Revista Visa em debate
sociedade, ciência & tecnologia

BRIEF COMMUNICATION
https://doi.org/10.22239/2317-269x.01341

Quantitative determination of Paraquat in apples of Gala, Fuji, Argentina and Verde types

Determinação de Paraquate em maçãs do tipo Gala, Fuji, Argentina e Verde

Renata Antunes Estaiano de Rezende[I]

Maria Aparecida Nicoletti[II,*]

Allan Gabriel de Angeles Pereira[I]

Bruno Oliveira Serafim[I]

Danilo Francisco da Paixão[I]

Joyce dos Santos Diniz[I]

Leticia Clug Bastos Gama[I]

Luan Passos da Silva[I]

Juliana Weckx Peña Muñoz[III]

André Rinaldi Fukushima[IV]

[I] Universidade São Judas Tadeu, São Paulo, SP, Brasil

[II] Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, SP, Brasil

[III] Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, SP, Brasil

[IV] Faculdade de Ciências da Saúde IGESP (FASIG), São Paulo, SP, Brasil

* **E-mail:** nicoletti@usp.br

Received: Jun 26, 2019
Approved: Dec 12, 2019

ABSTRACT

Introduction: Brazil is one of the world leaders in consumption of agrochemicals, generating billions of reais/year and this fact is increasing. The herbicide Paraquat has a highly hydrophilic molecule and it is used in the control of weeds in many types of plantations. **Objective:** To determine the presence and content of Paraquat in apples using the following types from the national market: Gala, Fuji, Argentina and Verde, with or without peel and to analyze the residual Paraquat content according to the Brazilian and European legislation. **Method:** An adapted methodology (UV/VIS spectrophotometer) was used in apples' types with or without peel. **Results:** Considering the studied types of apples, although Paraquat was present in all of them, only the Verde and Gala types would be approved by European regulations. Argentina type would be approved only by the limits stated by Anvisa and Fuji type would not be approved by none of the regulations. **Conclusions:** There is a need for constant inspection by analyzing the content of pesticides in food as well as legislation that establishes mandatory information about the type of agrochemical used and the corresponding residual limit allowed.

KEYWORDS: Herbicides; Apple; Paraquat; Agrochemical; Maximum Allowable Concentration; Public Health

RESUMO

Introdução: O Brasil é um dos líderes mundiais no consumo de agrotóxicos, gerando bilhões de reais por ano em valores crescentes. O herbicida Paraquate é uma molécula considerada altamente hidrofílica utilizada em plantações de milho, soja, batata, maçã, entre outros. **Objetivo:** Determinar a presença e o teor de Paraquate em maçãs do tipo Gala, Fuji, Argentina e Verde, em presença e ausência de casca e discutir seus limites aceitáveis nas legislações vigentes brasileira e europeia. **Método:** Determinar a quantidade de Paraquate por meio de metodologia adaptada utilizando um espectrofotômetro UV/VIS em amostras de maçãs já mencionadas com e sem casca. **Resultados:** Dos tipos estudados de maçãs considerando a fruta com a casca, embora o Paraquate estivesse presente em todas, somente os tipos Verde e Gala estariam aprovados pela regulamentação da Comissão Europeia, a Argentina seria aprovada pelos limites da Anvisa e a Fuji seria reprovada pelas duas regulamentações. **Conclusões:** Há a necessidade de constante fiscalização por meio da análise de teor de agrotóxicos em alimentos, bem como de novas legislações que estabeleçam a obrigatoriedade da informação do tipo de agrotóxico utilizado no produto e o limite residual permitido.

PALAVRAS-CHAVE: Herbicida; Maçã; Paraquate; Agrotóxico; Limite Máximo de Resíduo; Saúde Pública

INTRODUCTION

According to data from the Brazilian Association of Public Health (Abrasco), from 2002 to 2011, the consumption of pesticides in Brazil increased 42% (599.5 million liters in 2002 and 852.8 million liters in 2011), which is an alarming percentage[1].

Among the countless pesticides found in food in Brazil, paraquat[2] stands out. Its molecule, 1,1'-dimethyl-4,4'-bipyridine dichloride, acts on the human body causing intense oxidative stress, which causes damage to cells and tissues due to the amount of reactive species generated by the redox mechanism in the presence of nicotinamide and adenine dinucleotide phosphate (NADPH)[3]. This damage is caused by poisoning through the oral route, cutaneous absorption or inhalation[4,5].

In most cases, the cause of death because of paraquat poisoning is pulmonary fibrosis[5,6], since it spreads quickly throughout the body via bloodstream and fixes quickly and early in tissues with intense vascularization[5]. Because of its high poisoning capacity, paraquat has been undergoing toxicological reassessments since 2008, although it was banned by the Brazilian National Health Surveillance Agency (Anvisa) only in 2017. Today, farmers are in a transition process with a legal term of three years to adapt to the established measure[7].

