exposure to pesticides and body mass index (BMI). The self-reported history of cancer was also included in this study. To classify the BMI results, publications from the World Health Organization (WHO) were used. All participants signed the informed consent form (TCLE) approved by the Human Research Ethics Committee (COPEP), CAAE No. 65018017.7.0000.0104, opinion No. 6,209,432. Data collection was carried out through interviews using a pre-validated questionnaire. **Results**: Data showed that 34.8% of the participants were obese (BMI above 30), 42.8% were overweight (BMI above 25), and 22.4% were within the range considered ideal (BMI between 18.5 and 24.9). Out of these 112 farmers, 4.5% reported previous cases of cancer. Among these cases, 1.8% are classified as obese, while the remaining 2.7% are overweight. The types of cancer reported were thyroid, skin, and rectal cancer, of which 2.7% had no family history. However, it is worth highlighting that all 112 workers in question reported exposure to and direct contact with pesticides for a minimum period of 12 months and a maximum of 65 years. This indicates that the population has been exposed to pesticides for a significant duration of time. **Conclusion**: Occupational toxicology is a complex study field due to the amount of substances to which workers are exposed in their daily lives. The potential health risks of these substances and the lack of knowledge about their interaction with comorbidities and unhealthy habits presented by workers can further compromising their well-being. Obesity, for example, is a pressing concern. According to the collected data, the majority of workers are obese or overweight. Many classes of pesticides have been associated with obesity because they are endocrine disruptors. Organochlorines and organophosphates are among the most commonly associated with this issue. Although the exact mechanism of their interaction is not fully explained, these substances, in addition to acting as hormone receptor agonists and antagonists, can also interact with proteins involved in the synthesis of steroid hormones. This interaction can potentially increase the risk of both obesity and neoplasms. This creates a cascade effect in which each factor enhances the other. Overall, some workers have developed cancer even without a family history. Therefore, the correlation could exist and should serve as the basis for raising awareness on a large scale. Moreover, in addition to being encouraged to use using personal protective equipment (PPE), these workers need to know the risks that certain habits can pose to their health in their current situation. **Acknowledgments:** I thanks to the State University of Maringá (UEM), the Universidade Sem Fronteiras Extension Program, the Ingá University Center - UNINGÁ, the Toxicology (LATOX) and Pathology Laboratories at UEM, and all the collaborators who have participated and are currently participating in the project.

Development and validation of a method for toluene quantification in urine by gas chromatography CG-FID, in compliance with the update of regulatory norm NR-7

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Background/Introduction: Toluene is a flammable and volatile solvent characterized by its high liposolubility. It plays a significant role in various industrial applications, including the production of rubber and its use as a diluent in paints, varnishes, glues, and adhesives. This compound is naturally present in petroleum and can be released during fuel refining processes. It requires special attention due to the potential health risks associated with exposure. In the context of workers' health, exposure to toluene can result in a variety of adverse effects, from irritation in the eyes, nose, and throat to symptoms such as dizziness and headaches. Prolonged or high-level exposures raise additional concerns about causing damage to the kidneys, liver, and impacts on the central nervous system. Therefore, it is crucial to implement continuous monitoring to ensure that exposure levels remain within established safe limits. The NR-7, a regulatory standard updated in 2020, plays a crucial role in this scenario, bringing significant changes to the biological indicator and monitoring values for assessing occupational exposure to toluene. Notably, hippuric acid, previously widely used, has been replaced by the measurement of toluene in urine, proving to be the most selective and appropriate approach for the biological monitoring of solvent exposure. This change reflects an advancement in the accuracy and specificity of evaluation methods, contributing to the safety and well-being of workers exposed to toluene. **Objective:** Develop and validate a highly sensitive method that complies with the low limits established by the current Brazilian standard for the determination of toluene in urine. **Methods:** The validation was performed using the Thermo Trace 1310 Gas Chromatograph with Flame Ionization Detector (GC-FID). To ensure analytical analysis, Toluene with a purity of 99.9% was employed as the standard, acquired from Sigma Aldrich. The sample preparation procedure involved a 2 mL aliquot of urine, placed in a headspace vial, followed by the addition of 500 µL of n-propanol internal standard at a concentration of 0.01%, and 8 g of sodium sulfate. Subsequently, the samples were eluted on a BP5 capillary column of 0.25 µm (30 m x 0.25 mm). Helium gas was employed as the carrier gas at a flow rate of 15 mL/min. Quantification was achieved by comparing the responses of a specific sample with those of calibrators of known concentrations. Linearity was observed in the expected concentration range of 0.02 to 0.15 mg/L, and urine samples were evaluated at seven different concentrations, each repeated six times during the same study period. A white urine sample, free from the studied analyte, was used as the biological matrix for the study. **Discussion/Conclusion:** The linearity coefficient (R) was 0.99222. The method showed 100% selectivity and a residual interference effect of less than 5%. %. In order to determine the average inter-assay CV%, three different concentrations were analyzed over three days, resulting in values of 8.79%, 8.04%, and 6.31% for low, medium, and high concentration levels, respectively. The precision of the method was evaluated by analyzing samples with known concentrations, expressed as a percentage. A retention time (RT) of 3.1 minutes was obtained, and the total analysis time was 5.0 minutes. The method proved to be fast and efficient for determining toluene in urine. The effectiveness and selectivity, combined with technical robustness, can be utilized as monitoring tools for occupational exposure. **Acknowledgements:** Associação Fundo de Incentivo à Pesquisa, Laboratório de Química Clínica.

Evaluation of use of pesticides, protection equipment, and prevalence of symptoms on tobacco rural workers in the south region of Brazil

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Introduction: Health issues among tobacco planters are a distinctive concern within the agricultural sector. As tobacco cultivation heavily relies on the use of pesticides to control pests and diseases, besides the dangers of tobacco exposition, the health implications for those involved in its production are notable. **Objective:** To evaluate the usage of pesticides and protection equipment, and presence of symptoms of intoxication on tobacco rural workers. **Methods:** A questionnaire was applied directly to the tobacco farmers with questions about manifestation of 45 symptoms and 13 pesticides use, and use of the 5 adequate protection equipments. **Results:** 64 farmers participated in the study. The most common symptoms described were headache, backpain, leg pain, nervousness, auditory problems, and dizziness appearing on above 50% of participants, with highest prevalence of headache, with 75% of participants presenting the symptom. Only two symptoms were not reported by any participant, bronchitis and convulsions. 81,25% of participants showed more than three symptoms, which can be classified as signs of toxicity. Of the 13 pesticides included in the questionnaire, 10 were used by more than 50% of participants, and all were used by more than 33%. The most used pesticide was RoundUp® (glifosate), used by 92% of participants, followed by Gamit® (Clomazone) and PrimePlus (flumetramine), both used by 87,5% of participants. 48,14% of participants reported usage of full protection equipment, and 17,18% claimed to use 4 out of the 5 pieces of equipment. **Conclusion:** Tobacco farmers in southern Brazil showed a high prevalence of toxicity profile despite a high report of adequate use of protection equipment. The reported number of proper protections might be questionable due to the high number of intoxications. Rural workers should be educated on the responsible use of pesticides and use of protection equipment to reduce the cases of toxicity. **Acknowledgements:** CAPES and CNPq.

Importance of establishing biomarkers of exposure to atmospheric pollution and pesticides

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Introduction: Occupational exposure to air pollution and pesticides is a relevant topic that involves risks and challenges associated with each profession. Additionally, the absence of biomarkers in Brazil to monitor the levels of these exposures is a significant gap that still needs to be filled to enable early detection of diseases after exposure to these chemicals. **Objective:** The aim of this study was to investigate workers occupationally exposed to different chemical substances, quantifying exposure biomarkers and analyzing the updated NR7 (2020-2022). **Methods:** Participants in this study included workers exposed to air pollution (n=85), farmers (n=68), those exposed to benzene (n=67), and a group not exposed to these substances (n=67). This study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Sul/RS (CAAE: 69865417.1.3003.5346), and all participants signed informed consent forms. Whole blood (heparin) and urine were collected from all participants. For the quantification of 1-hydroxy-pyrene, the urine sample was diluted and subjected to enzymatic treatment using β-glucuronidase. Subsequently, it underwent solid-phase extraction using specific cartridges, finally reconstituted in methanol. The sample injection was performed in a high-performance liquid chromatography system equipped with a fluorescence detector. Results were expressed in ng/g of creatinine. Acetylcholinesterase (AChE) was quantified in whole blood, determined by visible spectrophotometry, and results expressed per UL–1. Butyrylcholinesterase (BuChE) was analyzed using a commercial kit, in plasma, using the BS-120 biochemical analyzer. Urinary trans, trans-muconic acid (TTMA) was extracted in specific solid-phase extraction cartridges and quantified by liquid chromatography with ultraviolet detection (UV). **Results/Discussion:** Individuals exposed to air pollution (1.30 µmol/mol creatinine) showed significantly higher ($p<0.0001$) mean results of 1-hydroxy-pyrene than the non-exposed group (0.50 µmol/mol creatinine). However, NR7, the occupational health regulatory standard, does not include any biomarkers for exposure to air pollution. Individuals exposed to pesticides had significantly lower AChE ($p=0.0002$) and BuChE ($p=0.007$) values than the non-exposed group, resulting in enzyme inhibition. Organophosphates and carbamates, mainly, inhibit cholinesterase activity but are nonspecific and do not reflect exposure to different types of pesticides used in agriculture. Additionally, exposure to pesticides also lacks established biomarkers in NR7. Those exposed to benzene had mean TTMA values of 458.15 µg/g of creatinine, significantly higher than the non-exposed group (85.83 µg/g of creatinine). TTMA is considered a biomarker for benzene exposure by NR7, with biological exposure indicators (BEI/EE) above 750 µg/g creatinine, a value higher than that established by the American Conference of Governmental Industrial Hygienists (ACGIH), which determines BEI=500 µg/g creatinine. Although still within the reference value, exposed individuals had results 5 times higher than non-exposed. **Conclusion:** For the substances discussed in this study, it was observed that only benzene exposure has an established biomarker in Brazilian regulations, but with a BEI value higher than determined by ACGIH. It is of utmost importance that regulatory authorities establish biomarkers and verify BEI concentrations that are genuinely safe for controlling these exposures and thus prevent health damage to these workers. **Acknowledgments:** The authors express their gratitude to all the subjects who volunteered to participate in this study and collaborators.

Nickel: allergic contact dermatitis and occupational aspect

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Background: Occupational dermatoses represent most occupational diseases, with 90% being cases of occupational allergic contact dermatitis (OACD). Its prevalence is complex to assess, due to self-treatment and underreporting. Globally, nickel is the main allergen. **Objectives:** To estimate the prevalence of nickel ACD among patients in Rio de Janeiro, co-sensitization, sex, affected anatomical portions and associated occupations. **Methods:** Hospital-based cross-sectional study, using medical records from two dermatology outpatient clinics in the city of Rio de Janeiro, from 2000 to 2008. The inclusion criterion was being 18 years old or over, complete Brazilian path test by these health centers. Exclusion criteria were incomplete results for contact testing with the Brazilian set test and lack of information on occupation. study approved by the human research ethics committee opinion 1.828.833. **Results:** 1465 valid protocols were analyzed. The prevalence of nickel CAD was 28%. Nickel ACD was associated with women over 40 years old, with hand disease, four times more likely to be sensitized to cobalt. Six occupations were analyzed for ACD, one of which showed a protective effect. **Conclusions:** assessment of nickel as an occupational allergy is complex, affected by multiple exposures and lack of knowledge about the composition of work instruments. The results highlight the influence of wet work and access to technology on nickel OACD.

