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Evaluation of Acute Toxicity of Cigarette Butt Leachates in Fish: Relationship with Nicotine Concentrations Monitored by Molecular Fluorescence

Maria Carolina Talio[1,2], Fernando Ángel Giannini[1,3*]

[1]Área de Química General e Inorgánica, Facultad de Química, Bioquímica y Farmacia (FQByF), Universidad Nacional de San Luis (UNSL), San Luis, Argentina
[2]Instituto de Química de San Luis (INQUISAL-CONICET), San Luis, Argentina
[3]Instituto Multidisciplinario de Investigaciones Biológicas de San Luis (IMIBIO-CONICET), San Luis, Argentina
Email: *fagian3@gmail.com

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Abstract

Cigarette butts are among the most abundant urban residues worldwide and constitute an emerging source of aquatic pollution due to the release of nicotine and other toxic compounds into the environment. In the present work, the acute toxicity of nicotine solutions and cigarette butt leachates was evaluated using fish, specifically juveniles of *Poecilia reticulata*, under controlled and standardized laboratory conditions. Acute toxicity assays of nicotine were performed, exposing fish to increasing concentrations ranging from 0.5 to 8.0 $mg \cdot L^{-1}$ for 96 h. Mortality increased with nicotine concentration, obtaining an LC50 value of 2.0 $mg \cdot L^{-1}$ for the studied species. Additionally, cigarette butt leachates were prepared under standardized laboratory conditions and tested at different dilution percentages (in a range of 2.5% to 20 %). The highest leachate concentrations produced elevated mortality and behavioral alterations, including severe mobility impairment in exposed organisms. Nicotine concentrations in the tested solutions were determined by a previously developed molecular fluorescence methodology based on the use of a luminescent metal-organic framework sensor. The analytical results demonstrated that nicotine concentrations in cigarette butt leachates were significantly lower than the LC50 experimentally determined for pure nicotine, suggesting that the observed toxic effects are associated not only with nicotine but also with the combined action of additional toxic substances released from cigarette residues. The present study highlights the ecotoxicological relevance of cigarette butt leachates as emerging aquatic pollutants and demonstrates the usefulness of combining

biological models with fluorescence-based analytical methodologies for environmental monitoring applications.

Subject Areas

Public Health

Keywords

Cigarette Butts, Nicotine, Ecotoxicology, Molecular Fluorescence, *Poecilia reticulata*, Aquatic Pollution

1. Introduction

Tobacco consumption remains one of the leading preventable causes of disease and mortality worldwide, with more than one billion smokers globally and a continuous increase in tobacco-derived waste generation [1] [2]. Cigarette butts (CBs), the residual filters discarded after smoking, constitute one of the most abundant forms of urban litter and represent an emerging environmental problem due to their persistence and inappropriate disposal into terrestrial and aquatic ecosystems [3].

Cigarette filters are mainly composed of cellulose acetate fibers, a plastic-derived material that may persist in the environment for years before complete degradation occurs [4]. During environmental weathering and leaching processes, cigarette butts release a complex mixture of toxic substances into surrounding waters, including nicotine, polycyclic aromatic hydrocarbons (PAHs), aromatic amines, phenolic compounds, volatile organic compounds, heavy metals, and microplastic fibers [5]. Recent studies have demonstrated that cigarette butt leachates may produce adverse effects on microorganisms, plants, invertebrates, amphibians, and fish, affecting survival, mobility, oxidative stress responses, metabolic pathways, and ecosystem balance [6] [7].

Among the compounds released from cigarette residues, nicotine (NCT) is one of the most relevant due to its high abundance and recognized biological activity [8]. Nicotine is a natural alkaloid responsible for the addictive properties of tobacco products and has been detected in environmental waters impacted by tobacco waste [9]. Besides its neuroactive effects in mammals, nicotine may produce toxic effects in aquatic organisms, including behavioral alterations, developmental toxicity, physiological stress, and mortality [10]. However, several investigations suggest that the toxicity associated with cigarette butt leachates cannot be exclusively attributed to nicotine concentration alone, indicating the contribution of multiple contaminants to the observed toxic effects [11].