The ban on paraquat after the transition period established by Anvisa[8] is seen with great concern by Brazil's agricultural sector, since it is used on a large scale in crops of grains, vegetables and fruit, including apples.

In Brazil, apple production concentrates on two cultivars: Gala and Fuji, which account for about 90% of the planted area[9]. Among the other cultivars, there is the so-called Argentine apple[10], and the Green apple, known as Granny Smith[9].

The objective of this study was to determine the residual amount of paraquat in apples of the Gala, Fuji, Argentine and Green types (Brazil-grown), with and without skin, marketed in the Mooca district, in the city of São Paulo (SP). The limits established by Anvisa for the domestic market and by the European Commission – which were stricter – were adopted as reference values, considering that a large share of Brazil's apple exports goes to the European market.

METHOD

The determination of paraquat content in apples (Gala, Fuji, Argentine and Green) was adapted from the method recommended by Pereira and Dantas[11]. This method is based on the complexation reaction between paraquat and sodium dithionite, which results in the formation of a bluish colored compound, whose absorbance was determined in a spectrophotometer (Shimadzu UV/VIS mini 1240 – calibrated), at 600 nm of wavelength. Sample preparation/dilution was performed according to the recommendations of Zweig[12]. To obtain the standard curve, a solution was prepared with 0.021 g of paraquat in one liter of ultrapure water (Milli Q system) (solution 1). From solution 1, 10 ml were transferred to a 500 ml volumetric flask and the volume was topped up with ultrapurified water (Milli Q system), corresponding to the concentration of 0.4 mg/L (solution 2); from it, 5 ml were transferred to a final volume of 250 ml, obtaining a 0.008 mg/L solution (solution 3). Afterward, the volume of 10 mL of solutions 2 and 3 and aliquots of 1, 2, 4, 6, 8 and 10 mL of solution 1 were pipetted (equivalent to 8 solutions with 0.00008; 0.004; 0.02; 0.04; 0.08; 0.12; 0.16 and 0.2 mg of paraquat), which were transferred to 100 mL volumetric flasks. In each of the flasks, a volume of 10 mL of 1% sodium dithionite solution in 0.1N NaOH was added, and the volume was topped up with ultrapurified water (Milli Q system). The juices from the apple samples were prepared with the introduction of the whole fruit, that is, with skin (six units of each type), in a Walita Juicer RI1861 centrifuge. This was also done with peeled apples (six units of each type). The samples had their skin manually peeled with the aid of a knife in a thickness of approximately 1.5 mm – 2.0 mm. They were then washed in running water and introduced in the same device, according to each type used in the study. To perform the matrix effect, 10 mL of each sample juice was added, totaling 80 mL, the so-called mix. For spectrophotometer reading, we used 2 mL of the mix + 2 mL of all previous aliquots. The analyses were performed in triplicate.

To ensure the reliability of the proposed analytical method to quantify paraquat by spectrophotometry, three analytical curves were built (with six concentration points – Figure). Some method validation parameters were determined, such as linear range, linearity (Table 1), sensitivity, detection limit and equipment quantification limit. Linear range: analytical curves were built for paraquat in the concentration range from 0.40 to 200.0 μgL-1 and, when the line started to have a linearity deviation, this concentration value was taken as the maximum value to be determined.

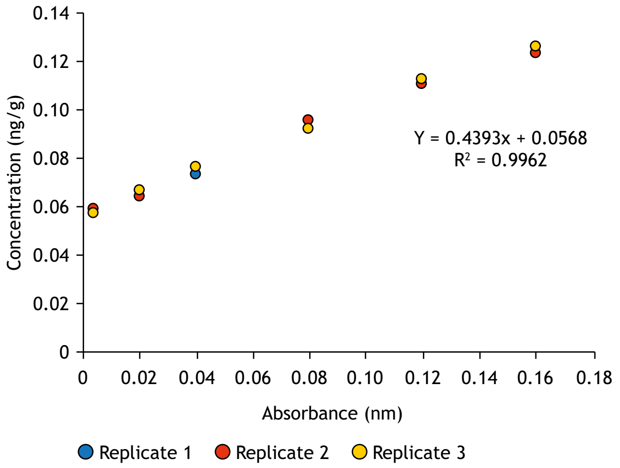
RESULTS AND DISCUSSION

For the analysis of the results, we used the means obtained for each type and condition. Each item had its analysis done in triplicate, standard deviation (SD) and coefficient of variation (CV), as shown in Table 2.

For reference values, we considered those determined by the European Commission guidelines[13] (international standard, with a stricter limit), which is 0.02 mg/kg, and also by Anvisa[14] (domestic standard), which is 0.05 mg/kg, that is, the domestic limit is higher and may have an impact on apple exports depending on the purchasing country.

