BRIEF COMMUNICATION
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Analysis of paraquat in cigarette samples

Análise de paraquate em amostras de cigarro

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ABSTRACT

Introduction: The use of herbicides in different crops may leave residues, which can compromise the safety of the people who consume the derived products, as well of those environmentally and/or occupationally exposed. Paraquat is an herbicide of the bipyridyl class and it is used in diverse types of food culture besides the planting of tobacco. **Objective:** The objective of this work is to determine, by quantitative methods, the presence of Paraquat in tobacco samples in cigarettes from four high consumption brands. **Method:** A method was utilized to detect Paraquat waste by means of a colorimetric technique, since this active ingredient is reduced to a blue colored radical in the presence of 1% sodium dithionite ($Na_2S_2O_4$) in basic medium, which intensifies as much as the concentration of the product increases. The samples were subjected to spectrophotometer reading (600 nm) and compared with a standard. **Results:** The quantitative evaluation revealed residual levels of the herbicide in all samples analyzed. **Conclusions:** There is a potential for toxicity in the use of Paraquate in the population.

KEYWORDS: Paraquate; Cigarettes; Toxicity; Tobacco

RESUMO

Introdução: A utilização de herbicidas nas diferentes culturas pode deixar resíduos e comprometer a segurança das pessoas que consomem os produtos derivados, assim como aquelas expostas ambiental e/ou ocupacionalmente. O paraquate é herbicida da classe dos bipiridílicos utilizado em diversos tipos de cultura de alimentos, além do plantio de fumo. **Objetivo:** Determinar, por método quantitativo, a presença de paraquate em amostras de tabaco em cigarros de quatro marcas de grande consumo. **Método:** Foi utilizado método para detectar resíduos de paraquate por meio de técnica colorimétrica, visto que este ativo é reduzido a radical de cor azul na presença de ditionito de sódio ($Na_2S_2O_4$) a 1%, em meio básico, que se intensifica à medida que a concentração do produto aumenta. As amostras foram submetidas à leitura em espectrofotômetro (600 nm) e comparadas com um padrão. **Resultados:** A avaliação quantitativa revelou níveis residuais do herbicida em todas as amostras analisadas. **Conclusões:** Há potencial de toxicidade no uso do paraquate na população.

PALAVRAS-CHAVE: Paraquate; Cigarro; Toxicidade; Tabaco

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INTRODUCTION

Brazilian legislation defines agrochemicals as products and agents of physical, chemical or biological processes, intended for use in production, storage, protection of crops, pastures and other ecosystems, and urban, water and industrial environments. The purpose of agrochemicals is to change the composition of the flora or fauna in order to preserve them from the hazardous action of other potentially harmful living beings. Substances and products used as defoliants, desiccants, growth inhibitors and stimulants are also considered agrochemicals[1].

The increase in herbicide use in soybean crops makes Brazil the largest buyer of agrochemicals in the world. This may lead to harmful situations concerning food safety, health and the environment[2].

Paraquat is a bipyridyl herbicide commonly used in agriculture. Its denomination is due to its molecule and its atom redistribution, quaternary nitrogen atoms in the para position (para + quaternary)[3,4]. Several countries have already banned or restricted its use due to the large number of cases of accidental poisoning or suicides, and there is no antidote to its effects[5]. However, paraquat is still used in various nations, particularly in developing countries, and it is associated with high morbidity and mortality rates[6,7]. It is known and marketed as Gramoxone®, Gramocil®, Agroquat®, Gramuron® and Paraquol®.It is currently produced and marketed by Syngenta (Zeneca) worldwide.

It was first synthesized in 1882, but its herbicidal property was discovered in 1955. It started being used in 1962[4]. It is chemically known as 1,1'-dimethyl-4,4'-biperidine-dichloride and is a post-emergent toxic herbicide, i.e. it is applied after weed growth, when the crop is already developed. It is used in several food crops and also in tobacco, as it acts as a drying agent, blocking seed germination and seedling establishment[6].

Tobacco crops stand out because of the hard work they require and because they receive a large volume and variety of agrochemicals at different growth stages. With that in mind, cigarettes may be one of the products with accumulation of paraquat residues. Another potential health problem due to the use of agrochemicals is the reuse of the land used for tobacco growing for other crops; farmers plant vegetables and fruit both in the trays of tobacco seedlings and in rows along the plantation[8].