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TOXICOLOGIA POSTMORTEM

Analytical insights into psychoactive substance presence in postmortem blood: a three-year study in Porto Alegre, Brazil

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Background: The Division of Postmortem Inspection, a vital component of the National Public Health System, plays a key role in clarifying deaths occurring under suspicious conditions, whether natural or not. The expansion of this service in 2020, with the establishment and improvement of units, including one in Porto Alegre, Brazil, underscores its growing significance. Postmortem investigations often rely on toxicological examinations, especially in cases where the cause of death is inconclusive. Analyzing biological samples in postmortem situations poses a significant challenge for toxicologists, requiring the use of sensitive and specific methodologies. Concurrently, analytical toxicology is crucial in forensic cases, particularly those involving psychoactive substances. Examining the presence of toxicants demands advanced analytical methods, such as liquid chromatography coupled with mass spectrometry, to thoroughly scan samples for various substances linked to fatalities. This emphas the essential need for sophisticated methodologies to unravel the complexities of investigating deaths under suspicious circumstances. **Objective:** Detect, identify, and quantify psychoactive substances in postmortem blood specimens collected from the Associação Hospitalar Vila Nova in Porto Alegre. Investigate the influence of these toxicants on the causes of death. Additionally, analyze the toxicological data generated over the past three years of findings. **Methods:** These analyses were made in postmortem blood samples, prepared through a simple protein precipitation method, and injected in a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system. For sample preparation, 100 µL of postmortem blood was mixed with 90 µL of NH4Cl 0.15 M. Subsequently, 400 µL of cold acetonitrile was introduced into the mixture. The resulting solution was subjected to vortexing, followed by centrifugation, drying, and eventual resuspension for subsequent analysis using LC-MS/MS instrumentation. A Nexera UFLC system coupled to a LCMS-8045 triple quadrupole mass spectrometer (Shimadzu, Japan) was used for the analysis. The analytical method was developed for the detection of 67 substances, including drugs of abuse and pharmaceuticals such as anesthetics, antidepressants, sedatives, and anxiolytics. **Results:** A total of 178 samples were successfully analyzed using this simple and reliable analytic methodology (n=75 in 2021, n=63 in 2022, n=40 in 2023). Over the three-year period, 25.84% of the samples tested positive for at least one psychoactive substance (n=14 in 2021, n=22 in 2022, n=10 in 2023). Thirty one substances were identified as follows: aminoclonazepam (n=2, 0.070 - 0.519 µg/mL); acetaminophen (n=1, 0.058 µg/mL); AEME (n=1, 0.002 µg/mL); alprazolam (n=1, 0.138 µg/mL); amitriptyline (n=9, 0.031 - 0.203 µg/mL); benzoylecgonine (n=13, 0.004 - 1.750 µg/mL); carbamazepine (n=6, 0.031 - 5.622 µg/mL); chlorpheniramine (n=4, 0.001 - 0.138 µg/mL); chlorpromazine (n=2, 0.121 - 0.128 µg/mL); cocaethylene (n=4, 0.006 - 0.359 µg/mL); cocaine (n=12, 0.003 – 0.166 µg/mL); desipramine (n=1, 0.770 µg/mL); diazepam (n=5, 0.062 – 0.460 µg/mL); diphenhydramine (n=1, 0.026 µg/mL); Ecgonine methyl ester (n=7, 0.027 – 0.261 µg/mL); phenobarbital (n=1, 27.41 µg/mL); fluoxetine (n=4, 0.026 – 0.286 µg/mL); haloperidol (n=2, 0.003 – 0.009 µg/mL); hydroxycocaine (n=1, 0.001 µg/mL); imipramine (n=1, 1.29 µg/mL); lidocaine (n=7, 0.025 – 1.597 µg/mL); midazolam (n=1, < 0.001 µg/mL); norcocaine (n=1, 0.002 µg/mL); nordiazepam (n=6, 0.010 – 0.270 µg/mL); norfluoxetine (n=3, 0.030 – 0.653 µg/mL); nortriptyline (n=9, 0.023 – 0.198 µg/mL); promethazine (n=5, 0.013 – 0.093 µg/mL); and zolpidem (n=1, 0.067 µg/mL). **Discussion/Conclusion:** Considering these findings, there can be an influence of the use of drugs of abuse and pharmaceuticals in deaths that occur without traumatic events. Nevertheless, these data imply that the prevalence of the use of these substances in the population may be underestimated, as epidemiological data typically relies on information from poison control centers and forensic medical institutes.

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Development and optimization of a green analytical toxicology (GAT) method: supramolecular solvent (SUPRAS) microextraction for the analysis of benzodiazepines in vitreous humor

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Background/Introduction: The toxicological analysis of post-mortem samples in forensic casework is essential to investigate the presence or absence of drugs in instances of suspected intoxication. The vitreous humor (VH), a gelatinous substance that fills the posterior segment of the eye, has stood out as an alternative biological matrix in forensic investigations due to the existence of few interfering compounds for the analytical process and its stability over time, even after death. In this context, the analysis of psychoactive substances like benzodiazepines (BZs) through vitreous humor is crucial for determining the cause of death and assessing the extent of the abuse issue. BZs are one of the most prescribed drugs used as anticonvulsant, anxiolytic, muscle relaxant, and sedative-hypnotic agents. Faced with challenges in forensic drugs analysis, the approach of supramolecular solvent (SUPRAS) microextraction could be highlighted as a Green Analytical Toxicology (GAT) method, and also offer intrinsic advantages, including high sensitivity, selectivity, and efficiency in extracting analytes. The SUPRAS microextraction occurs with the combination of the salt (sodium sulfate) and the alcohol (1-hexanol) that promotes the extraction of the sample with the formation of two phases, a continuous phase and a dispersed phase, generated from the association of different molecules in a self-organizing system, being able to extract the benzodiazepines (alprazolam, nordiazepam, 7-aminoclonazepam, bromazepam, clonazepam, diazepam, flunitrazepam, flurazepam, nitrazepam, temazepam, lorazepam, oxazepam and midazolam) from the VH. **Objective:** To develop and optimize a fast, green, and reliable SUPRAS microextraction method to identify BZs in VH. **Methods:** Initially, a Plackett-Burman (PB) experimental design was carried out, for parameters as sodium sulfate mass, agitation time, alcohol volume, and matrix volume. The absolute area of diazepam at different conditions through analysis by LC-MS/MS were evaluated and the best conditions were selected. Thus, a rotational composite central design (RCCD) was set up, with two replicates and five central points (2^3). The parameters were agitation time, alcohol volume, and matrix volume. Chromatographic separation was performed with a Raptor Biphenyl (2.7 µm, 100 x 2.1 mm, Restek, Bellefonte, PA, EUA) column in a LCMS8045 (Shimadzu®, Kyoto Japan). The mass spectrometer was equipped with an electrospray ionization source, operating in negative mode. The total run time of analysis was 4.3 minutes. **Results/Discussion:** With PB optimization, it was possible to verify which parameters presented significant differences in the results, at a confidence level of 95%: agitation time, alcohol volume, and matrix volume. Then, considering that sodium sulfate mass was presented as not significant, the RCDD was performed and the best results for agitation time, alcohol volume, and matrix volume were 7 minutes, 150 µL, and 300µL, respectively. Then, for extraction, following the optimized parameters, 300 µL of water was used to mimic VH and was transferred to a 2 mL polypropylene tube and 20 µL of diazepam-d5 2000 ng/mL (internal standard) was added. The tube was vortexed for 10 seconds. Then, 150 µL of 1-hexanol was added, homogenized for 7 minutes at 2500 RPM, centrifuged for 5 minutes at 2500 RPM, and 100 µL of the supernatant was transferred to another tube. Subsequently, the solution was dried under nitrogen flow at 40°C and resuspended with 100 µL of a MeOH:water (70:30 v/v) solution. Finally, 2 µL was injected into the LCMS8045. **Conclusion:** A GAT method was developed using SUPRAS microextraction for the analysis of BZs in VH. Posteriorly, the method will be validated as recommended by the AAFS Standard Practices for Method Validation in Forensic Toxicology and applied for the analysis of authentic post-mortem samples. **Acknowledgements:** This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - 130300/2022-5).

Development and validation of qualitative analytical method for multi-substances in postmortem blood samples based on 2,021 scope of the ToxCrim system

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Introduction: The ToxCrim system, a pilot of a national database of criminal toxicological information, provided an important knowledge of the scope of intoxication cases in the Brazilian scenario, initially from year 2,021. Based on those results and because currently in Brazil there is no standardized scope for the detection of multi-substances in postmortem blood samples one qualitative method might be developed to meet that suggested scope and postmortem toxicology requires low limits of detection to expand the detection power. The aim of this study was to develop and validate a qualitative method for analysis of 46 substances, metabolites, and relatives (cocaine, Δ9-THC, antidepressants, benzodiazepines, antihistamines, opioids, anesthetics and miscellaneous) in postmortem blood samples, using liquid-liquid extraction (LLE) and LC-MS/MS. **Methods:** The list of substances selected to this study was based on the 2,021 scope recommended by ToxCrim system. Postmortem blood samples were extracted with methyl-t-butyl ether and acetonitrile. After agitation, centrifugation and evaporation of solvent, samples were resuspended with 100 µL mobile phase and 2 µL was injected into LC-MS/MS system (LCMS8045, Shimadzu) with electrospray ionization. The chromatographic separation was performed with a RaptorTM biphenyl column (100×2.1mm, 2.7µm). The mobile phase consisted of ultrapure water and methanol, both containing 0.1% formic acid (v/v) and 2mmol/L ammonium formate, eluted in gradient mode. The qualitative method was validated according to ANSI/ASB Standard 036 recommendations. **Results:** The method was developed with limits of detection ranging from 1 ng/mL to 1,000 ng/mL. The matrix effects observed 0 to +14% (sertraline). Neither carryover nor interferences were observed. Autosampler stability study showed 45% (norsertraline) to 106% (oxazepam) for 24 h at 4 oC. **Conclusion:** A qualitative method based on simple LLE and LC-MS/MS analysis was developed and validated to screening 46 multi-substances in postmortem blood samples, showing low and suitable limits of detection. The method could be applied to A) determine the contributing role or impact of substances for cases with a known anatomical cause of death, B) to provide information for the determination whether substances contributed to the cause and/or manner of death, and C) to provide information for impaired and injured driving investigations in Paraiba state, Brazil.

