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Ultratrace detection of organophosphate pesticide in maize flour: risk assessment and food safety

Detecção por ultratraços de pesticida organofosfato em farinha de milho: avaliação de risco e segurança dos alimentos

Maria Larisse Pinheiro Uchoa [a], Séfura Maria Assis Moura [b], Renata Chastinet Braga [c], Luana Guabiraba Mendes [c], Jéssica Roberta Pereira Martins [c], Yago Queiroz dos Santos [c], Maria Aparecida Liberato Milhome [c,*]

[a] Nutrition Course, Federal Institute of Education, Science and Technology of Ceará (IFCE), 62930-000, Limoeiro do Norte, Ceará, Brazil
[b] Specialization Course in Health and Food Safety, Federal Institute of Education, Science and Technology of Ceará (IFCE), 62930-000, Limoeiro do Norte, Ceará, Brazil
[c] Postgraduate Program in Food Technology, Federal Institute of Education, Science and Technology of Ceará (IFCE), 62930-000, Limoeiro do Norte, Ceará, Brazil

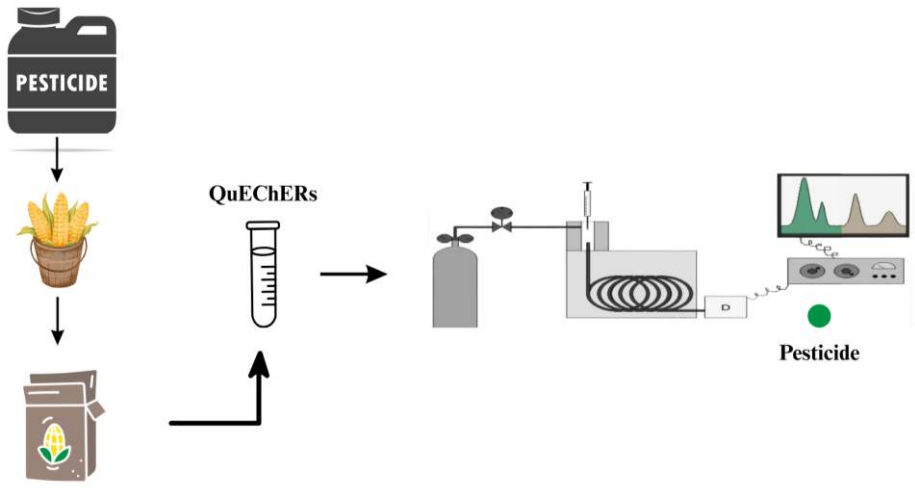
Abstract
The expansion of agriculture plays an important role in the Brazilian economy. Maize represents one of the country's main commodities. However, the intensive use of pesticides to increase productivity has generated environmental and human health impacts due to the toxicity of these products. This study aimed to use a validated methodology by gas chromatography coupled to mass spectrometry (GC-Q/MS) for analyzing pesticides in maize flour and estimating the risk of contamination in this product. The results showed that, among the compounds investigated in the samples, only the Fenitrothion residue was detected, at levels below the FAO/ANVISA maximum residue limits (MRLs). Although this compound belongs to the group of organophosphate pesticides, capable of causing serious damage to the central nervous system, when associated with prolonged exposure in people with low body weight, the values found for the risk quotient (RQ) in this study (1.32 and 1.52 for men and women, respectively) did not present a potential risk to the consumer. Based on the results obtained in the present study, it is suggested to expand the toxicological monitoring of maize products to ensure food security.

Keywords: Food Matrices. Cereals. Chromatography. Multiresidue. Risk quotient. Toxicity.