In recent years, increasing attention has been focused on the ecotoxicological evaluation of cigarette butt leachates in aquatic environments. Fish constitute highly valuable biological models for toxicity studies because they allow rapid assessment of lethal and sublethal effects under controlled experimental conditions [12]. In particular, *Poecilia reticulata* has been extensively employed in ecotoxicological assays due to its sensitivity to pollutants, easy laboratory maintenance, short reproductive cycles, and suitability for acute toxicity testing [13]. Furthermore, behavioral responses such as loss of mobility or abnormal swimming patterns are considered sensitive indicators of aquatic contamination [14].

Specifically, our working group has used this biomarker in multiple evaluations of different compounds [15]-[20].

On the other hand, monitoring nicotine concentrations in environmental waters represents an important analytical challenge, especially at trace levels. Conventional analytical methodologies such as high-performance liquid chromatography and gas chromatography coupled to mass spectrometry provide adequate sensitivity but frequently require expensive instrumentation, complex sample treatment, and high operational costs [21]-[23]. In this context, molecular fluorescence methodologies have emerged as promising alternatives because of their simplicity, sensitivity, rapid response, and lower implementation costs [24]. Recently, luminescent metal-organic frameworks (MOFs) have attracted considerable attention as fluorescent sensors for environmental and toxicological analysis due to their remarkable optical properties and high selectivity toward target analytes [25] [26].

Considering these aspects, the aim of the present work was to evaluate the acute toxicity of nicotine solutions and cigarette butt leachates using juveniles of *P. reticulata* as biological models under controlled and standardized laboratory conditions. Additionally, nicotine concentrations in the evaluated systems were monitored employing a previously developed fluorescence methodology based on a luminescent MOF sensor [14]. The relationship between nicotine concentration and the observed toxicological effects was analyzed in order to contribute to the understanding of the environmental impact associated with cigarette butt residues as emerging aquatic pollutants.

2. Materials and Methods

2.1. Chemicals

Nicotine analytical standards and reagents employed for fluorescence measurements were the same as those previously described in the developed MOF-based methodology for nicotine determination [14].

2.2. Biological Models and Maintenance Conditions

Juveniles of *P. reticulata* (0.5 - 1.0 cm in length and up to 10 days old) were used as biological models for acute toxicity assays. Fish were obtained by reproduction in our laboratory from adult specimens maintained under routine aquarium conditions commonly employed for ornamental freshwater fish. Juveniles were maintained in aquarium water at approximately 25˚C under the natural light/dark cycle of the laboratory and were acclimated under these conditions prior to the experiments. During the acclimation period, fish were monitored daily to ensure normal health and behavior. During the 96 h acute toxicity assays, organisms were not fed in order to avoid alterations in water quality and potential interference with toxicity measurements. No artificial aeration was provided during the exposure period.

For the acute toxicity assays, a static exposure procedure based on the methodology recommended by the U.S. Fish and Wildlife Service was used, with modifications to reduce the volume of test solutions and the number of organisms required. Fish were exposed to the corresponding nicotine solutions or cigarette butt leachate dilutions for 96 h. Mortality and behavioral alterations were evaluated at 24, 48, 72, and 96 h. Dead organisms were counted and removed at each observation time. The final mortality percentage recorded after 96 h exposure was used to estimate the LC50 values for nicotine and cigarette butt leachates.

2.3. Preparation of Cigarette Butt Leachates

Cigarette butt leachates were prepared from commercially available filtered cigarettes containing blond tobacco, representative of conventional cigarette products widely consumed by young and adult smokers. Cigarettes were smoked to the filter under standardized laboratory conditions in order to minimize variability associated with smoking conditions and puffing behavior. After smoking, all residual tobacco and burnt paper were carefully removed, and only the cigarette filters were used for leachate preparation.

Four cigarette filters were placed in 500 mL of bidistilled water and maintained at room temperature for 48 h to obtain the corresponding leachates. After the extraction period, the resulting solutions were used to prepare leachate dilutions of 20, 10, 5, and 2.5% (v/v) for acute toxicity assays. All solutions were prepared immediately before biological exposure experiments.

2.4. Acute Toxicity Assays

Acute toxicity assays were performed using juveniles of *P. reticulata* under controlled laboratory conditions. Experimental systems consisted of 10 fish per container, and all assays were carried out in duplicate.

For nicotine toxicity evaluation, organisms were exposed to nicotine concentrations ranging from 0.5 to 8.0 $mg \cdot L^{-1}$ for a period of 96 h. Mortality and behavioral alterations were recorded at 24, 48, 72, and 96 h during the exposure period.