Nicotine and cotinine intoxication: two cases of suicide by ingestion of tobacco honey

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Introduction: Nicotine and cotinine are alkaloids produced naturally in the nightshade family of plants, most predominantly in tobacco (Nicotiana tabacum), and widely used for recreational purposes as a stimulant and anxiolytic in cigarette consumption. However, in the northeast region of Brazil it is very common to use a type of tobacco extract called "tobacco honey" as a pesticide to eliminate pests and insects in agriculture. Accidental or intentional ingestion of tobacco based products can to conduct to a severe poisoning or death. The main symptoms of intoxication related to nicotine and continine ingestion are nausea, salivation, tachycardia, increased bronchial secretions, hypertension, seizures, and muscle spasms. This work presents two cases of suicide due to nicotine intoxication after ingesting "tobacco honey". In the first case, an 82-year-old woman with depression and psychiatric disorder, was found dead at home without signs of violence. The second case is related to a 31-year-old man found unconscious at home, next to a bottle containing a dark liquid, reported to be a possible poison he had ingested. **Objective:** The aim of this work was to investigate two cases of suicide involving the ingestion of "tobacco honey" resulting in possible fatal intoxication. **Methods:** A comprehensive screening of 160 illicit drugs, pharmaceuticals and metabolites by LC-MS/MS was performed in order to evaluate a possible intoxication. In sample preparation, 500 µL of femoral blood was transferred to a 5 mL polypropylene tube, followed by 500 µL of saturated sodium tetraborate solution, 2 mL of MTBE and 25 µL of IS (nicotine-d4 and cotinine-d3 10 µg/mL). The tube was homogenized by vortexing (5 min) and centrifuged at 4500 rpm/10 min in a refrigerated centrifuge (10°C). The uper layer was transferred to a microtube (2 mL), dried under a gentle flow of nitrogen (37°C), reconstituted with 80 µL of methanol, and placed in a glass insert. After this step, 1 µL was injected on LC-MS/MS system Shimadzu® LCMS-8040. Chromatographic separation was performed in a Restek® Raptor Biphenyl column (100mm x 2.1mm, 2.7µm) maintained at 40°C with mobile phase composed by ultrapure water (A) and methanol (B), both containing 0.1% formic acid and 2 mmol/L ammonium formate in a gradient elution starting at 5% MeOH/95% H2O to 85% MeOH/15% H2O. The flow rate was set to 0.4 mL/min with 4.5 min total run time. The mass spectrometer was equipped with an electrospray ionization (ESI) source operating in positive ionization mode. The analyses were performed in multiple reaction monitoring mode (MRM), in which two MRM transitions were chosen, one for quantitation and one for confirmation. **Results:** Nicotine and cotinine were identified in both cases, in addition to zolpidem and desvenlafaxine in case 1 and 7-aminoclonazepam in case 2. The calibration curves were linear from 10 - 1000 ng/mL, reaching an R2>0.99 and LOD of 5 ng/mL. These substances were quantified in femoral blood samples from both cases using a diluting factor and the concentrations found were as follows: case 1 (nicotine 9,2 µg/mL and cotinine 537 ng/mL) and case 2 (nicotine 2,2 µg/mL and cotinine 481 ng/mL). **Conclusion:** A rapid and sensitive method based on LLE and LC-MS/MS was applied to quantify nicotine and cotinine in postmortem femoral blood in two cases of suicide by ingestion of "tobacco honey". Nicotine is highly toxic and death can occur within minutes because respiratory failure results from paralysis of the respiratory muscles. The concentrations obtained were high considering the toxic range for nicotine (400 - 1000 ng/mL) and cotinine (300 - 1000 ng/mL) where the cause of death was concluded as fatal poisoning.

Optimization of micro-QuEChERS extraction for quantification of designer benzodiazepines in postmortem blood samples

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Background and aim: QuEChERS is a sample preparation technique initially developed to extract pesticide residues in food samples and is used as a green analytical toxicology alternative for sample preparation. The aim of this work was to develop and validate an analytical method for detection and quantification of designer benzodiazepines (8- aminoclonazolam, bromazolam, clonazolam, desalkylflurazepam, deschloroetizolam, flualprazolam, etizolam, flubromazepam and flubromazolam) using micro-QuEChERS and LC-MS/MS. **Methods:** Initially, design of experiments was conducted to screen variables for the extraction procedure, evaluating the partitioning salts, extraction solvent, agitation mode and sorbent clean-up. These factors were evaluated by 2^4 full factorial design, experiments were conducted in random order and responses evaluated were the individual chromatographic peak areas of each designer benzodiazepine obtained from LC-MS/MS analysis. The statistical analysis of the experimental design was performed using the Statistica software package (v.14.0.1.25) with statistical significance $p<0.05$. Thus, a composite central design was set up to optimize the sample volume, solvent volume, agitation time and sorbent clean-up effect. **Results:** The best conditions for the extraction procedure used 100 µL postmortem blood, fortification with 10 µL diazepam-d5, 500 ng/mL internal standard. Acetonitrile (300 µL) was added with 100 mg NaAc:MgSO4 (1:4, w/w) partitioning salts, followed by agitated at 2500 rpm for 7 min and centrifugation at 14000 rpm/5min. The supernatant (200 µL) was evaporated, resuspended in 100 µL methanol, and 5 µL analyzed by LC-MS/MS. The method was linear from 1 to 200 ng/mL ($r^2>0.99$), with adequate imprecision (<9.8%) and accuracy (<11.1%) evaluated at three different concentrations of quality control. Matrix effects and recovery were better than 58% and 77.5%, respectively. Neither carryover nor interferences were observed. The method was applied to two authentic post-mortem blood samples positive for bromazolam. Bromazolam concentrations were 31 and 40 ng/mL. **Conclusion:** A micro-QuEChERS extraction method was succefully developed and validated for the analysis of designer benzodiazepines in postmortem blood samples. **Acknowledgments:** This work was supported by Sao Paulo Research Foundation (FAPESP) - (Process Number 2021/04768-7), Coordination for the Improvement of Higher Education Personnel (CAPES) and National Council for Scientific and Technological Development (CNPq).

Postmortem investigation of a case series involving methamphetamine consumption in Rio Grande do Sul state

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Background: Methamphetamine is a synthetic drug utilized recreationally for its stimulant effects. Seizures of methamphetamine by the Brazilian Federal Police surged by 300% in 2021 (n=24) compared to the years 2020, 2019, and 2018 (n=6). Consequently, investigating the presence of this substance in fatalities and comprehensively characterizing these cases becomes crucial to monitoring the regional trends in methamphetamine consumption. **Objective:** The aim of this study was to provide a detailed description of postmortem cases associated with the identified consumption of methamphetamine in the state of Rio Grande do Sul. **Methods:** Five postmortem blood samples, provided by the laboratory department of the Instituto Geral de Perícias do Rio Grande do Sul (DPL-IGP), from the years 2021 and 2022 were subjected to evaluation. The samples underwent analysis using a previously validated methodology, involving protein precipitation in sample preparation. In this process, an aliquot of 100 µL of postmortem blood was added with 10 µL of amphetamine-d5 and 90 µL of a 100 mM ammonium chloride solution, followed by agitation and the addition of 400 µL of cold acetonitrile. After agitation and centrifugation, 100 µL of the supernatant were collected, and 0.5 µL was injected into the LC-MS/MS system. The method lower limit of quantification (LLOQ) is 5 ng/mL, and it exhibits linearity up to 1000 ng/mL. Furthermore, data related to the circumstances of each case and individual information (gender and age) were compiled to thoroughly characterize methamphetamine consumption. **Results:** Following the analysis, the data from each case were organized as follows: Case 1: A 50-year-old male involved in a traffic collision in 2021. The methamphetamine concentration in the sample was 99.52 ng/mL; Case 2: A 31-year-old male who was a victim of a homicide (shooting) in 2021. The detected methamphetamine concentration was 158.26 ng/mL; Case 3: In 2022, a 36-year-old male was involved in a homicide (shooting), with a methamphetamine concentration of 85.22 ng/mL; Case 4: A 23-year-old male in 2022, whose sample was provided for obit verification without an obvious cause of death identified. The methamphetamine concentration was 64.52 ng/mL; Case 5: Another male, aged 30, experienced a homicide (shooting) in 2022, with a determined methamphetamine concentration of 6.09 ng/mL. **Discussion/Conclusion:** Concerning methamphetamine concentrations, it's crucial to acknowledge significant variability attributed to various factors such as putrefaction, postmortem redistribution, methamphetamine stability, and individual characteristics. These variables can contribute to considerable fluctuations in detected concentrations. In terms of gender and age, all cases involved male individuals around the age of 30 (except for case 1), aligning with the prevalent demographic profile in violent deaths. Additionally, the correlation between methamphetamine consumption and exposure to violent scenarios, established in previous research, is evident in this study, given that the majority of deaths resulted from traumatic events. In conclusion, it is imperative to empha the ongoing necessity for investigating methamphetamine intoxication and related deaths. This continuous effort is vital for promptly identifying potential increases in consumption trends and establishing effective health policies targeted at addressing issues associated with this specific substance. **Acknowledgments:** The authors would like to acknowledge the collaboration of the toxicology division team from Instituto Geral de Perícias do Rio Grande do Sul (IGP-RS) and the financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Suicide of a nurse by self-administration of a mixture of fentanyl and benzodiazepines

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Introduction: The use of polysubstances is a common practice among people who abuse medications or illicit drugs. The recent increase in mortality and overdose cases associated with the consumption of multiple substances has revealed many cases of suicide caused by acute intoxication. The case of a 38-year-old nurse found dead in her apartment is presented. According to the case history, the nurse worked in a hospital with a high workload and had access to controlled pharmaceuticals, being addicted on benzodiazepines and painkillers, motivated by personal problems. She was found dead with an intravenous catheter attached to her arm. Several bottles and blisters of tablets, broken ampoules of midazolam, used bottles of Rivotril® (clonazepam) and Fentanest® (fentanyl) were recovered at the scene. A comprehensive screening of 160 illicit drugs, some NPS, pharmaceuticals and metabolites by LC-MS/MS and volatile substances by HS-GC-FID in femoral blood was performed in order to evaluate a possible intoxication. **Objective:** The aim of this work was to investigate a case of suicide involving the use of polysubstances resulting in acute intoxication. **Methods:** Different sample preparation procedures and analytical methods already established in the laboratory routine were used in the quantification step. A comprehensive screening of 160 substances including common illicit drugs, some NPS, pharmaceuticals and metabolites was performed using micro-QuEChERS and LC-MS/MS while volatile substances was determined using HS-GC-FID in femoral blood. Two quantitative methods were used in order to quantifiy benzodiazepines and opioids employing LC-MS/MS. A method to quantify benzodiazepines was used employing 100 µL of whole blood and micro-QuEChERS while opioids quantitation was performed using 500 µL of whole blood and LLE (with MTBE). Chromatographic separation was performed in a Restek® Raptor Biphenyl column (100mm x 2.1mm, 2.7µm) maintained at 40°C with mobile phase composed by ultrapure water (A) and methanol (B), both containing 0.1% formic acid and 2 mmol/L ammonium formate. The mass spectrometer was equipped with an electrospray ionization (ESI) source operating in positive ionization mode. The analyses were performed in multiple reaction monitoring mode (MRM), in which two MRM transitions were chosen, one for quantitation and one for confirmation. **Results:** Screening for illicit drugs, some NPS, pharmaceuticals, and metabolites in femoral blood detected 7- aminoclonazepam, midazolam (158 ng/mL) and fentanyl (6,4 ng/mL). No volatile compounds such as ethanol or inahalants were detected. **Conclusion:** Sensitive and selective LC-MS/MS analytical methods were applied to quantify midazolam (158 ng/mL) using micro-QuEChERS and fentanyl (6.4 ng/mL) using LLE (with MTBE) in postmortem femoral blood sample. Quantification of 7-aminoclonazepam in femoral blood was not performed because it is an inactive metabolite, but detection indicates recent use of clonazepam. Although the concentration of midazolam was found in the therapeutic range (40 - 250 ng/mL), it is important to highlight that fentanyl was identified above the therapeutic range (0.5 - 2 ng/mL) in concentration in the toxic range (2 - 20 ng/mL) which contributed decisively to intoxication. The cause of death was determined by the medical examiner to be fatal intoxication with fentanyl and midazolam and the manner of death was respiratory depression, followed by cardiorespiratory arrest.