Resumo
A expansão da agricultura desempenha um papel importante na economia brasileira. O milho representa uma das principais commodities do país. No entanto, o uso intensivo de pesticidas para aumentar a produtividade tem gerado impactos ambientais e à saúde humana devido à toxicidade desses produtos. Este estudo teve como objetivo utilizar uma metodologia por cromatografia gasosa acoplada à espectrometria de massas (GC-Q/MS) validada para análise de pesticidas na farinha de milho e estimar o risco de contaminação neste produto. Os resultados mostraram que, dentre os compostos investigados nas amostras, apenas o resíduo de Fenitrotiona foi detectado, em níveis abaixo dos limites máximos de resíduos (LMRs) da FAO/ANVISA. Embora este composto pertença ao grupo dos pesticidas organofosforados, capazes de causar sérios danos ao sistema nervoso central, quando associado à exposição prolongada em pessoas com baixo peso corporal, os valores encontrados para o quociente de risco (QR) neste estudo (1,32 e 1,52 para homens e mulheres, respectivamente) não apresentaram risco potencial ao consumidor. Com base nos resultados obtidos no presente estudo, sugere-se ampliar o monitoramento toxicológico de produtos de milho para garantir a segurança de alimentos.

Palavras-chave: Matrizes Alimentares. Cereais. Cromatografia. Multirresíduo. Quociente de Risco. Toxicidade.

Graphical Abstract



*Corresponding author: Maria Aparecida L. Milhome. Email Address: maria.milhome@ifce.edu.br
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ROYALDATASET

1. Introduction

Agriculture has been expanding worldwide and assuming a decisive role in the gross domestic product of several countries. Within this context, maize monoculture stands out as a strategic component of the global agricultural economy, being cultivated on a large scale and generating important social and commercial impacts (Sandhu et al., 2020). As a highly consumed cereal in different regions of the world, maize has broad technological applicability and participates in the formulation of a variety of processed foods, in addition to serving as an energy-dense ingredient for nutritional purposes (Euan-Pech et al., 2024).

Its versatility allows the production of healthy flours and derived products, making maize the second most cultivated cereal for human consumption worldwide. However, maintaining productivity in large-scale farming requires intensive management practices and the recurrent use of chemical inputs to control pests and reduce crop losses (Hasnaki et al., 2023). In Brazil, this scenario is even more expressive due to the country's agricultural vocation and its consolidated position among the world's largest food exporters (Souza et al., 2023).

As a result of this expansion, Brazil has become one of the leading global consumers of agrochemicals. Between the main national commodities, maize production showed a growth of more than 193% between 1990 and 2018, placing the country in the third position of the world production ranking (Artuzo et al., 2019; Mariuzzo, 2019). While this increase reinforces Brazil's economic importance, it also raises concerns about chemical input residues that may remain in grains and derived foods.

Pesticide applications are efficient for preventing agricultural losses; however, they are recognized sources of contamination in food matrices, including cereals. These compounds can remain stable after harvest, persist through processing steps, and reach the final consumer, posing risks to human health (Zhu et al., 2024). Previous studies have demonstrated that processed plant foods can retain detectable levels of pesticides, even after heat treatment and storage (Costa et al., 2023; Milhome et al., 2019). González-Curbelo et al. (2017) evaluated dimethoate, terbufos, disulfoton, and pirimiphos-methyl in roasted maize flour (gofio) and showed that degradation during three months of storage ranged from 34% to 86%, indicating that dissipation varies according to matrix composition and pesticide class. Similarly, Mahugija et al. (2017) detected twelve pesticides in maize grains and eight in maize flour using GC-MS, reinforcing that contamination can persist even after processing and reach levels associated with public-health concern.

Considering the continuous growth in maize consumption in Brazil and the potential exposure of consumers to pesticide residues through derived products, it becomes essential to monitor food quality. Therefore, the present study aimed to investigate the presence of pesticide residues in maize flour marketed in Brazil and to estimate the potential health risks associated with the consumption of these products.

2. Materials and Methods

2.1. Reagents and solutions

Certified pesticide standards (purity >99.0%) of 20 pesticides were purchased from Sigma-Aldrich (Brazil) and Dr. Ehrenstorfer (Brazil). The chromatographic grade solvents were purchased from Merck (Brazil) and J.T. Baker (USA). Anhydrous magnesium sulfate was purchased by Vetec (Brazil), as well as the reagents sodium chloride, trisodium citrate dihydrate. Sodium hydrogen citrate sesquihydrate was purchased from Sigma-Aldrich (Brazil) and Bondesil Primary Secondary Amine (PSA) 40 µm from Supelco (USA).