We noticed the presence of residual levels of paraquat in all samples analyzed.

However, considering the legislation in force in Brazil, only Fuji apples (with skin) had residues above the limits allowed by Anvisa's regulation, which determines a Maximum Residue



Source: Prepared by the authors, 2019.

Figure. Determination of linearity and linear equation.

Limit (MRL) of 0.05 mg/kg for apples[14]. They could not, therefore, be marketed in Brazil; in Fuji apples without skin, we observed that the limit found is in compliance only with the domestic standard. Apples of the Green type (with and without skin), Gala (with and without skin) and Argentine without skin met the limits set by the European Commission and by Anvisa.

A report resulting from the Program for Analysis of Pesticide Residues in Food (PARA)[15] presented the results of the monitoring of pesticide residues in food from 2013 to 2015. In all, 12,051 samples of 25 food products of plant origin, representative of the Brazilian population's diet, were analyzed: 764 apple samples were analyzed. Of these, 683 samples were considered satisfactory, with ten showing no residues of the researched pesticides and 673 showing residues in concentrations equal to or less than the MRL. In total, 47 different pesticides were detected among the 185 researched. Of the analyzed samples, 68 presented pesticides not authorized for apple growing[15].

There is a need for greater control over the use of pesticides like paraquat, which in addition to the health problems that may be triggered by exposure or ingestion, can also cause the death of farmers because of pesticide poisoning[16].

According to Anvisa, there can only be a healthy diet if the food is safe for consumption, that is, free from contamination[17]. Therefore, food consumers must have information

Table 1. Values obtained to determine the linearity of the method.

Mean (of the three determinations)	Standard deviation	Coefficient of variation (%)
0.057333333	0.000577350	1.007006283
0.064666667	0.001154701	1.785619389
0.074333333	0.001154701	1.553408796
0.094000000	0.001732051	1.842607242
0.111333333	0.000577350	0.518578086
0.124666667	0.001527525	1.225287619
0.134333333	0.000577350	0.429789282
0.094380952	0.001043004	1.194613814

Source: Prepared by the authors, 2019.

Table 2. Results obtained from the four types of apple samples, with and without skin.

Apple samples	Weight (g)	1st analysis (mg/kg)	2nd analysis (mg/kg)	3rd analysis (mg/kg)	Mean (mg/kg)	SD	CV
Green without skin	217.51	0.012	0.012	0.012	0.012	0.00037425	2.999915834
Green with skin	217.51	0.018	0.019	0.018	0.018	0.00052412	2.796472982
Gala without skin	113.12	0.01	0.010	0.010	0.010	8.16326E-05	0.80693854
Gala with skin	113.12	0.012	0.012	0.012	0.012	4.36102E-05	0.36112158
Argentine without skin	159.02	0.012	0.012	0.012	0.012	0	0
Argentine with skin	159.02	0.026	0.028	0.025	0.026	0.000119267	4.44653877
Fuji without skin	128,80	0.040	0.037	0.038	0.039	0.000162628	4.15639109
Fuji with skin	128,80	0.051	0.055	0.055	0.054	0.000249158	4.57990743

SD: standard deviation; CV: coefficient of variation.
Color legend: red: failure to comply with both legislations; yellow: approval according to the limits established by Anvisa; blue: approval by both legislations, considering that, for the European Commission, the limit established is lower and, therefore, once it complies with the maximum allowed value, it would also be approved by the Brazilian legislation.
Source: Prepared by the authors, 2019.

shared by suppliers/dealers on the type of pesticide used and the MRL in the food to be purchased. However, we need legislation to make this come about and enable consumers to make informed purchases.

The consumer's right to information and the right to health cannot be harmed and, therefore, additive regulations must be established in that industry[18].

CONCLUSIONS

Among the apple samples, only three were above the limit established for exports and one sample was unsuitable for both exports and domestic distribution. Discussion is necessary to create legislation that establishes mandatory information regarding the pesticide used in the food, as well as the MRL established in the product to be purchased and the indication of the amount present in the food.