Additionally, acute toxicity assays were performed using cigarette butt leachates at different dilution percentages (20%, 10%, 5% and 2.5%). Behavioral alterations and mortality were evaluated during exposure periods up to 96 h. Organisms were considered dead when a complete absence of mobility and response to external stimulation was observed.

2.5. Fluorescent Nicotine Determination

Nicotine concentrations in cigarette butt leachates were determined using a previously developed and validated molecular fluorescence methodology based on the fluorescence enhancement of a Eu-Tb-PSA metal-organic framework sensor [14]. Fluorescence measurements were performed at $\lambda em = 611$ nm using $\lambda exc = 280$ nm under the optimized experimental conditions previously reported.

The analytical method exhibited a limit of detection (LOD) of 12.7 ng·L^{-1}, a limit of quantification (LOQ) of 38.5 ng·L^{-1}, and a linear working range between 38.5 and 8.95×10^3 ng·L^{-1} ($R^2 = 0.9956$). Method accuracy was previously evaluated by recovery studies in water samples contaminated with cigarette butt leachates, obtaining recoveries between 97 and 104%, demonstrating adequate applicability for nicotine determination in this type of matrix [14].

2.6. Statistical Analysis

Mortality results were expressed as percentages obtained from duplicate experiments. LC50 values were estimated after 96 h of exposure by Probit analysis using OriginPro software. The LC50 was calculated from the concentration–mortality relationship, and the corresponding confidence interval was considered when applicable. Graphical representations and data treatment were also performed using OriginPro software.

3. Results and Discussion

3.1. Acute Toxicity of Nicotine in *Poecilia reticulata*

The acute toxicity of nicotine was evaluated by exposing juveniles of *P. reticulata* to increasing concentrations of the alkaloid over a 96 h period. Mortality results demonstrated a clear concentration-dependent toxic effect. No mortality was observed at nicotine concentrations of 0.5 and 1.0 mg·L^{-1}, whereas exposure to 2.0 mg·L^{-1} resulted in 50% mortality. Complete mortality was observed at concentrations of 4.0 and 8.0 mg·L^{-1}.

Probit analysis of the mortality data yielded a 96 h LC50 value of 2.0 mg·L^{-1} for nicotine in juvenile *P. reticulata* (See Table 1).

Table 1. Acute toxicity of nicotine in juveniles of *Poecilia reticulata* during 96 h exposure.

Nicotine Concentration (mg·L^{-1})	Dead Fish (n = 10)	Mortality (%)
0.0	0	0
0.5	0	0
1.0	0	0
2.0	5	50
4.0	10	100
8.0	10	100

The experimental behavior observed agrees with previous reports describing the toxicological effects of nicotine on aquatic organisms, including fish embryos and juveniles [10]. Nicotine is known to interfere with neurotransmission processes through interaction with nicotinic acetylcholine receptors, potentially affecting mobility, respiration, and survival in exposed organisms [8] [10].