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TOXICOLOGIA REGULATÓRIA

Advancements in skin irritation assessment for agrochemical formulations: alternative methods and their effectiveness

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The OECD Guidance Document 203 offers insights into testing and assessment methods for skin corrosion and irritation. However, these methods may not provide accurate predictions for the skin irritation potential of agrochemical formulations. This study sought to evaluate the effectiveness of the Globally Harmonized System (GHS) threshold approach and the in vitro skin irritation test (SIT; OECD 439) as alternatives to the in vivo Draize skin irritation test for assessing agrochemical formulations. Additionally, the study considered other factors that can influence the accuracy of these alternative methods when evaluating agrochemicals. To achieve this, a retrospective analysis was conducted on 84 agrochemical formulations with available in vivo skin irritation data, utilizing the GHS threshold approach. This method involves assigning a skin hazard classification based on the components' skin irritation classification if the sum of the components exceeds specific concentration limits. Additionally, available SIT data for 56 formulations in this dataset, using the EpiDermÔ model, were evaluated. This test is based on the in vitro test system of reconstructed human epidermis and may be used to identify irritant chemicals without differentiating between Categories 2 and 3. The prevalence of GHS hazard categories for skin irritation in this dataset was as follows: 57.1% (48/84) Not Classified (NC), 27.4% (23/84) Category 2, and 15.5% (13/84) Category 3. All formulations had a pH that fell within the range of 2 to 11.5. The data was analyzed using a bottom-up approach. The focus was on comparing the ability of both the alternative methods and the in vivo method to independently predict formulations as NC, in comparison to the combined prediction of Categories 1, 2, and 3. The results of the comparison between the GHS threshold approach and the in vivo test for 84 formulations showed an overall accuracy of 64.29%. However, when considering formulations where the active ingredient(s) were not classified for skin irritation (58 formulations), the overall accuracy improved to 75.86%. The specificity was found to be 76.92% and the sensitivity was 73.68%. The negative predictive value (NPV) was determined to be 85.75%. These results indicate that the GHS threshold approach is a reliable method for predicting the skin irritation potential of non-irritating agrochemical formulations that do not contain active ingredient(s) classified for skin irritation in their composition. When comparing the SIT method to the in vivo test for 56 formulations, the overall accuracy was found to be 64.29%. To understand how different formulation types affected the predictive performance of the SIT method, the data was divided into three clusters: 51.8% (29/56) liquid water-based, 37.5% (21/56) liquid solvent-based, and 10.7% (6/56) solid formulations. Interestingly, the results showed a significant improvement in the predictive performance of the SIT method for liquid solvent-based formulations. The overall accuracy was 76.19%. The specificity was determined to be 57.14% with a NPV of 66.67%, and the sensitivity was found to be 85.71% with a PPV of 80%. In contrast, when combining the results for liquid water-based and solid formulations, the overall accuracy decreased to 54.29%. The specificity was 90%, but the sensitivity was very low at 6.67%. Based on the results, it can be inferred that the SIT method is effective in predicting the irritation potential of liquid solvent-based formulations, but not for liquid water-based and solid formulations. The findings in this study have important implications for the development of alternative testing strategies and can contribute to more efficient and ethical regulatory decision-making processes in the assessment of agrochemical formulations. **Acknowledgments:** Natalia Tamachiro.

Approvals and non-approvals of pesticides in different countries

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Introduction: One of the most controversial topics about pesticides frequently mentioned in the media concerns the registration of certain active ingredients (AIs) in some countries and its non-authorization or ban in other territories. In addition to the hazard or risk assessment parameters, the diversity of the incidence of pests and diseases on each continent and the commercial strategies of registrants, there are administrative procedures of each country or economic bloc to consider a substance unauthorized or prohibited. **Objective:** The present study aimed to compare the chemical active ingredients registered in Brazil for use as agriculture pesticides with their regulatory situation in the European Union (EU), United States of America (USA), Australia, Japan and Canada, as well as those registered in these countries and not in Brazil. **Method:** The data were collected until August 31, 2023, using the following open databases: EU Pesticides Database - Active Substances; Registered Pesticides Products (USEPA); Australian Pesticides and Veterinary Medicines Authority (APVMA); List of Agricultural Pesticide Active Ingredients of Japan - Food and Agricultural Materials Inspection Center (Famic); Pesticide Product Information Database (Health Canada); and Portal de Monografia de Agrotóxicos (Anvisa/Brazil). Only chemical (including inorganic) agricultural pesticides were considered. **Results:** The first observation is that the amount of registered and unregistered active ingredients is similar between agricultural- producing and commodity-exporting countries, the exception being the European Bloc, which has 254 chemical pesticides authorized for agricultural use, less than all others, while Brazil has 305, USA 364, Canada 271, Australia 343, and Japan 414 chemical active ingredients authorized by 08/31/2023. Below it is showed the comparison of Brazil with the other countries. Brazil vs USA The comparison between these countries revealed there are 62 chemical AIs authorized in Brazil and not authorized in the USA, while there are more than twice as many in the opposite situation: 133 chemical AIs authorized in the USA and not authorized in Brazil. Among them, it is worth highlighting some pesticides still used in that country that were banned in Brazil after a toxicological reevaluation process, such as Aldicarb, Phorate and Paraquat. Brazil vs Austrália: The comparison indicated that there are 56 chemical AIs authorized in Brazil and not authorized in Australia, while 121 are authorized there and not in Brazil. Carbendazim, Omethoate and Trichlorfon are examples of AIs not allowed in Brazil and still authorized in Australia. Brazil vs Canada: The survey showed that the number of chemical AIs authorized in Brazil and not authorized in Canada is 109, while there are 99 in the opposite situation. Phorate can be cited as an example of AI banned in Brazil and still authorized in Canada. Brazil vs Japan: There are 90 chemical AIs authorized in Brazil and not authorized in Japan, compared to 194 authorized in Japan and not authorized in Brazil. Among the AIs banned in Brazil that remain authorized in Japan are Benomyl, Prochloraz and Trichlorfon. **Discussion/Conclusion:** The study results demonstrate it is unfounded the claim that the amount of active ingredients registered in Brazil is much more than in developed countries. Besides, considering the 10 most used pesticides in the country in 2021, the last year compiled by Ibama (Glyphosate, 2,4-D, Mancozeb, Chlorothalonil, Atrazine, Acephate, Malathion, Clethodim, Sulphur, and S-Metolachlor), all of them are authorized in Australia, Canada, Japan and USA, and all but four (Mancozeb, Chlorothalonil, Atrazine and Acephate) are authorized in the EU, contrasting with the vague narrative frequently disseminated by non-experts that Brazil uses a lot of active ingredients “banned in other countries”, to disqualify the regulatory work practiced in the country.

Critical appraisal of the results of sisagua database

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Introduction: The Drinking Water Quality Surveillance Information System (SISAGUA) is an Information System managed by the Ministry of Health -MH and available on the internet. In its database are inserted the results of monitoring the quality of water for human consumption, carried out by collective water supply service providers, state and municipal companies, local authorities and others. The analyses carried out aim to monitor the presence of biological, organic and inorganic contaminants, including heavy metals, pesticides, as well as organoleptic parameters and radioactivity. The results are manually entered into SISAGUA at the municipal level. The System has currently more than 11 million results, 3.6 million of which refer to the analysis of pesticides in water, in the period of 2014 to 2022, collected in approximately 3,500 Brazilian municipalities. The SISAGUA data are available in the Federal Government's open database, and since the end of 2022 on a MH portal. The database is frequently used by the lay public, society organizations and journalists to support articles about contamination of water by pesticides. **Objective:** To analyze the consistency of SISAGUA data and the incidence of results with the presence of pesticides detected at any level, as well as above the maximum permitted values. **Methods:** With the aid of programming and data analysis techniques, the authors analyzed all the information relating to pesticides from 2014 to 2022 and established minimum criteria for validating the data, such as: stating the Limit of Detection (LOD) or quantification used when the results are expressed as < LOD or < LOQ; checking the existence of information on the numerical result, as in several cases the result is left blank or filled with zeros or dashes; identifying gross errors such as results higher than the commercial concentration of certain pesticide products due to the misuse of decimal keys, and detection or quantification limits higher than the maximum permitted value (MPV). **Results:** Inconsistent results in some years reach more than 60% of the total samples for the period. After discarding inconsistent samples, the results of the presence of pesticides in the water supply for each year in the database were analyzed, in terms of the sample collection location (intra- household/intra-building treatment station, collection point, distribution system and alternative solution), geographic distribution, month of collection and the most frequent pesticides with result above MPV. As a result, less than 0.001% of the validated SISAGUA samples presented values above the MPV. In 2022, for instance, 21 samples were identified with results higher than the MPV in a total of 324 thousand samples tested for the presence of pesticides. The active ingredients most frequently related to results above the MPV belong to the group of organochlorines, banned from agriculture use in Brazil since the 1980s (aldrin, dieldrin, chlordane and endrin). In the carbamate class, there was identification of aldicarb, a pesticide without any use in the country since 2012. **Conclusion:** The results of this research demonstrate that there is a misuse of SISAGUA data to publicize water contamination, when in fact there is no indication for such a conclusion. The result of samples above the MPV is statistically insignificant, in addition to the fact that many samples are picked up at collection points or treatment stations, not in the distribution system. The high risk perception of laypeople concerning pesticides largely explains the controversy surrounding this issue, which is also addressed in the study.

Dermal absorption data with extended observation - period to evaluate the availability of the skin depot

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The skin is the most relevant route of exposure for individuals applying pesticides. Dermal absorption data is important for the occupational risk assessment as external dermal exposure can be transferred into internal exposure. Human in vitro data alone can be used to derive dermal absorption values for risk assessment purposes. According to OECD test guideline 428, human skin is exposed to a finite dose of the product for 6-10 h in diffusion cells and followed for a total 24-h observation period. Although dermal absorption can be simply defined as the transport of a substance from the surface into the inner layer of the skin and systemic circulation, different approaches are adopted to decide whether to consider the amounts detected in the different skin layers as absorbable. The European Food Safety Authority (EFSA) relies on absorption at half of the study duration (t0.5) to determine inclusion of stratum corneum (SC) as absorbed or not. OECD Guidance No 156 mentions additional ways to assess whether absorption is nearly complete within the experimental observation period. In case of the latter the amounts in the SC or even the complete skin depot may be considered not available for absorption. These refinements are mainly possible for in vivo studies as they allow longer observation periods (e.g. 168 h), thus providing additional information on the potential fate of the skin depot. This work presents an analysis of human in vitro skin experiments with extended 72-h observation periods providing additional data on mobility of the SC in comparison to typical 24-h experiments. Two suspension concentrate formulations containing the same active ingredient (AI) with expected slow penetration were tested in two different studies comprising six spray dilutions (SDs 1-6) for each. All experiments included four different skin donors and minimum eight replicates at each concentration. Data from in vitro 72-h experiments were compared against standard 24-h observation data to evaluate the availability of the SC for dermal absorption. Mean absorption between 24 and 72 h showed no or only slight increases (up to 0.18% of dose) in all tested concentrations. These slight increases were not associated with corresponding decreases from the overall skin depot – mostly amounted to less than 5% of the skin depot after 24 h. They are therefore not considered to indicate relevant absorption from the skin depot. Absorption rates decreased towards the end of the observation periods in both the 24 and 72 h experiments. While not reaching zero, all were very low in the last 4 hours of the respective observation period indicating completeness of absorption. Absorption rates can also be used to estimate the time required for absorption of the amounts remaining in the SC or the whole epidermis and then compared with available information of the time for SC desquamation (about 14 days) and whole epidermal turnover (35-42 days) in humans. For most tested scenarios the estimated time for absorption from the SC and whole epidermis were longer than 14 and 35-42 days, respectively. The extended 72-h data collected showed no or only non-relevant increases in absorption that are not considered due to absorption from the epidermis/SC, while amounts in the dermis are expected to be absorbable over time. Measured penetration rates also support a decline of absorption over time. The remaining material in the epidermis/SC is therefore expected to be lost due to epidermal turnover in vivo rather than completely absorbed. The additional data after 72 h demonstrate that relying on t0.5 to conclude the fate of the skin depot can overpredict absorption for AIs that show very slow absorption and tend to be retained in the skin.