Determining agrochemical residues in tobacco is essential for us to understand human exposure and thus reduce the occurrence of these situations, enabling regulatory decisions to establish the safety of products of agricultural origin[9].

Paraquat is considered one of the agents of higher toxicity specific to the lungs. It may be absorbed through ingestion, inhalation or skin contact. It causes liver, kidney and irreversible pulmonary fibrosis, and it can also lead to death from respiratory failure within two weeks of exposure in severe cases[10].

When it comes to its pharmacokinetics, most of the agent is eliminated by the kidneys (about 80%) within the first days. After this period, the elimination route is not fully understood[11].

Its toxic action has not been fully elucidated, but there is a possibility that it may lead to the formation of unstable free radicals that are harmful to cells and lead to the breakdown of antioxidant enzyme systems that catalyze reactions to neutralize free radicals and the formation of reactive oxygen species, including catalase, vitamins C and E, and glutathione peroxidase. We do not yet know what the specific target of free radicals is - deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or other cellular constituents[11,12].

Chronic effects affect workers in contact with paraquat for long periods of time, and there are records of Parkinson's disease as a consequence of this exposure[13].

Through the analytical method, we can quantitatively check the substances found in tobacco – paraquat, in particular. Depending on the results, this can guide the approach to the assessment of toxicity due to residual contamination by herbicides used in the cultivation of tobacco.

This research aims to verify the residual presence and amount of paraquat in cigarette samples through quantitative analysis.

METHOD

Four commercially available cigarette brands were used as samples. In this study they were called A, B, C and D. The analyses were performed during the month of October 2016. The steps for developing the method were as follows.

Sample preparation

The sample was obtained from five cigarettes of each of the four popularly known brands selected for the study. The filters and the paper wrapping of the cigarettes were removed, and we considered only the internal content as samples. The experiments were carried out in two stages: first, after being macerated and mixed with water, the contents were vortexed, filtered and centrifuged to obtain the extract. A simplified colorimetric method was used to detect residues: the paraquat is reduced to a blue color radical in the presence of 1% sodium dithionite ($Na_2S_2O_4$) in a basic medium of sodium hydroxide (NaOH) 0.1N that intensifies as the product concentration increases[14].

A 1% solution of $Na_2S_2O_4$ was then prepared. Immediately afterward, 10 ml of the sample (extract) was pipetted and 50 ml of the $Na_2S_2O_4$ solution were added with 0.1N NaOH solution in a volumetric flask, filled with purified water to the volume of 100 ml. The same procedure was repeated for all samples. After this procedure, the possible occurrence of colorimetric change (change to the bluish color) in the samples was verified. In the second step, the samples were read in a spectrophotometer - UV/VIS mini 1240 - Shimadzu® (at 600 nm wavelength), in which their respective absorbances were measured.

Standard curve preparation

We prepared a paraquat standard solution (Paraquat-Gramonone® secondary standard) in $Na_2S_2O_4$ with the combination of the internal content of the cigarettes (four brands), which were subjected to the same procedures of maceration, addition of water, vortexing, filtration and centrifugation so we could obtain an extract at a 0.2 mg/l concentration.

From this solution, we performed serial dilution in order to obtain the concentration points (0.2 mg/l, 0.4 mg/l, 0.8 mg/l, 1.2 mg/l, 1.6 mg/l and 2.0 mg/l). A share was taken from each concentration so we could read the absorbance rates in a spectrophotometer. Each scan was done in triplicate.

The absorbance results were subsequently interpolated in the standard curve for the determination of the residue concentration through the equation of the line, where y = absorbance in UA and x = concentration in parts per million (ppm). The blank was zeroed with $Na_2S_2O_4$.

As quality control we used positive control and negative control. The negative control was done in a previously tested tobacco sample in which no paraquat concentration was detected. Positive control was performed by intentional contamination of previously analyzed tobacco (negative control), added with standard paraquat.

RESULTS AND DISCUSSION

For the elaboration of the standard curve (Figure), we used the data described in Table 1.

The Figure represents the standard curve resulting from the absorbance values obtained from the serial dilution and the corresponding equation of the line.

From the equation of the line (y), we analyzed the paraquat concentrations of the cigarette samples (Table 2) as a function of the absorbance we had obtained.

From the absorbance values we could determine the concentrations (ppm) of paraquat in the analyzed cigarette brands (Table 3).