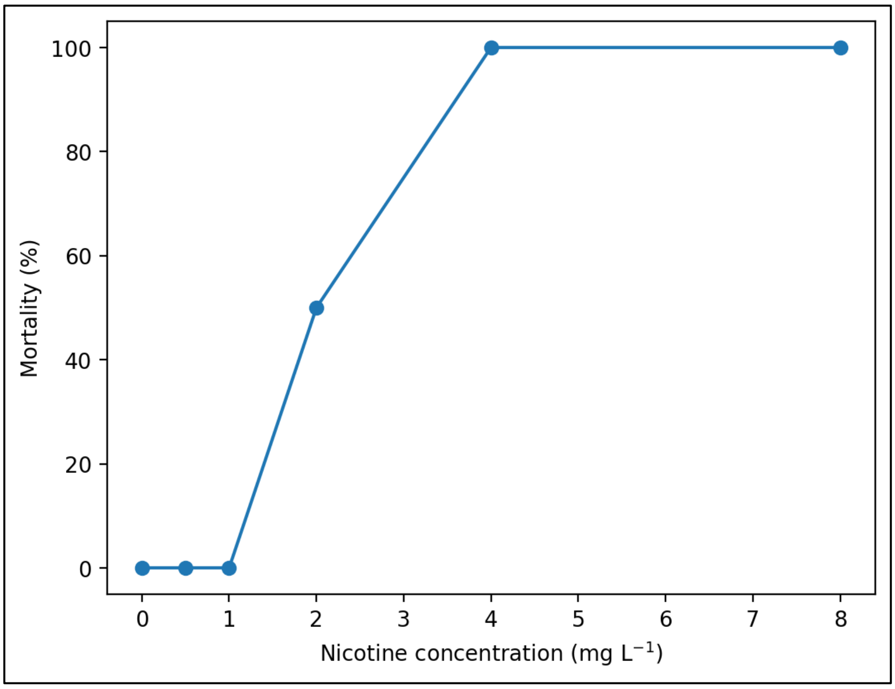

Figure 1. Acute toxicity of nicotine in juveniles of *Poecilia reticulata* after 96 h exposure. Mortality percentage increased as nicotine concentration increased, showing a concentration-dependent toxic effect. A LC50 value of 2.0 mg·L^{-1} was experimentally determined for the studied species.

The obtained LC50 value indicates that nicotine exhibits considerable acute toxicity toward *P. reticulata*, confirming its relevance as an environmental contaminant in aquatic systems impacted by tobacco-derived residues (See **Figure 1**). However, environmental cigarette butt leachates contain complex mixtures of contaminants whose toxicological effects may exceed those expected from nicotine concentration alone [3]-[5].

3.2. Acute Toxicity of Cigarette Butt Leachates

Acute toxicity assays employing cigarette butt leachates revealed elevated toxicity even at relatively low dilution percentages. The systems containing 20% leachate produced complete mortality within the first 24 h of exposure, while 10% systems showed progressive mortality reaching values of 60% after 96 h. Lower toxicity was observed for 5% leachates, whereas no mortality was detected for the 2.5% systems.

For clarity, leachate samples were identified according to their dilution level as follows: A and Ad (20% leachate), B and Bd (10% leachate), C and Cd (5% leachate), and D and Dd (2.5% leachate), where "d" indicates the corresponding duplicate experiment (See **Table 2**).

Table 2. Mortality (%) of *P. reticulata* exposed to cigarette butt leachates during 96 h.

Sample	Leachate Concentration (%)	24 h	48 h	72 h	96 h
Control	0	0	0	0	0
A	20	100	100	100	100
Ad	20	100	100	100	100
B	10	40	60	60	60
Bd	10	20	40	60	60
C	5	0	10	10	20
Cd	5	0	5	10	20
D	2.5	0	0	0	0
Dd	2.5	0	0	0	0

Ad, Bd, Cd, and Dd correspond to duplicate experiments.

In addition to mortality, important behavioral alterations were observed during exposure experiments. Fish exposed to 10% leachates exhibited severe mobility impairment after prolonged exposure periods. Interestingly, surviving organisms recovered normal swimming behavior after replacement with clean water within approximately 12 h, suggesting reversible neurotoxic or physiological effects associated with the exposure conditions (See Figure 2).