Guidance for determining reference doses in human health risk assessment of agrochemical products

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The goal of toxicology and human risk assessment is to ensure we have sufficient information to reliably support regulatory decisions that protect public human health. Determining a reference dose (RfD) that is representative of the respective uses and exposure scenarios is essential for robust dietary and non-dietary risk assessments of agrochemical products. Therefore, we describe a systematic approach based on scientific criteria and sound judgement to define the most suitable RfDs to be used in human health risk assessment. The initial part of the risk assessment is the problem formulation, which involves the identification of the individual, population(s) and life stage(s) of concern, and potential exposure to a certain product, such as frequency, duration, and magnitude of exposure. In a second step, the hazard should be identified based on all available toxicological data for that product's active ingredient, and this includes to evaluate which specific data is indeed necessary to outline its safety profile based on what is already known. The most relevant toxicological outcome for the human species should be determined also considering the data from toxicokinetic studies and/or physiologically based kinetic models that may help to understand the relevance of the findings in potentially more sensitive species compared to humans. Also, the mode of action elucidation, if feasible, combined with an overall weight of evidence assessment is recommended rather than simply concluding whether an isolated effect is of concern or not. Once the critical effect(s) of concern and the exposure conditions to trigger such effect(s) have been identified, the most representative study(s) of the actual human exposure scenarios should be considered to set the point of departure (POD). The concern for exposure to residues in food commodities during a lifetime by the overall population could be typically addressed by using the most critical data from chronic and carcinogenicity studies as POD for establishing the acceptable daily intake. On the other hand, if the concern is for operators handling the product during an eight-hour working day for a couple of months, the most representative studies would be those evaluating the short-term exposure, ideally covering the exposure routes of interest (dermal and/or inhalation). However, if the most relevant toxicological outcome is observed only by the oral route, the repeated dose oral studies (e.g., 90-day, prenatal development, multi-generation studies) would be appropriate surrogates for establishing the acceptable operator exposure level. After selecting the most appropriate POD (e.g., No Observed Adverse Effect Level, Low Observed Adverse Effect Level, Benchmark Dose), uncertainty factors related to interspecies (10x) and intraspecies (10x) variability are commonly applied to derive a RfD for humans. These two factors consider toxicodynamic and toxicokinetic data which, when known, may reduce the uncertainties. Additional uncertainty factors may be needed depending on the extension of the database, the quality of available studies and scientific judgment. In conclusion, considering that there are different possibilities in risk management, a clear guidance establishing a systematic approach based on scientific criteria to set RfDs is essential to increase the consistency of the risk assessment process.

Non-mutagenic mechanism of micronucleus induction of a Protoporphyrinogen-Oxidase Inhibitor Herbicide

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Protoporphyrinogen-oxidase inhibitors (PPOi) are a class of herbicides that acts on chlorophyll synthesis. Likewise, it affects heme synthesis resulting in anemia in mammals to varying degrees. A series of experimental PPOi candidates showed, despite their clear negative in vitro mutagenic profile, enhanced micronucleus formation in rodent bone marrow in vivo. Since increased erythropoiesis may result in increased micronucleus formation, a series of investigations were performed to determine whether the micronucleus findings were attributed to a mutagenic effect or linked to the observed anemia. One of these PPOi candidates was tested in vivo by oral gavage in rats; micronucleus assays in the liver and bone marrow were performed on day 14. The data showed an increase in the micronucleus counts in the bone marrow whereas, despite clear indication of toxicity, the hepatocyte micronucleus frequency was not affected. In another study, 24 hours after a single gavage administration in mice a portion of the bone marrow was assessed for micronucleus formation while the rest was subjected to a subpopulation separation process isolating erythroid (selected via their Ter119 surface expression) and non-erythroid (selected by their CD45 surface expression) cells, which were then assessed in a comet assay. Results showed a dose dependent increase in micronucleus frequencies with a No Observed Genotoxic Effect level (NOGEL) < 100 mg/kg body weight. However, increases in % DNA tail intensities in the comet assay were confined only to the mature erythroid Ter119+ subpopulation. In conclusion, it can be stated that the observed induced micronucleus frequencies in vivo using the PPOi candidate is most probably a product of enhanced erythropoiesis and not linked to a direct DNA damaging effect.

Pesticide safety: regulatory and toxicological overview in Brazil

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The aim of the present study is to provide a regulatory and toxicological overview of pesticides in Brazil, based on the technical analysis of current regulations by the regulatory bodies responsible for the registration of such products. Pesticides play a fundamental role in agriculture, driving productivity, promoting large-scale food supply, and contributing to the country's economic health. To ensure the safe use of these products and maximize their benefits, it is necessary to establish reliable analysis procedures and parameters through regulations and controls. In Brazil, pesticides must undergo analysis and approval in three different spheres: environmental (by IBAMA), toxicological (by ANVISA), and agronomic (by MAPA). Together, these three agencies are responsible for the registration and approval process of these products in the Brazilian market. Regarding toxicology, ANVISA conducts safety assessments of pesticides through a meticulous process, involving rigorous evaluation criteria of studies submitted by applicants regarding irritation, sensitization, acute toxicity, mutagenicity, and chronic health damage (such as carcinogenicity, reproductive toxicity, and effects on specific organs). From an environmental perspective, the technical analysis conducted by IBAMA is based on laboratory, semi-field, and field tests, involving multidisciplinary areas such as statistics, chemistry, biology, agronomy, soil science, toxicology, among others. Based on this information, the physicochemical and ecotoxicological properties of the chemical substance are known, as well as information regarding persistence, bioaccumulation, transport in national soils, and residues in environmental matrices, which will support the understanding of the behavior of pesticides in various environmental compartments. With this data, it is possible to establish criteria and parameters in legislation to ensure the safety of pesticide use for the environment. MAPA is responsible for evaluating the agronomic efficiency of pesticides and, in conjunction with the analyses of other regulatory bodies, approving and granting registration numbers to products for regular commercialization and use in agriculture. Faced with the significant challenges of balancing the risks and benefits of pesticide use in agriculture, regulatory and toxicological analyses of these products are essential to achieve this milestone. Exploring technological and scientific innovations opens perspectives for continuously improving existing processes and strengthening safety in food production, such as replacing animal tests with equivalent in vitro tests. Considering the need to maintain effective and updated regulations to ensure the safe use of pesticides, this study presents an overview of the regulatory and toxicological landscape in which these products are embedded in Brazil.

PFAS: regulatory advances and toxicological challenges

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Introduction: Recently, regulatory agencies around the world have proposed new standards for the regulation of per- and polyfluoroalkyls (PFAS), which are a group of emission synthetic chemicals widely used in consumer products to make them non-stick, waterproof and stain resistant. The toxicological issue is what underpins this uprising of proposals, considering that they are very persistent substances, that they do not decompose in the environment and that, despite scientifically based care, they have been linked to various health problems, such as changes in the thyroid, cancer, high cholesterol, obesity, effects on the immune system, greater propensity for breast cancer, among others. **Objective:** Present and discuss proposals for new regulations at international and national levels regarding the use of PFAS in the production of materials for consumption and related risks. **Methods:** Proposals and regulations in force in the United States (US), European Community (EU) and Brazil were consulted and critically evaluated to map progress on the subject in different regulations. **Results:** The US via the "Environmental Protection Agency" (EPA), planned for the period 2021 to 2024 points, adopting an approach to regulating the life cycle of PFAS in order to obtain significant progress, thinking not only about the control of contamination by existing PFAS, but also by new PFAS produced. In parallel, EPA continues to pursue a scientific agenda to better characterize toxicities, understand exposure pathways, and identify new methods to prevent and remediate PFAS pollution. In EU, through the "European Chemicals Agency" (ECHA), some most widely used PFAS are already included in the Stockholm International Convention to eliminate their use, however, as they are a group of synthetic substances that have in common a molecular structure containing fluorine-carbon bonds, the efforts and proposals received from countries in the bloc, such as Germany, Denmark, the Netherlands, Norway and Sweden, to address the issue not by restricting the use of individually identified substances, but rather by restricting which covers a wide range of PFAS and their uses. Therefore, the proposal evaluated in the EU is to include these substances in the list of substances of very high concern (SVHC) based on their persistence, mobility, and toxicity, which were considered a threat to human health and wildlife when exposed. through the environment. These PFAS have been identified as being of equivalent concern to carcinogens, mutagens, and toxic to reproduction (CMR) and persistent, bioaccumulative, and toxic/very persistent and very bioaccumulative (PBT/vPvB) chemicals. In Brazil, PFAS are products that can be considered as not regulated at the national level, however, the "Companhia Ambiental do Estado de São Paulo" (CETESB) participates in a monitoring plan in Latin America for these compounds and in the absence of value standards monitoring system in Brazil uses criteria from American legislation, where only some are regulated. **Conclusion:** Notably, there are efforts aimed at regulating, restricting and possibly eliminating PFAS in consumer products due to their toxicity to human and environmental health around the world, with the discussion apparently most advanced in the European Community and the United States, given that these have proposals prepared and/or even already submitted to the plenary for consideration, while in this regard Brazil is lagging behind on the world stage, as it does not have any proposal for discussion on the topic in national regulatory bodies.