Based on the results, we could verify the presence of paraquat in the analyzed samples. Its presence in tobacco means greater toxicity risk for consumers, in addition to other cigarette components that are known to cause damage to the lungs.

Considering the harmfulness of paraquat, the authors of this study draw attention to the risks to which professionals who work directly with tobacco are subject when continuous exposed this herbicide and, therefore, the risks to the health of these workers. Furthermore, raising the population's awareness of the possible residues in cigarettes could strengthen the fight against smoking.

Chronic smoking, as well as passive smoking, is a current public health problem. Despite the large number of anti-smoking campaigns and warnings about chemical hazards in cigarette packs, there is still a very high number of smokers (10.8% of the population in 2015)[15]. The presence of paraquat suggests there is another toxic agent being consumed involuntarily by the smoking population.

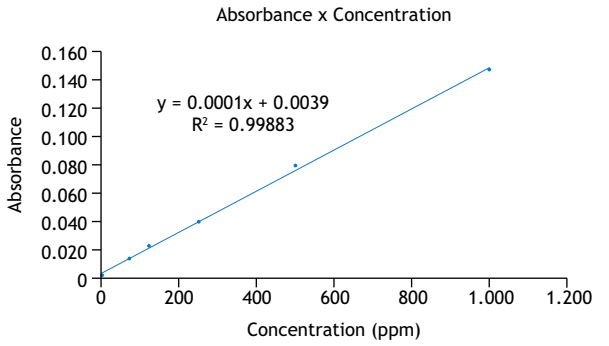

Figure. Standard curve based on the proposed dilutions

Tabela 2. Valores de absorbância, determinados em triplicada, referentes a cada marca de cigarro

Sample	Reading 1	Reading 2	Reading 3	Mean	Standard deviation
A	0.061	0.063	0.062	0.0620	0.0010
B	0.085	0.081	0.087	0.0843	0.0031
C	0.092	0.096	0.092	0.0933	0.0023
D	0.099	0.098	0.097	0.0980	0.0007

Table 3. Paraquat values in the analyzed samples

Sample	Amount of paraquat per cigarette brand
A	118.2 ppm
B	116.2 ppm
C	186.2 ppm
D	192.2 ppm

Table 1. Results of the readings obtained in spectrophotometer for the construction of the standard curve

Determination	Concentration used					
	0.2 mg/l	0.4 mg/l	0.8 mg/l	1.2 mg/l	1.6 mg/l	2.0 mg/l
	Absorbance based on the concentration					
Mean	0.00609	0.007126667	0.030833333	0.032956667	0.064333333	0.148541333
Standard deviation	0.000157162	0.000123423	0.001572588	0.003077862	0.002766231	0.008867369
Coefficient of variation	2.580662339	1.731853001	4.100285731	4.339117463	4.299840761	4.969630385

Through agriculture agents, health professionals and other public stakeholders, public administration should join forces with farmers and civil society organizations to discuss, identify and develop cross-sector actions to encourage the reduction of tobacco use. Because it is a vulnerable group, historically neglected by public policies of labor, agriculture, health or welfare, we must think of collective intervention strategies that include rural workers and their families in actions and practices of protection and promotion of health and in the planning and implementation of feasible forms of physical and social replication[8].

It should be noted, however, that there are no scientific papers reporting the presence of paraquat in cigarettes. With that in mind, we highlight a study carried out with fruits and vegetables that shows a high percentage of irregularities in the use of agrochemicals. The presence of residues that are prohibited or above permitted limits, with consequent harmful effects on the environment and public health, indicates the need to create more efficient public policies to control and monitor the use of agrochemicals[16].

CONCLUSIONS

Paraquat is potentially toxic for the population; it is therefore necessary to identify and quantify the presence of this herbicide both in food and in other crops, to avoid contact contamination during product handling.

Surveillance agencies must rigorously assess human exposure in crops and the levels of contamination in tobacco before it is marketed, since these levels may worsen lung damage caused by smoking.

The management and use of this herbicide should be reassessed. We should also look for alternatives to control spontaneous species in tobacco crops without using poison and avoiding its irregular and undue use in tobacco fields.

We should also note that the validated method was adequate for the analyses we performed. In addition to being inexpensive, fast, simple and applicable to determining residues in cigarettes, it can be used in small and medium-sized toxicology laboratories.

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Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.