Individual stock solutions of pesticides (1,000 µg mL^{-1}) in methanol or acetone were prepared for the analytical process. A stock solution (1 µg mL^{-1}) containing the 20 pesticides evaluated in the present study was used for validation tests. The calibration standards were used by diluting the multicomponent solution in ethyl acetate: cyclohexane (1:1). Finally, matrix-matched curves (0.01 to 1.0 mg kg^{-1}) were obtained by spiking blank matrix extracts with known concentrations of pesticides and stored at 4 ºC.

2.2. Sample preparation

The study was developed using processed maize flour, purchased in Limoeiro do Norte, Ceará, Brazil. Samples were received, processed, and stored, as recommended by the SANTE/11312/2021 Guidelines (European Commission, 2021). Initially, the samples are ground, homogenized, and weighed. After extraction, partitioning, and cleaning steps were performed.

The processed maize flour samples were prepared for the extraction and analysis of pesticide residues. Initially, extraction was carried out using the QuEChERS multiresidue method from Alcântara et al. (2019), briefly exemplified in **Fig. 1**. Buffer salts were added to the crushed sample to extract the substances of interest from the matrix, followed by a clean-up and injection directly into the chromatograph. The analyses by QuEChERS and GC-Q/MS were performed (in duplicate) at the Ceará Center for Industrial Quality and Technology (NUTEC).

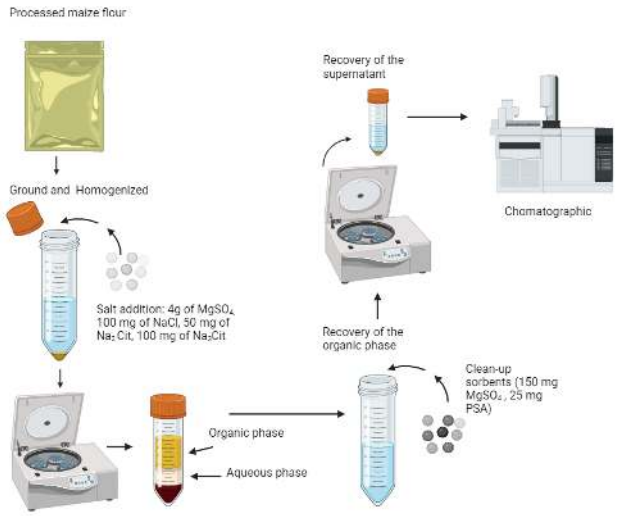

Fig. 1 Schematic illustration of the steps of the QuEChERs method for pesticide analysis in processed maize flour

2.3. Chromatographic conditions

Chromatographic analyses were carried out with a gas chromatograph coupled to a single quadruple mass spectrometer (GC-Q/MS, DSQII model, Thermo, USA). **Table 1** shows the analytical parameters of 20 pesticides studied. Separation of the pesticides was performed using the RTX-5ms (30 m × 0.25 mm × 0.25 µm) capillary column and helium (99.999%) carrier gas at a constant flow of 1 mL min^{-1}. The injection temperature was 250 °C, and 1 µL volume was injected in splitless mode (1 min). Oven temperature program was as follows: initial temperature of 100 °C (1 min), 15 °C min^{-1} to 180 °C, then 4 °C min^{-1} rate to 280 °C (14 min) (Milhome et al., 2019). The mass spectrometry of 20 pesticides was analyzed to identify the presence of the

components present in maize flour, as well as to determine their quantification in the sample.

Table 1 List of 20 pesticides analyzed by gas chromatography coupled to a single quadrupole mass spectrometer (GC-Q/MS), chemical groups, and their respective fragment masses of the present study.

Pesticide	Chemical Group	Fragments (m/z)		
Esfenvalerate	Pyrethroid	125	152	167
Molinate	Thiocarbamate	126*	187	98
Trifluralin	Dinitroaniline	306*	264	290
Lambda-cyhalothrin	Pyrethroid	198	141	181*
Atrazine	Triazine	200*	215	173
Parathion-methyl	Organophosphate	263*	125	109
Ametryn	Triazine	227*	212	170
Fenitrothion	Organophosphate	125	260	277*
Malathion	Organophosphate	173*	125	127
Chlorpyrifos	Organophosphate	97	197*	199
Buprofezin	Non-Classified	105*	172	106
Kresoxim-methyl	Strobilurin	116*	131	206
Triiazophos	Organophosphate	161*	162	172
Cis-propiconazole	Triazole	173	259	175
Trans-propiconazole	Triazole	173	259	175
Bifenthrin	Pyrethroid	181	165*	166
Pyriproxyfen	Non-Classified	136*	96	226
Cis-permethrin	Pyrethroid	183*	163	127
Trans-Permethrin	Pyrethroid	183*	163	127
Boscalid	Carboxamide	140*	112	142