The development of a new herbicide in the context of the mechanistic model-based approaches proposed by Transforming the Evaluation of Agrochemicals (TEA)

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Currently, more than 10,000 vertebrate animals are expended in the development of a new pesticide active ingredient. Reducing and replacing vertebrate animals in toxicity studies that inform the regulatory risk assessment for agrochemical is a global interest as reflected in the most recent literature and also in the 2019 Directive from USEPA to eliminate vertebrate testing for regulatory approvals by 2035. The Transforming the Evaluation of Agrochemicals (TEA) is an initiative proposed by HESI to reframe the safety evaluation of crop-protection. Knowing that and in an effort to deliver faster, more sustainable agrochemical registrations, Syngenta is developing an approach to generate the information necessary to meet the regulatory requirements for a new pesticide registration without the use of chemical-specific vertebrate tests. Our first case study is for a new acetyl CoA carboxylase (ACCase) inhibitor herbicide. The workflow we established for the prediction of human safety endpoints resembles a traditional approach to safety evaluation in that we defined both exposure and hazard and then combined them for the risk assessment. Exposure was based on proposed uses of the new ACCase, using established tools to estimate residues and dietary intake. Dietary exposure was calculated with the Dietary Exposure Evaluation Model (DEEM), incorporating both residues in food and feed as well as drinking water, based on surrogate residue values from comparable herbicides. Operator exposures were based on EPA's Handling Scenario-based Unit Exposure model. For hazard, we utilized a comparative assessment for 14 registered ACCase herbicides and insecticides, working on the hypothesis that a new ACCase will be no more toxic than any existing ACCase. Risk assessment endpoints were collected from EPA Human Health Risk Assessment documents and used to establish the range of possible endpoints for the 14 chemicals. For chronic dietary risk assessment, the range of EPA-assigned points of departure was 0.23 to 30 mg/kg/day. The estimated chronic dietary exposures for all populations of interest ranged from 0.000074 to 0.000283 mg/kg/day for the new ACCase. Therefore, we can conclude that if the new ACCase has an endpoint as low as the most toxic existing ACCase, a risk assessment will show nearly 1000-fold difference between hazard and exposure. In other words, the new ACCase would pass the chronic dietary risk assessment with up to 1000X safety factor added to the hazard endpoint. Similarly favorable risk assessments were predicted for all other exposure scenarios, thus demonstrating the success of this approach and ability to conduct a human health-protective risk assessment without performing new mammalian toxicity studies.

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TOXICOLOGIA REPRODUTIVA E DE DESENVOLVIMENTO

Development of human stem cell-based model for in vitro teratogen screening

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Background/Introduction: Exposure to teratogenic compounds during pregnancy can harm embryonic development, including embryo death, growth retardation, and severe congenital disorders. Currently, the gold standard for the assessment of developmental and reproductive toxicity (DART) is the OECD 414 test, which utilizes an average of 784 rats and 560 rabbits per study. In addition to the ethically questionable methodology, OECD 414 is resource-intensive, time-consuming, and may not accurately mimic human responses, leading to false predictions and tragic outcomes like the thalidomide case. As an alternative to in vivo assays, many in vitro models for DART rely on animal-origin cells, including the mouse embryonic stem cell (ESCs) test, whole embryo culture (WEC), and micromass (MM) embryotoxicity assay. These assays exhibit limited predictability (70%) for detecting weakly or non-embryotoxic compounds, and they are based on animal cells rather than human cells. Consequently, there is a strong demand for new in vitro model approaches to reduce animal testing and enhance the prediction of developmental toxic hazards. Human dental stem cells emerge as a potential solution, being a source of adult stem cells with high plasticity and multipotential capabilities. Furthermore, they are easily obtained as discarded byproducts, involving a minimally invasive collection method. The ethical dilemma is restricted to donations made willingly and with a clear understanding. **Objective:** Development of a teratogen screening platform using dental stem cells as a human-based model. **Methods:** Dental stem cells derived from apical papilla (SCAP) were isolated, cultured, and characterized to CD90, CD105, STRO-1, Nanog, OCT ¾, Nestin, and CD34 by flow cytometry (Ethical protocol: #44067021.0.00005083). Cellular viability was evaluated with an MTT assay at 24 and 48h, using classic teratogenic compounds 5-fluorouracil (0.1 – 360 µg/mL), cisplatin, and cyclophosphamide (0.1 – 100 µg/mL) and methotrexate (0.1 – 1000 µg/mL), and non-teratogenic compound folic acid (0.16 – 250 µg/mL), to determine CV80. In addition, the expression of CD90, CD105 and STRO-1 biomarkers was evaluated by flow cytometry after 48h exposure to the drugs. **Results:** SCAPs exhibited a positive phenotype to mesenchymal biomarkers CD90, CD105, and STRO-1, as well as pluripotency markers Nanog and Oct ¾, along with the neural progenitor biomarker Nestin. On the other hand, they were negative for the hematopoietic biomarker CD34. No cytotoxicity was observed for any concentrations after 24h of exposure to 5-FU, cyclophosphamide, methotrexate, and folic acid. However, the CV80 value for cisplatin was determined to be 16.01 µg/mL after 24h of exposure, and it decreased to 13.23 µg/mL after 48h. The CV80 value for 5-FU was established at 13.47 µg/mL after 48h of exposure. No cytotoxicity was observed for any concentration within the 48h exposure to the teratogenic compounds cyclophosphamide and methotrexate, as well as to the non-teratogenic compound folic acid. Preliminary Results: suggest a decrease in CD90 and CD105 expression by the presence of teratogenic compounds, while the non-teratogenic compounds, folic acid, did not modify the mesenchymal biomarkers. **Discussion/Conclusion:** This study demonstrates the potential of human dental stem cells as a promising source of cells to develop new NAMs. Moreover, this represent a substantial step forward in decreasing reliance on animal testing, simultaneously improving our ability to predict human developmental toxicity through modulation of specific biomarkers. **Acknowledgments:** Coordination for the Improvement of Higher Education Personnel and Laboratory of Research and Education in In Vitro Toxicology (Tox In).

Exposure to chlorpyrifos during peripubertal period impairs spermatic parameters and the number of Leydig and Sertoli cells in rats

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Chlorpyrifos is an organophosphate insecticide largely used as plague control in fields. Thus, humans are exposed to it through food and/or water consumption, inhalation of contaminants and through skin. The juvenile and peripubertal period comprise a development window of the reproductive system, with great sensitivity to toxic agents. Considering the lack of data on the exposure to the insecticide during this period, the aim of this study was to evaluate the effects of chlorpyrifos on the male reproductive system during peripuberty. Thirty male Wistar rats with an initial age of 25 days were divided in three groups: control, which received corn oil (vehicle); CPS5 and CPS15, which received 5 mg/Kg and 15 mg/Kg of chlorpyrifos, respectively. The groups were treated daily, via gavage, during 40 days and, in the 41º experimental day, the animals were anesthetized and subjected to euthanasia. Blood was collected to obtain plasma for testosterone dosage. The testis were collected for the sperm count, histopathological an oxidative stress analysis. The sperm from vas deferens were collected for the spermatic morphology and acrosomal integrity analysis. All procedures and protocols were approved by the Ethics Committee on the Use of Animals of the State University of Londrina (OF. CIRCA. CEUA Nº 034/2021). Both concentrations of chlorpyrifos caused a reduction in the number of Leydig and Sertoli cells, as well as the daily sperm production. The number of abnormal spermatozoa and spermatozoa with acrosomal damage was increased in the CPS5 and CPS15 groups. It was observed a reduction in the lipid peroxidation and total glutathione in the CPS5 and CPS15 groups, and a reduction in glutathione-S-transferase activity in the CPS5 group, but no alterations in the reduced glutathione concentration. The morphometric analysis showed a decrease in the seminiferous epithelium height in group CPS5, but the histopathological and spermatogenic kinetics analysis did not show statistical differences. In light of these results, we conclude that the exposure to chlorpyrifos during peripuberty impairs sperm production, as well as their quality, in addition to causing a disturbance in the redox balance of testis. I extend my gratitude to State University of Londrina for the support and to CAPES/PROEX for providing the financial support to carry out this work.

Exposure to cyantraniliprole during pregnancy and lactation impairs the male reproductive system of offspring

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Introduction: Brazil has led the world in pesticide consumption for a decade, using 7.3 liters per inhabitant annually, resulting in 11 daily intoxications in 2017. Adverse effects include infertility, abortions, cancer, among others. Due to the high toxicity of pesticides and their harmful effects on human health and the environment, less harmful insecticides are constantly developed. Like cyantraniliprole, a diamide that modulates ryanodine dependente calcium channels, essential for cellular processes, embryogenesis, and fertility. **Objective:** Evaluate whether exposure to cyantraniliprole during gestation and lactation may cause impairments to the genital system of pubertal and adult male rat offspring. **Methods:** Male and female Wistar rats from the central breeding unit of UEL were paired overnight. Pregnant females were divided into three groups: Control (C), Cyantraniliprole 1 mg/kg/day (C1), and Cyantraniliprole 10 mg/kg/day (C10). Cyantraniliprole was administered via gavage from the 5th day of gestation until day 21 of lactation, while the control group received only water. Controlled conditions were maintained throughout the experiment (12h light/dark cycle; temperature 22°C ± 3°), with standard diet and ad libitum water. Obtention of biological material for reproductive parameter analysis occurred at Postnatal Day 55 for pubertal animals and at DPN 80 for adult animals. Project approved by the CEUA of the State University of Londrina, according to OF. CIRC. CEUA n. 20/2020. **Results:** The study in adult animals showed that the C10 group presented small quantity of normal spermatozoa compared to the control group. The C1 group showed a reduction in spermatozoa labeled by DABI, increasing those labeled by DABII. There were fewer spermatids in the testis and less daily sperm production in the C1 group in adult animals. In pubertal animals, there was no significant change in Sertoli cells, but Leydig cells decreased in both doses. In adults, the C10 group showed more Sertoli cells and fewer Leydig cells. Seminiferous tubules increased in diameter in the exposed groups, but epithelial height decreased in exposed pubertal animals. Seminiferous tubule thickness increased in both C1 and C10 groups in pubertal and adult animals. There was no significant difference in spermatogenesis kinetics at both ages. In histopathology, only the C10 group of pubertal animals showed alterations, while in adults, the C1 group exhibited cells in the lumen and the C10 group showed cells in the lumen and vacuoles. Stereological analysis of the epididymis revealed structural changes in C10 prepubertal and adult animals, decreasing the epithelial layer and increasing the lumen and interstitial regions in both the head and tail of the organ. **Discussion/Conclusion:** Exposure to cyantraniliprole during gestation and lactation had significant impacts on the genital system of prepubertal and adult rats. The results indicated that the pesticide distinctly influenced the reproductive health of the experimental groups, affecting sperm parameters, testicular histology, and epididymal structure, with distinct responses depending on the developmental phase and pesticide dose. These findings highlight the importance of further investigations into the potential risks of cyantraniliprole for reproductive health.

Acknowledgments: We would like to express our gratitude to CAPES/PROEX and CNPq for the financial support granted for the accomplishment of this study.

In vitro exposure to malathion impairs testosterone biosynthesis and oxidative profile in TM3 Leydig cells

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Currently, insecticides are being widely used. Among them, Malathion plays a prominent role within the class of organophosphates, being used in agricultural and urban environments to control arboviruses, caused by the Aedes aegypti mosquito vector. As a result, the population is exposed to this compound, including children and adolescents, through both ingesting contaminated food and inhaling insecticide droplets. Given the scarcity of literature on Malathion's relationship with the male reproductive system and the clinical, political, and social relevance of the subject, the aim of the study was to evaluate whether exposure to different concentrations of Malathion could compromise Leydig cell functionality in vitro. For this purpose, TM3 murine Leydig cells were exposed to concentrations of 1, 10, or 100 µM of Malathion for 24 hours. Subsequently, both cell viability and the cells' redox profile were evaluated, along with assessments of testosterone concentrations and the cytokines IL-1β, IL-6, IL-10, and TNF-α. Under these experimental conditions, Malathion was able to compromise cell viability and decrease testosterone production by Leydig cells at the lowest experimental concentrations (1 and 10 µM). Although IL-1β levels increased and TNF-α levels decreased at certain Malathion concentrations, they did not appear to be related to the alteration of testosterone production. Furthermore, different concentrations of Malathion induced oxidative stress by increasing superoxide anion and compensatorily enhancing antioxidants. Thus, it can be concluded that Malathion compromised Leydig cells' function by reducing testosterone biosynthesis through alteration of the redox state. I extend my gratitude to the State University of Londrina for their support and to CAPES/PROEX and CNPq for the funding necessary to carry out this project.