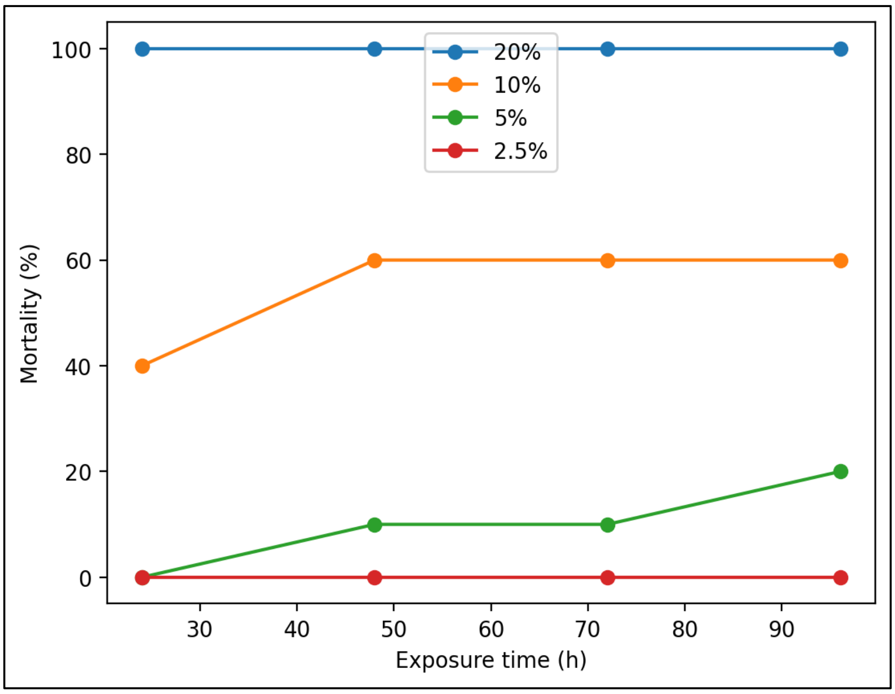

Figure 2. Mortality of *P. reticulata* exposed to cigarette butt leachates during 96 h. Different leachate concentrations (20%, 10%, 5% and 2.5%) produced distinct toxicological responses. The highest mortality values were observed for 20% and 10% leachates, while lower concentrations produced reduced or negligible mortality.

The observed toxicological effects are consistent with previous investigations demonstrating that cigarette butt leachates may adversely affect aquatic organisms due to the release of multiple hazardous substances [5]-[7]. Besides nicotine, cigarette residues contain polycyclic aromatic hydrocarbons, phenolic compounds, aromatic amines, volatile organic compounds, and toxic metals capable of producing acute and chronic toxicity in aquatic ecosystems [3]-[5].

The rapid mortality observed at high leachate concentrations confirms the ecotoxicological relevance of cigarette butt residues as emerging aquatic pollutants. Considering the enormous global production of cigarette waste and its widespread disposal into urban drainage systems, rivers, and lakes, cigarette butts constitute an important source of environmental contamination requiring increased monitoring and public awareness [1] [3].

3.3. Fluorescent Monitoring of Nicotine in Cigarette Butt Leachates

Nicotine concentrations present in cigarette butt leachates were determined employing the previously developed molecular fluorescence methodology based on the Eu-Tb-PSA luminescent MOF sensor [14]. The analytical methodology allowed sensitive quantification of nicotine in all tested systems.

Table 3. Nicotine concentrations determined in cigarette butt leachates by molecular fluorescence methodology.

Sample	Nicotine Concentration ($mg \cdot L^{-1}$)
A	0.14
Ad	0.15
B	0.07
Bd	0.06
C	0.028
Cd	0.025
D	0.015
Dd	0.013

The obtained concentrations ranged between 0.013 and 0.15 $mg \cdot L^{-1}$, depending on leachate dilution percentage. As expected, higher leachate concentrations produced higher nicotine levels. However, the experimentally determined nicotine concentrations were substantially lower than the LC50 value obtained for pure nicotine (See **Table 3**).

The comparison between experimentally determined nicotine concentrations and observed mortality constitutes one of the most relevant findings of the present work. Although nicotine was successfully quantified in all leachates, the measured concentrations were significantly lower than the toxic concentration required to produce equivalent mortality effects when nicotine was evaluated individually (See **Figure 3**).