* Quantitative fragments. Source: PPDB: Pesticide Properties DataBase (University of Hertfordshire, 2025) and ANVISA (2025).

2.4. Validation tests

Validation of the analytical method is an essential requirement to guarantee data reproducibility and comparison. In particular, despite the high levels of sensitivity and specificity widely recognized by the gas chromatograph coupled to a single quadruple mass spectrometer, validation is necessary to demonstrate that a method is fit for purpose. Therefore, validation must demonstrate the identity and concentration of the analyte, taking into account the effects of the matrix that constitutes the sample, providing a statistical characterization of the recovery results and other performance criteria of the analytical method (Maestroni et al., 2018). To ensure efficiency, both quality assurance (QA) and control (QC) procedures have been incorporated into the analytical method.

Thus, to meet the method validation parameters, the SANTE 2021 guidelines (European Commission, 2021) were followed, such as LOD (limit of detection), LOQ (limit of quantification), linearity, precision, and accuracy, for the 20 pesticides under study. The linearity range was established by injection, adopting an n = 3. Subsequently, a standard solution containing the selected 20 pesticides in a range of concentrations between 0.01 to 1.0 mg kg^{-1}, calibration curves (combined matrix), and correlation coefficients (R^2). To check the influence of interferers on the pesticide sample, selectivity was studied.

To determine the limit of quantification (LOQ), the samples were injected with successively diluted solutions, calculated by the signal-to-noise ratio (S/N) 10:1. Accuracy and precision were determined from analysis of samples with recovery experiments enriched with pesticides at concentrations 0.05, 0.1, and 0.8 mg kg^{-1} (n=7). Intermediate precision (interday precision) was determined through analysis of spiked samples in concentrations 0.05, 0.1, and 0.8 mg kg^{-1} (n=7), carried out by the same analyst on three different days. The results found were expressed as relative standard deviation-RSD (%).

2.5. Dietary risk assessment

Risk assessment for human health aims to estimate the nature and probability of adverse effects resulting from consumption or exposure to pesticides in the long term. The Risk Quotient (RQ) was determined considering the Estimated Daily Intake (EDI) of pesticide residues present in maize flour and the legally permitted daily intake (ADI - Acceptable Daily Intake), according to **Eqs. 1** and **2**:

$$EDI\ (mg/(kg.day)) = [C(kg)*PF*R(mg/kg)]/BW(kg) \quad \textbf{Eq. 1}$$

$$RQ = EDI/ADI*100 \quad \textbf{Eq. 2}$$

Where *C* is the estimated national consumption (kg) of processed maize flour (SUAS, 2020); *PF* is processing factors for Fenitrothion (FAO/WHO, 2007), *R* the concentration of pesticide residue found in the sample analyzed and *BW* the Average Body Weight of a certain population portion (kg) according to the Household Budget Survey, carried out in Brazil between 2008 and 2009, by the Brazilian Institute of Geography and Statistics (IBGE, 2010). Processing factors (PF) represent the ratio between the levels of residues in the processed and unprocessed product. These allow us to determine if residues increase (> 1) or decrease (< 1) during the process. In general, these depend on the physical and chemical characteristics of the residues, especially on their solubility in water and their octanol-water partition coefficient (Cámara et al., 2020).

The RQ risk quotient is then obtained by **Eq. 2**, using the EDI value determined by **Eq. 1** and the ADI for the pesticide Fenitrothion (University of Hertfordshire, 2025). This is a widely used guideline value for long-term daily ingestion exposure. RQ ≤ 100 represents an acceptable risk to human health, and RQ > 100 indicates that the risk of a pesticide to humans is unacceptable, *i.e.*, higher RQ values indicate higher risks to human health.