Influence of in utero and/or lactational exposure to the antidepressant venlafaxine: late repercussion on reproductive parameters in male rats

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Introduction: Clinical depression is commonly found in women of childbearing age, especially during pregnancy and the postpartum period. Venlafaxine is the primary option for treating maternal depression. It is classified as a Serotonin Norepinephrine Reuptake Inhibitor (SNRI), enhancing the action of these neurotransmitters that play a crucial role in the prenatal development of the central nervous system. In the context, exposure to SNRI during different critical periods may impact the hypothalamus-pituitary-gonad axis, potentially compromising hormone-dependent processes, such as hypothalamic sexual differentiation. Additional data comparing exposure to this medication are necessary, since the safety for the fetus is not fully elucidated. **Objective:** Evaluate the effects resulting from maternal exposure during pregnancy and/or lactation to the antidepressant venlafaxine on sexual development and the possible late repercussions on reproductive parameters in male rats. **Methods:** Pregnant wistar rats were divided into four experimental groups and treated during gestation and lactation (CGL and VENGL) or only during lactation (CL and VENL). All groups were treated daily, via gavage, with distilled water or venlafaxine (4.5 mg/kg/day). Male offspring were investigated at different ages (22, 50, and 90 days postnatal) using the following parameters: determination of body weight, anogenital distance (AGD), preputial separation and testicular descent ages, organ weights, and evaluation of spermatic parameters. **Results:** Females treated with venlafaxine during lactation exhibited higher initial weight and lower weight gain compared to those treated during gestation and lactation. On postnatal day (PND) 1, exposure to venlafaxine in both groups increased AGD, with a significant increase in offspring weight (VENGL). At PND 22, there was an alteration in the weight of the prostate and testicle. At PND 50, venlafaxine exposure increased the weight of the adrenal gland, spleen and liver. In adulthood (PND 90), male offspring showed an increase in the weight of the liver (VENGL), testis, vas deferens (VENGL and VENL) and epididymis (VENGL). There was a reduction in post- implantation loss in the group exposed to venlafaxine (VENGL) when compared to the control group (CGL). **Conclusion:** Exposure to venlafaxine at different periods caused moderate toxicity, compromised sexual development and altered reproductive parameters in male offspring. Approved by the ethics committee, n° 874407042022.
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Malathion exposure during peripuberty later impairs placentation and fetal development in wistar rats

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The DOHaD hypothesis states that injuries suffered in early life and key developmental windows, such as during fetal development and early childhood, may lead to programming and cause long-term effects on an individual's health and disease risk later in life. These injuries could be caused by maternal nutrition, exposure to toxins, stress, and other environmental influences. Programming can be passed across generations. The maturation of the reproductive system and establishment of the hypothalamic-pituitary-gonadal (HHG) axis occurs during the developmental window known as puberty. Malathion is a pesticide of the organophosphate class widely used since the 1980's, and in Brazil it has been used in the control of the Aedes aegypt mosquito. According to the EPA, more than 30 million pounds of malathion are used in rice and cotton cultures per year. Thus, the aim of this study was to evaluate if malathion exposure during the juvenile and peripubertal periods impairs placentation and fetal development in female Wistar rats. To do so, 36 Wistar rats were equally distributed into 3 experimental groups. Animals in M10 and M50 groups received respectively 10 or 50 mg/kg of malathion diluted in saline 0,9% via gavage daily from PND 22 to 60. Animals in the control group received the same volume of vehicle. Beginning on PND 28, observations of vaginal opening day and the first estrus were made. Once exposure was ceased, the females were left overnight with a sexual experienced male and considered pregnant when sperm cells were found in their vaginal smear (GD 0). At GD 18, they were euthanized by heart puncture following anesthesia. The fetuses were delivered, weighted and their crown-rump was measured. The placentas were also harvested, measured, weighted, and destined to evaluation of oxidative stress. The experimental protocol followed the ethical principles and was approved by the Ethics Committee on Animal Use (CEUA) of State University of Londrina (OF. CIRC. CEUA 17/2023). Regarding the puberty onset of the dams, the vaginal opening day occurred earlier in M50 animals, in comparison to the control group, however, no differences were observed amongst experimental groups on the day of the first estrus. Fetal weight was not altered; however, the crown-rump was diminished in both groups which the dams were exposed to malathion prior to pregnancy. Regarding the placentas, neither placental eccentricity, nor placental thickness were altered between experimental groups. However, placental weight was diminished in M10 and the placental area was diminished by both malathion doses, in comparison to the control group. Lipid peroxidation in the placentas, measured by TBARS, was increased in M50 group. GSH levels were not altered between experimental groups, although GST activity was diminished in M10, in comparison to the control group. These partial results indicate that malathion exposure during the juvenile and peripubertal periods influences placentation and fetal development in pubertal female Wistar rats, with oxidative stress being one of the damage mechanisms involved. I would like to extend my gratitude to State University of Londrina for all the support and to CAPES for the financial fomentation.

Malathion exposure impairs uterine development, but does not alter fertility in pubertal wistar rats

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Infertility is a disease of the reproductive system that impairs reaching pregnancy after a year of regular intercourse. The most common factors that alter fertility in women of reproductive age are STIs, stress, lifestyle, and environmental contaminants. Malathion is an organophosphate pesticide widely used in agricultural cultivation since 1980's and, in Brazil, it has been used in the control of the Aedes aegypt mosquito. More than 30 million pounds of malathion are used in rice and cotton cultures annually. Puberty is the developmental window in which the maturation of the reproductive system and HHG axis occurs. In this context, children and teenagers growing up are constantly being exposed to this compound while developing. Thus, the aim of this study was to evaluate if exposure to low doses of malathion during the juvenile and peripubertal periods impairs uterine development and fertility in female rats. For that, 66 female Wistar rats were distributed equally in 3 experimental groups: rats in M10 and M50 groups received malathion in the doses of 10 or 50 mg/kg, respectively, daily via gavage from PND 22 to 60. Rats in the control group received saline 0,9% (vehicle) in the same volume. Following exposure, during the estrous phase, half of the animals were euthanized by heart puncture following anesthesia, and had blood collected from the hepatic portal vein and destined to estradiol dosage, their uteri were harvested and destined to histological processing or destined to evaluation of oxidative stress. The other animals were submitted to a mating protocol, in which during the proestrus phase of the cycle, they were left overnight with a sexual experienced male and considered pregnant when sperm cells were found in their vaginal smear (GD 0). At GD 18, they were euthanized and had blood samples collected as previously described, their uterus and ovaries were harvested, the number of corpora luteum, fetuses and implantation sites were counted, tissue samples from the uterus were collected and destined to evaluation of oxidative stress. The experimental protocol followed the ethical principles and was approved by the Ethics Committee on Animal Use (CEUA) of State University of Londrina (OF. CIRC. CEUA 01/2020; OF. CIRC. CEUA 17/2023). Estradiol levels were not altered amongst experimental groups, neither in peripubertal rats nor during pregnancy. The myometrium thickness increased in M10 animals, while the perimetrium diminished and the glandular epithelium increased in M50 animals. The luminal epithelium height increased in both M10 and M50. In a histopathological analysis, both doses impaired tissue integrity. Cell desquamation in the glandular lumen, hyperplasia in the glandular epithelium, and the presence of vacuoles were observed in M10 and M50. Also, vascular congestion was observed in the vessels of M50 animals. The fertility potential, preimplantation and post implantation rates were not altered. Regarding the oxidative stress analysis, lipid peroxidation (LPO) was diminished, and GST activity was increased in the uterus of M50 animals following malathion exposure. GSH levels were not altered. In the pregnant uterus, LPO, GST activity and GSH levels were not altered. These results indicates that exposure to low doses of malathion during the juvenile and peripubertal periods impairs uterine postnatal development, given histopathological abnormalities and oxidative profile observed in peripubertal females. However, these doses were not sufficient to impair the physiology of this system, as shown by the puberty onset, estradiol levels, oxidative profile in the pregnant uteri and fertility, that were not altered between experimental groups. Thus, malathion exposure impairs postnatal uterine development, however, does not alter fertility.

I would like to extend my gratitude to State University of Londrina for all the support and to CAPES and CNPq for the financial fomentation.

N-nitrosodimethylamine: developmental, toxicological and reproductive effects of direct and indirect exposure of F0 and F1 generation of Wistar rats

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N-nitrosamines are potential mutagenic and carcinogenic agents that can be found in water, food, drugs, cosmetics and cigarettes. Although potential carcinogenic effects are well established, few studies have investigated the reproductive effects of N-nitrosamines, especially in low dosages, considered acceptable by regulatory agencies. Environmental interferences are among the main causes of infertility, but data on reproductive and developmental effects following exposure to NDMA are limited. This study aims to investigate consequences of N-nitrosodimethylamine (NDMA) exposure in developmental, toxicological and reproductive parameters of F0 and F1 generation of Wistar rats. In the F0 generation, male and female Wistar rats (n=20/group/sex) were exposed to distilled water (vehicle) or 7.2 ng/kg/day orally (gavage). Males were exposed during the preconceptional period, postnatal day (PND) 60 to 90, and mating period, PND 90 to 104. Females were exposed during the preconceptional period, PND 60 to 90, mating period, from PND 90 until pregnancy, and the gestational/lactational period, gestational day 0 to lactational day 22. During the mating period, animals were paired (1:1) and divided into four experimental groups (n=10/sex/group): Control (control males X control females); Maternal NDMA (NDMA females X control males); Paternal NDMA (control females X NDMA males); and Combined NDMA (NDMA females X NDMA males). The animals were euthanized after the exposure periods to collect organs and blood. In F0 males, there was an increase in the platelet count of the exposed group [839.1 ± 58.21 vs. 1019 ± 48.64], as well as an increase in the relative weight of the prostate [104.7 (89.88-118.5) vs. 122.5 (105.6-152.9)], when compared to the control group. In F0 females, there was a significant reduction in mean corpuscular volume (MCV) [58.80 ± 0.3988 vs. 57.12 ± 0.3645, p=0.0036] and mean corpuscular hemoglobin (MCH) [19.17 ± 0.1659 vs. 18.45 ± 0.1214, p=0.001]. F1 males had a delay of testicular descent in the combined group compared to the control [25 (23 - 26) x 23 (22 - 24)], but no change in preputial separation. In F1 females, an increase in body weight was observed at PND 1 in the paternal group [6.81 (6.38 - 7.10) x 6.40 (5.91 - 6.81)], and a reduction in anogenital distance (AGD) (7.36 ± 0.12 x 7.75 ± 0.08) and relative AGD (2.48 ± 0.04 x 2.61 ± 0.03) at PND 13 of the maternal group. At PND 60, females in the paternal and combined groups showed a reduction in MCVC (57.18 ± 0.55 x 59.58 ± 0.33). So far, it is possible to hypothe that N-nitrosodimethylamine has a potential toxic effect on normal systemic and reproductive functioning and potential to alter the sexual development of offspring. However, these data are derived from preliminary results and ongoing further analysis are essential to validate this hypothesis. **Acknowledgments:** FAPESP (2022/15364-7; 2022/15849-0; 2023/04536-4); CAPES (88887.806581/2023-00). Ethics Committee: 4704260423