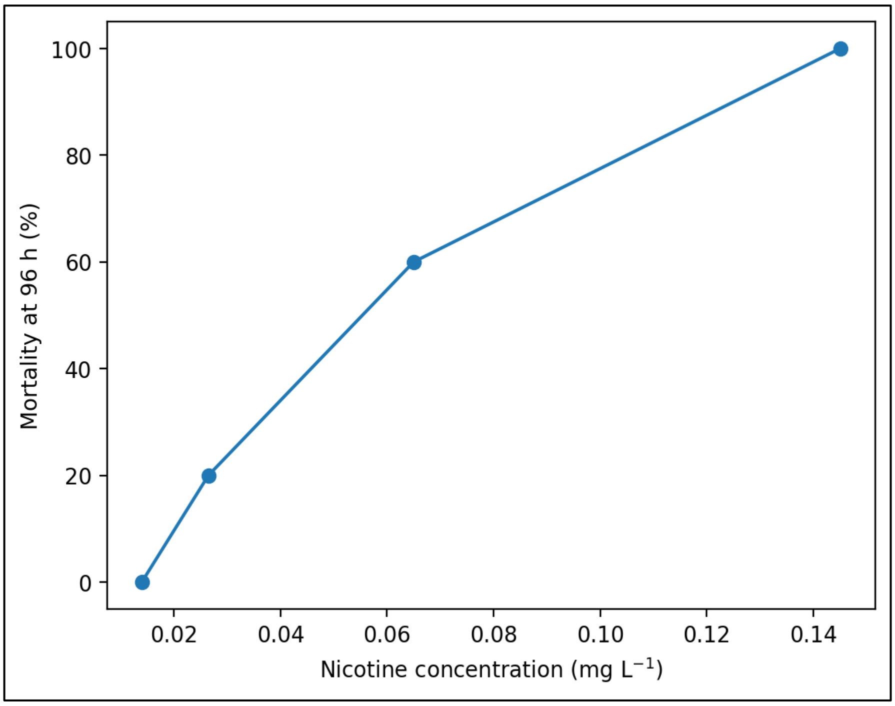

Figure 3. Relationship between nicotine concentration quantified in cigarette butt leachates and observed mortality in *P. reticulata*. Although nicotine was detected in all leachate systems by molecular fluorescence methodology, the quantified concentrations were substantially lower than the LC50 value experimentally obtained for pure nicotine, suggesting that nicotine alone cannot account for the observed mortality and that additional toxic compounds contribute to the ecotoxicological effects.

[Nicotine] leachates = 0.013 - 0.15 mg·L^{-1} vs. LC50 (nicotine) = 2.0 mg·L^{-1}.

These results strongly suggest that the toxicity associated with cigarette butt leachates cannot be exclusively attributed to nicotine concentration. Instead, the observed effects likely arise from the combined action of multiple toxic substances released during the leaching process. Similar conclusions have been proposed by other authors investigating cigarette butt toxicity in aquatic systems [5] [7] [11].

Furthermore, the present study demonstrates the usefulness of combining biological assays with fluorescence-based analytical methodologies for environmental monitoring purposes. The developed analytical approach provides a rapid and sensitive tool for nicotine determination, while biological models allow direct evaluation of ecotoxicological effects under environmentally relevant conditions.

Overall, the obtained results reinforce the importance of considering cigarette butt residues as emerging environmental contaminants with significant potential impact on aquatic ecosystems. Additionally, the study highlights the necessity of implementing environmental education and waste management strategies aimed at reducing the inappropriate disposal of cigarette-derived residues into natural waters.

4. Conclusions

The present study demonstrates that cigarette butt leachates constitute an important source of aquatic toxicity capable of affecting the survival and behavior of bioindicator *P. reticulata* juveniles under short exposure periods. Acute toxicity assays revealed a clear concentration-dependent effect, with elevated mortality observed for the highest leachate concentrations evaluated.

The experimentally determined LC50 value for nicotine confirmed the intrinsic toxicity of this alkaloid toward aquatic organisms. However, nicotine concentrations quantified in cigarette butt leachates by the developed molecular fluorescence methodology were significantly lower than the toxic concentration required to produce equivalent mortality effects when nicotine was evaluated individually. This finding strongly suggests that the toxicity associated with cigarette butt leachates is not exclusively attributable to nicotine, but rather to the combined action of multiple toxic compounds released from cigarette residues.

The work also demonstrates the usefulness of combining biological assays with fluorescence-based analytical methodologies for environmental monitoring purposes. The previously developed MOF-based fluorescence methodology allowed sensitive determination of nicotine in complex aqueous systems, providing a valuable analytical tool for monitoring contamination associated with tobacco-derived residues.

Overall, the obtained results reinforce the ecotoxicological relevance of cigarette butt residues as emerging aquatic pollutants and highlight the necessity of implementing environmental education, waste management strategies, and monitoring programs aimed at reducing the environmental impact associated with the inappropriate disposal of cigarette-derived waste into aquatic ecosystems.

Furthermore, the present study provides a preliminary basis for future investigations involving additional biological models, chronic exposure assays, behavioral biomarkers, and the evaluation of other toxic compounds associated with cigarette butt leachates.

Ethical Statement

All experimental procedures involving animals were carried out according to institutional guidelines for laboratory animal care and handling and were approved by the CICUAL (Institutional Committee for the Care and Use of Laboratory Animals) of the Universidad Nacional de San Luis (UNSL), Argentina (Approval Resolution No. 291/2025).

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Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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