3. Results and Discussion

3.1. Validation tests and sample analysis

The use of selected ion monitoring (SIM) mode in the quantification of pesticides in multiresidue methods has been efficient, since it offers greater selectivity when compared to full-scan acquisition mode. By monitoring only specific ions of interest, SIM reduces matrix interference, increases the signal-to-noise ratio, and therefore allows more reliable detection of multiclass pesticides even at low concentrations. **Table 2** presents the results of linearity, LQs, precision (%), accuracy (%), the concentration of the pesticide present in the flour samples, and the Maximum Residue Limit (MRLs)

In the validation tests, linearity was assessed using the calibration curves of each component and the correlation coefficient (R^2) values (>0.99, except for esfenvarelate). Method LQs ranged from 0.0006 and 0.0330 mg kg^{-1} (<MRLs). The precision (relative standard deviation - RSD%) and accuracy (mean recovery %) for 19 pesticides analyzed showed satisfactory results, ranging from 5.50 to 11.9 and from 83.8 to 114.6, respectively. Thus, all pesticides studied reached the established standards, and no modifications to the method were necessary, except for esfenvarelate.

Table 2 Validation parameters and quantification of pesticides in a commercial sample of maize flour by GC-Q-/MS

Pesticide	Curves	R^2	LQ (mg kg^{-1})	Precision RSD (%)	Accuracy (%)	Sample Average (mg kg^{-1})	MRL (mg kg^{-1})	
							ANVISA (2025)	FAO (2024)
Esfenvalerate	y = 60556.9x + 32610.7	0.9862	0.0330	13.2	134.6	nd	1.0	ne
Molinate	y =16374424.6x + 110342.5	0.9993	0.0033	5.50	98.4	nd	ne	ne
Trifluralin	y = 5737016.7x – 37834.0	0.9978	0.0066	10.90	114.6	nd	0.05	ne
Lambda-cyhalothrin	y = 1789969.2x – 408.2	0.9990	0.0165	10.70	88.5	nd	1.0	0.02
Atrazine	y = 10084082.2x + 202943.7	0.9994	0.0033	9.57	111.4	nd.	0.25	ne
Parathion-methyl	y = 25029279.4x – 547405.7	0.9999	0.0016	10.22	99.2	nd	na	ne
Ametryn	y = 13793637.9x + 175174.8	0.9979	0.0016	9.70	99.2	nd	0.04	ne
Fenitrothion	y = 4598837.8x – 593.5	0.9994	0.0082	11.90	114.3	0.0125	1.0	ne
Malathion	y = 52109088.4x + 299558.2	0.9972	0.0006	9.70	103.13	nd	8.0	0.05
Chlorpyrifos	y = 6956179.9x + 2394357.6	0.9999	0.0006	11.40	107.7	nd	0.1	ne
Buprofezin	y = 5383221.0x – 52510.5	0.9979	0.0066	10.34	101.2	nd	0.01	ne
Kresoxim-methyl	y = 10685064.8x – 137460.1	0.9990	0.0066	11.43	94.8	nd	ne	ne
Triazophos	y = 9367299.5x – 187452.2	0.9999	0.0330	9.45	102.8	nd	na	ne
cis-Propiconazole	y = 4877018.2x – 1765.6	0.9994	0.0066	9.80	93.63	nd	0.1	0.05
trans-Propiconazole	y = 6113873.2x + 23989.0	0.9995	0.0066	8.93	93.60	nd	(*cis+trans)*	(*cis+trans)*
Bifenthrin	y = 11917675.8x + 834650.9	0.9988	0.0016	10.90	83.8	nd	0.02	0.05
Pyriproxyfen	y = 25225456.9x – 399749.1	0.9999	0.0016	6.83	111.6	nd	ne	ne
cis-Permethrin	y = 4117347.5x + 596089.7	0.9999	0.0033	8.93	108.3	nd	0.05	ne
trans-Permethrin	y = 9462852.5x – 220550.9	0.9999	0.0033	7.97	104.3	nd	0.05	ne
Boscalid	y = 8530138.9x – 170398.2	0.9998	0.0033	8.83	107.5	nd	ne	ne