Phthalate exposure during pregnancy and lactation impairs the epididymis in the offspring of rats in a transgeneral manner

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The DOHaD – Developmental Origins of Health and Disease – hypothesis suggests that environmental stimuli during development in the womb or in early life may cause the development of diseases later in life. Phthalate esters are used as plasticizers to a variety of items, such as household utensils and children's toys, to guarantee their flexibility and durability. When heated, these items release phthalates that contaminate food and drinks. Thus, the aim of this study was to evaluate the epididymal parameters of two generations (F1 and F2) of offspring of rats exposed to a mixture of phthalate esters during gestation and lactation. Pregnant Sprague–Dawley females were divided into four groups and gavaged daily from GD10 to PND21 with corn oil (Control:C) or the phthalate mixture at three doses (20, 200, and 200 mg/Kg). F1 males were subjected to euthanasia at 22PND and 120PND and the F1 females were mated at 90 PND with unexposed males to obtain the F2 generation. F2 male rats were subjected to euthanasia at 22PND. The epididymis were collected and destined to histological evaluation and gene expression evaluation. All experimental procedures and protocols complied with the Ethical Principles of Animal Research, adopted by the Brazilian College of Animal Experimentation, and were approved by the Ethics Committee on the Use of Animals (CEUA) of the Biosciences Institute of UNESP de Botucatu (Protocol 1040 / CEUA). Tissue remodeling of the epididymal compartments was observed at all doses in the 22PND animals of both generations. Treatment at 200 mg/Kg resulted in an increase in the lumen and a decrease in the epithelium in the cauda in F1 PND22. In the F2 22PND the lumen was increased and the interstitium was decreased in the caput and cauda at the same dose. In adult animals (F1 120PND) the lumen was reduced and the interstitium was increased at the two highest doses. There was a reduction of the expression of GPR30, GPX3, GSR, IL10, and TNFa in adult animals at the highest dose. GPR30 expression was increased at 200 mg/Kg dose in F1 PND22. TNFa expression was reduced at all doses in F1 PND22 animals and significantly increased in F2 PND22 animals at 200 µg/Kg and 200 mg/Kg doses. In light of these results, we conclude that phthalates modify the epididymal structure and impair gene expression across generations. I extend my gratitude to State University of Londrina for the support and to CAPES for providing the funding to carry out this work.

Risk of neural tube defects due to the exposure to pesticides during pregnancy: a systematic review

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Background: Exposure to pesticides during pregnancy has been associated with several serious congenital malformations, such as neural tube defects, therefore, is a cause for concern in terms of human health. **Objective:** This review aims to gather information related to maternal exposure during pregnancy and the risk of triggering neural tube defects in the offspring. **Methods:** The search strategy for the studies followed the PRISMA guidelines. We conducted a systematic search in the Science Direct, PubMed, Cochrane Library, Embase, Scopus, and Web of Science databases for all epidemiological studies that sought to associate exposure to pesticides during embryonic development with the risk of neural tube defects (NTDs). The keywords used were "pesticide", "herbicide", "congenital" and "neural". **Results:** Of the 229 articles, 8 eligible ones (7 case-control and 1 cross-sectional) evaluated pesticide exposure in pregnancy. Different methods were used, including analysis of biological samples and questionnaires. The pesticides studied included insecticides, herbicides, fungicides, and nematicides. Insecticides were the most studied, with variations in concentrations between tissues and studies. Distinct levels of pesticides have been detected in maternal serum, placenta, and umbilical cord. Confounding factors such as smoking and supplementation were statistically adjusted. Concentrations were measured in different exposure windows (periconception and prenatal), related to NTDs such as anencephaly and spina bifida. **Discussion:** Different data collection techniques, types of biological samples, and exposure windows were used, which made comparison difficult. The main pesticides studied included DDT, DDE, HCH, and endosulfan. Maternal serum showed the highest concentrations of pesticides, but detection in placental tissue and umbilical cord confirms embryonic exposure. Confounding variables were adjusted for in the analysis of the articles, but they may still contribute to the risk of NTDs. **Conclusion:** All the studies analyzed pesticide exposure and the relationship with NTDs. However, a more standardized survey would be ideal for better comparisons. **Acknowledgments:** The authors would like to thank the Instituto de Pesquisa Pelé Pequeno Príncipe for the scholarship granted to Karoline Felisbino and Nathalia Kirsten; the CNPq for granting a junior post-doctoral (PROFIX-JD) fellowship to Shayane da Silva Milhorini (process 421691/2022–0). We would also like to thank the Instituto de Pesquisa Pelé Pequeno Príncipe for the equipment and materials used for the development of the experiment.

Spinosad – mode of action and human relevance assessment of dystocia in rats

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Background/Introduction: Spinosad technical, a substance derived from natural fermentation that has insecticidal activity, was associated with treatment-related adverse pregnancy outcomes which manifested as dystocia in the rat. **Objective:** The objective of this work was to determine a robust mode of action (MOA) programme for dystocia in rats and relevance of this hazard to humans using the (WHO)/International Programme on Chemical Safety (IPCS) framework. **Methods:** Approximately 30 highly innovative mechanistic studies were conducted to characterize the rat mode of action and the human relevance of these findings using a variety of in vitro and in vivo toxicodynamic and toxicokinetic studies. In addition, a Physiologially-Based Kinetic (PBK) model to further investigate human relevance, following recently issued OECD guidance. **Results:** A number of dose-related key events have been identified that characterise the rat MOA for Spinosad-induced dystocia. Dystocia was characterised by prolonged parturition which was associated with peri-partum maternal death and other peri-partum effects. Using in vivo and ex vivo contractility experiments, it was concluded that parturition became protracted due to inhibition of uterine muscle contraction, arising due to a pharmacological/receptor-mediated inhibition of action potential generation in uterine smooth muscle cells (myometrial cells). By using competition binding experiments with receptor ligands, it is hypothed that the Spinosad receptor mediating uterine effects may be Translocator Protein (TSPO). The initial dynamic molecular initiating event of Spinosad binding to TSPO requires uterine exposure to Spinosad above a certain tissue concentration threshold. With pharmacokinetic studies, uterine exposure to Spinosad has been unequivocally demonstrated in the pregnant rat after Spinosad oral administration. It was concluded that non-dose proportional (supralinear) increases in uterine tissue concentrations between the rat NOAEL and the LOAEL for dystocia at parturition caused exceedance of the contractility inhibition thresholds and consequently dystocia at the higher dose level. This MOA is not relevant for humans due to measured quantitative toxicokinetic differences in uterine tissue exposure to Spinosyns (Spinosad and metabolites) between rats and humans. By using a variety of in vitro comparative pharmacokinetic determinations the following was demonstrated: A) Spinosyns have higher hepatic clearance (oxidative metabolism) in humans; B) GSH conjugation (a key process for Spinosyns excretion via the bile) is likely saturated in rats where GSH depletion is expected earlier/at lower dose levels; C) humans are expected to have a lower uterine partitioning of Spinosyn based on generic PBK modelling; and D) compound-specific PBK (R- based) modelling for two Spinosyns up to the rat dystocia LOAEL levels showed that uterine concentrations in humans are expected to be approximately one order of magnitude lower compared to rats. **Discussion/ Conclusion:** The rat Spinosad adverse effect of dystocia will not be triggered in humans as tissue concentrations remain below the effect threshold for the dynamic (receptor-mediated) molecular initiating effect. Since, the rat mode of action is not plausible in humans, Spinosad does not pose a reproductive hazard to humans.

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TOXINAS

Poisoning from multiple bee stings in the workplace

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Introduction: African bee Apis Mellifera scutellata was introduced in the southeastern region of Brazil in 1956. That year swarms of Apis Mellifera Scutettata escaped and a process of expansion of the potihybrids that are formed when crossed with European bees , known as Africanized bees, was detected in Uruguay in 1971 (Juri et al 2015). These new hybrids are very defensive and attack massively, causing serious injuries in humans. (Medeiros et al 2019) In Uruguay, the Toxicological Information and Advice Center (CIAT) reports 46 accidents in the last 3 years and of which 4 have been fatal. Poisoning by multiple stings from Africanized bees constitutes a public health problem in the Americas.The toxins present in the venom, which include melittin and phospholipase A2, cause injuries to various organs that can be fatal. **Objective:** To describe 2 clinical cases that were stung by more than 500 bees with a fatal outcome. **Methods:** descriptive observational and retrospective study of 2 clinical cases received at the CIAT in Uruguay in 2022. **Results:** Case 1 : Male, 63 years old, from the north of the country. The patient had experienced a bee swarm sting 4 hours before while working in the countryside. There were greater than 50 stings in his body including his scalp, with edema of the face, neck and sting areas. Stingers were partially removed. Treatment with hydrocortisone, chlorpheniramine and sodium bicarbonate was administered. Laboratory results: Leukocytes 21,400/mm3, pH 7.32. Serum Creatinine 1.77 mg/dl, Blood urea nitrogen (BUN) 0.39 mg/dl. After 24 hours in the intensive care unit (ICU) the patient developed rhabdomyolysis (total creatine phosphokinase – CPK 20160 UI) and acute renal failure (creatinine 3,65 mg/dl). He died 24 hours after admission with suspected cardiac ischemia. Case 2: Male, 24 years old,from the north of the country. The patient had experienced a bee swarm sting 4 hours before while working in the countryside. More than 100 bee syings in his body. He was admitted with multiple lesions, face, eyelid and neck edema. Stingers were partially removed. Laboratory results: Leukocytes 30,000/mm3,serum creatinine 4,6 mg/dl, CPK 14308 UI. After 36 hours in ICU the patient developed acute renal failure secondary to rhabdomyolysis.(BUN1.15 mg/dl , Creatinine 5.78 mg/dl, potassium 5.24 mEq/l, Total CPK 24773 UI. After 48 hours, Hemodialysis (HD)was started. On the 4th day after admission, he had bleeding at puncture sites, nostrils and digestive tract that required vitamin K and plasma. On the 18th day after admission, BUN 1,42 mg/dl, Creatinemia 7.1mg/dl, potassium 4.45 mEq/l. On the 24th day of admission, he died with systemic multiorgan failure. **Discussion:** Both cases were admitted 4 hours after the accident, they presented an increase in CPK at 12 hours with acute renal failure and leukocytosis. Case 1 presented mycocardial ischemia and case 2 associated multiorgan failure. All these clinical manifestations are described in severe cases of poisoning by bee venom. Treatment in these cases was symptomatic. It has been reported (Medeiros et al 2019) that the release of venom from the stinger occurs in the first minutes, therefore, it is recommended to remove the stingers immediately, to avoid local complications, but this does not change the course of poisoning. **Conclusions:** In Uruguay, occupational exposure and recreational activities are risk scenarios, especially in the northern region of the country where Africanized bees are present. Easy access to tertiary centers in our country (generally < 2 hours) would establish a promising scenario, in addition to the initial clinical measures, to have antiapid serum for these poisonings (Barbosa et al 2017), this could be a treatment that changes the fatal course of these accidents.

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