REFERENCES

1. Carneiro FF, Augusto LGS, Rigotto RM, Friedrich K, Búrigo AC, organizadores. Dossiê Abrasco: um alerta sobre os impactos dos agrotóxicos na saúde. Rio de Janeiro: Escola Politécnica de Saúde Joaquim Venâncio; 2015[acesso 2 maio 2018]. Disponível em http://www.abrasco.org.br/dossieagrotoxicos/wp-content/uploads/2013/10/DossieAbrasco_2015_web.pdf
2. Ferreira I. Estudo indica agrotóxicos mais frequentes em alimentos consumidos no Brasil. Jornal da USP. 6 dez 2016[acesso 2 maio 2018]. Disponível em https://jornal.usp.br/?p=58224
3. Martins T. Herbicida paraquat: conceitos, modo de ação e doenças relacionadas. Semina Cienc Biol Saude. 2013;34(2):175-86. https://doi.org/10.5433/1679-0367.2013v34n2p175
4. Schmitt GC, Paniz C, Grotto D, Valentini J, Schott KL, Pomblum VJ et al. Aspectos gerais e diagnóstico clínico laboratorial da intoxicação por paraquat. J Bras Patol Med Lab. 2006;42(4):235-43. https://doi.org/10.1590/S1676-24442006000400003
5. Serra A, Domingos F, Prata MM. Intoxicação por paraquat. Acta Med Port. 2003;16:25-32.
6. Elnga N, Merlin C, Le Guern R, Kom-Tchameni R, Ducrot YM, Pradier M et al. Clinical features and prognosis of paraquat poisoning in French Guiana. Medicine. 2018;97(15):1-7. https://doi.org/10.1097/MD.0000000000009621
7. Agência Nacional de Vigilância Sanitária – Anvisa. Voto da diretoria de regulação sanitária N° 56, Processo N° 25351.056773/2013-21: analisa a reavaliação toxicológica do ingrediente ativo paraquate. Brasília: Agência Nacional de Vigilância Sanitária; 2017[acesso 28 set 2018]. Disponível em http://portal.anvisa.gov.br/documents/219201/2782895/Voto+Renato+Paraquate/fa409d90-a520-4302-9815-f39b683da509
8. Agência Nacional de Vigilância Sanitária – Anvisa. Resolução RDC N° 190, de 30 de novembro de 2017. Altera a resolução da diretoria colegiada N° 177, de 21 de setembro de 2017, que dispõe sobre a proibição do ingrediente ativo paraquate em produtos agrotóxicos no país e sobre as medidas transitórias de mitigação de riscos. Diário Oficial União. 1 dez 2017.
9. Serviço Brasileiro de Apoio às Micro e Pequenas Empresas – Sebrae. O cultivo e o mercado da maçã. Brasília: Serviço Brasileiro de Apoio às Micro e Pequenas Empresas; 2018[acesso 10 jun 2019]. Disponível em http://www.sebrae.com.br/sites/PortalSebrae/artigos/o-cultivo-e-o-mercado-da-maca,ea7a9e665b182410VgnVCM100000b272010aRCRD
10. Bobbio PA, Bobbio FO. Pigmentos naturais: introdução à química de alimentos. 2a ed. São Paulo: Varela; 1995.
11. Pereira W, Dantas RL. Qualidade de tubérculos de batata-semente tratados com paraquat e o desenvolvimento de uma metodologia simplificada de detecção de resíduos do herbicida. Planta Daninha. 1995;13(1):32-8. https://doi.org/10.1590/S0100-83581995000100006
12. Zweig G. Analytical methods for pesticides, plant growth, regulators, and food additives. Nova York: Academic Press; 1963.
13. European Commission - EU. EU pesticides database: current MRL values. Brussels: European Commission; 2016[acesso 28 set 2018]. Disponível em http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.CurrentMRL&language=EN&pestResidueId=172
14. Agência Nacional de Vigilância Sanitária – Anvisa. Índice monográfico: paraquate. Brasília: Agência Nacional de Vigilância Sanitária; 2018[acesso 12 jun 2019]. Disponível em http://portal.anvisa.gov.br/documents/111215/117782/P01%2B%2BParaquate.pdf/0ec98b27-1b1f-49de-b788-7bbaa9dfb191
15. Agência Nacional de Vigilância Sanitária – Anvisa. Programa de análise de resíduos de agrotóxicos em alimentos para relatório das análises de amostras monitoradas no período de 2013 a 2015. Brasília: Agência Nacional de Vigilância Sanitária; 2016[acesso 13 jun 2019]. Disponível em http://portal.anvisa.gov.br/documents/111215/0/Relat%C3%B3rio+PARA+2013-2015_VERS%C3%83O-FINAL.pdf/494cd7c5-5408-4e6a-b0e5-5098cbf759f8
16. Santana VS, Moura MCP, Nogueira FF. Occupational pesticide poisoning, 2000-2009, Brazil. Rev Saude Publica. 2013;47(3):598-606. https://doi.org/10.1590/S0034-8910.2013047004306

17. Agência Nacional de Vigilância Sanitária — Anvisa. Alimentação saudável: fique esperto! 2a ed. Brasília: Agência Nacional de Vigilância Sanitária; 2008.

18. Teixeira, T. Saúde e direito à informação: o problema dos agrotóxicos nos alimentos. Rev Dir Sanit. 2017;17(3):134-59. https://doi.org/10.11606/issn.2316-9044.v17i3p134-159

Authors' Contributions

All authors participated in the conception, planning (study design), acquisition, analysis, interpretation of data and writing of the paper. All authors approved the final draft of the paper.

Conflict of Interest

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