nd, not detected; ne, not established; na, not authorized

3.2. Pesticide Residues in Maize Flour

Of the 20 pesticides analyzed, only fenitrothion was detected. This organophosphate pesticide is present in maize flour at an average concentration of 0.0125 mg kg^{-1} (12.5 µg kg^{-1}), detected in two study samples. A similar study by Ogah & Coker (2012) on pesticide residues in maize found the presence of contaminants chlorpyrifos (maximum 62.1 µg kg^{-1}), fenitrothione (maximum 15.8 µg kg^{-1}), and pirimiphos methyl (maximum 3185.1 µg kg^{-1}) at levels higher than those found in the present study.

A more recent study by Mahugija et al. (2017) on pesticide residues in raw maize grain and processed flour from selected areas of Tanzania found higher concentrations of organophosphate pesticides, including chlorpyrifos, pirimiphos methyl, and fenitrothione. Fenitrothione was detected at 132.1 µg kg^{-1}, while cypermethrin had its highest concentration at 14 µg kg^{-1}. In both studies, the detection technique by gas chromatography coupled with mass spectrometry (GC-MS) was used.

3.3 Health risk assessment

The human health risk assessment seeks to estimate the nature and probability of adverse effects resulting from exposure to pesticides. Therefore, the potential risk of the presence of fenitrothion residues found in the maize flour samples analyzed in this study was assessed by determining the EDI and comparing it with its respective ADI (Brasil, 2020). The EDI was calculated using the corresponding processing factors (PF) for Fenitrothion (FAO, 2024). The ADI value for Fenitrothion is equivalent to 5.0 µg. kg^{-1} d^{-1} (University of Hertfordshire, 2025).

The half-life of these insecticides varies greatly depending on the nature of the compound. Some metabolites are more toxic than their parent substance. Tests carried out on laboratory animals show that Fenitrothion is rapidly absorbed from the gastrointestinal tract, distributed, and metabolized. In context, it is of great importance to investigate the consequences that may occur with the exacerbated use of products containing this pesticide since organophosphates are known to have a history of harm to human health. The EDI and RQ results for consumers (men and women) are described in **Table 3**.

The binding of organophosphates with acetylcholinesterase (AChE) in the Central Nervous System leads to phosphorylation of the enzyme, and this reaction is not easily reversible. Phosphated AChE is relatively stable and, depending on the groups attached to the phosphorus atom, becomes irreversibly inhibited (Barboza et al., 2018).

Table 3 Estimated Daily Intake (EDI), Acceptable Daily Intake (ADI), and Risk Quotients (RQ) of the pesticide Fenitrothion per body mass of consumers (men and women).

Adult	C (kg)	PF*	R (µg. kg^{-1})	BW (kg)	EDI (µg kg^{-1} day^{-1})	ADI (µg kg^{-1} day^{-1})	RQ
Men	0.10	3.95	12.5	75	0.0658	5.0	1.3167
Women	0.10	3.95	12.5	65	0.0759	5.0	1.5192

Regarding the bioavailability of the pesticide Fenitrothion, although the Brazilian Ministry of Agriculture, Livestock and Supply refers to this molecule, when in tests carried out on animal models, with a low potential for bioaccumulation, it was also able to point out the speed at which this organophosphate is absorbed by the gastrointestinal tract, distributed in the circulation and metabolized in the most different organs.

Since recent studies have demonstrated the ability to inactivate AChE through covalent bonding between the pesticide and the enzyme (Barboza et al., 2018), studies suggest that even low-level exposures can generate cumulative effects, especially in vulnerable individuals with impaired regulation of action potentials in nerve tissue, leading to long-term deleterious effects. Since a good part of the excretion takes place in the first 24 hours after its absorption, mainly via urine (88 – 94%), Fenitrothion is a molecule that presents difficult traceability in a long period after exposure, which can difficult sampling and analysis of its prolonged effects difficult according to the ingested levels.

This difficulty could be circumvented with the use of highly sensitive analytical tools, among which mass spectrometry stands out, capable of pointing out the presence of the contaminant even at very low levels, as those detected in the present study.

Once the MRL of a given substance is known and the average daily consumption is verified, it becomes possible to verify how different anthropometric variables, such as weight or height, influence EDI values. While the relationship between EDI and height remains linear for the same Body Mass Index (BMI), with variation only on height (**Fig. 2**), the relationship between EDI and body weight shows an exponential graphic behavior.

In this way, it is possible to verify that, even in subtle differences in body weight, the EDI can reach considerably different values, being even more radical in situations of low weight, which occurs in cases of malnutrition. Thus, even a low MRL can produce a high EDI in low-weight individuals who may consume food associated with pesticides or other contaminants.

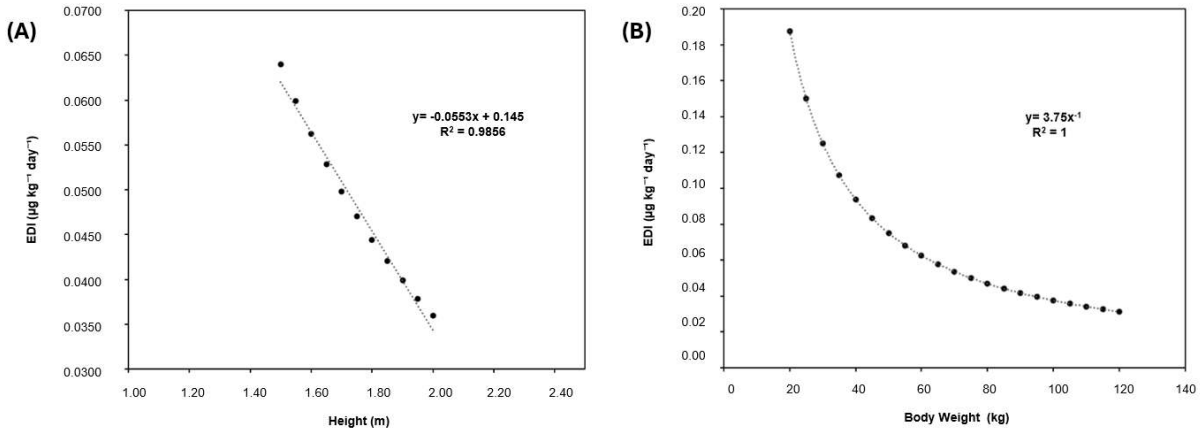

Fig. 2 Relationship between: (A) Estimated Daily Intake (EDI) and height, normalized for the same Body Mass Index (BMI); (B) Estimated Daily Intake (EDI) and body weight.

Once subjected to the toxicant more intensely, such malnourished patients may develop chronic and deleterious effects much more quickly, thus worsening the general health of the individual even more. When comparing the same MRL for different body weights, it becomes evident that even low levels of pesticide contamination can cause exponential effects of accumulation in daily consumption, which leads to being necessity a more detailed analysis, considering anthropometric parameters of the population directly involved, and not just a binary diagnosis of "above" or "below" a standard reference value.

4. Final Considerations

The QuEChERS method and GC-Q-MS were successfully validated for extracting pesticide residues present in processed maize flour, commonly consumed by the population. Furthermore, for the evaluation of 20 pesticides, it was possible to identify traces of Fenitrothion in samples of maize flour purchased in the countryside of Ceará, Brazil. The results showed an RQ value within acceptable limits. However, the study indicates the importance of continuous monitoring of fenitroniton levels in maize products, considering that organophosphates are harmful to human health and can cause damage to the Central Nervous System. Although the levels detected are below the maximum permitted limits, the results highlight the importance of continuous monitoring, especially considering more vulnerable populations. It is recommended to expand the surveillance of residues in corn derivatives in different regions of the country.

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Authors' Contributions

M.L.P.U.: Data Curation, Writing- Original preparation; S.M.A.M.: Writing- Original preparation; R.B.C.: Writing - Review & Editing, Supervision; L.G.M.: Writing- Original preparation; J.R.P.M.: Writing- Original preparation; Y.Q.S.: Writing- Original preparation; M.A.L.M.: Writing - Review & Editing, Supervision, Project administration.

Availability of data and materials

The data may be requested from the Corresponding Author at a reasonable request.

Conflicts of Interest

The authors declare that they have no competing interests.

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