Official Title:
Design of a multicomponent intervention to reduce the risk of exposure to high concentrations of fluoride in children in Huila, Colombia

NCT Number:
Not yet assigned

Document type:
Study Protocol with Statistical Analysis Plan and Informed Consent Form

Document Date:
September 22, 2025

Sponsor:
Universidad Antonio Nariño (UAN)

The design of this trial protocol complies with the SPIRIT statement (Standard Protocol Items: Recommendations for Intervention Trials).
A randomized controlled trial of parallel groups and two arms (intervention and non-intervention groups) is proposed, with a 1:1 allocation ratio to determine the effectiveness of the multicomponent intervention in reducing the risk of exposure to high concentrations of fluoride in children (Fig. 1).

Intervention and control conditions.

Intervention.
The program theory with changes and expected outcomes is presented in a logic model (Fig. 2).

Implementation of the intervention.
This intervention includes five components. Table 1 lists the components, thematic modules for implementation, and follow-up to the intervention.

1. *Installation of physical barriers:* a reverse osmosis water filter will be installed in the intervention group's kindergarten, and staff will be trained in its operation, maintenance, and care to ensure its effectiveness.

Educational sessions.
Designed under the Dialoguing Interestructuring pedagogical model, which works on the three dimensions of the individual: cognitive, socio-affective, and practical.

2. *Educational sessions for parents:* two weekly 45-minute meetings will be held over a period of six weeks to cover three thematic modules. The first aims to educate parents about the natural presence of fluoride in water and its relationship to adverse effects such as dental and skeletal fluorosis. The second consists of two sessions focused on identifying sources of fluoride intake such as food, salt, water, and dental products, and the adoption of safe practices in the home environment. The third module consists of two sessions that provide guidance on the use of fluoride in oral health, fluoridated toothpaste, adult supervision during brushing, oral hygiene habits, and regular dental visits (Table 2).

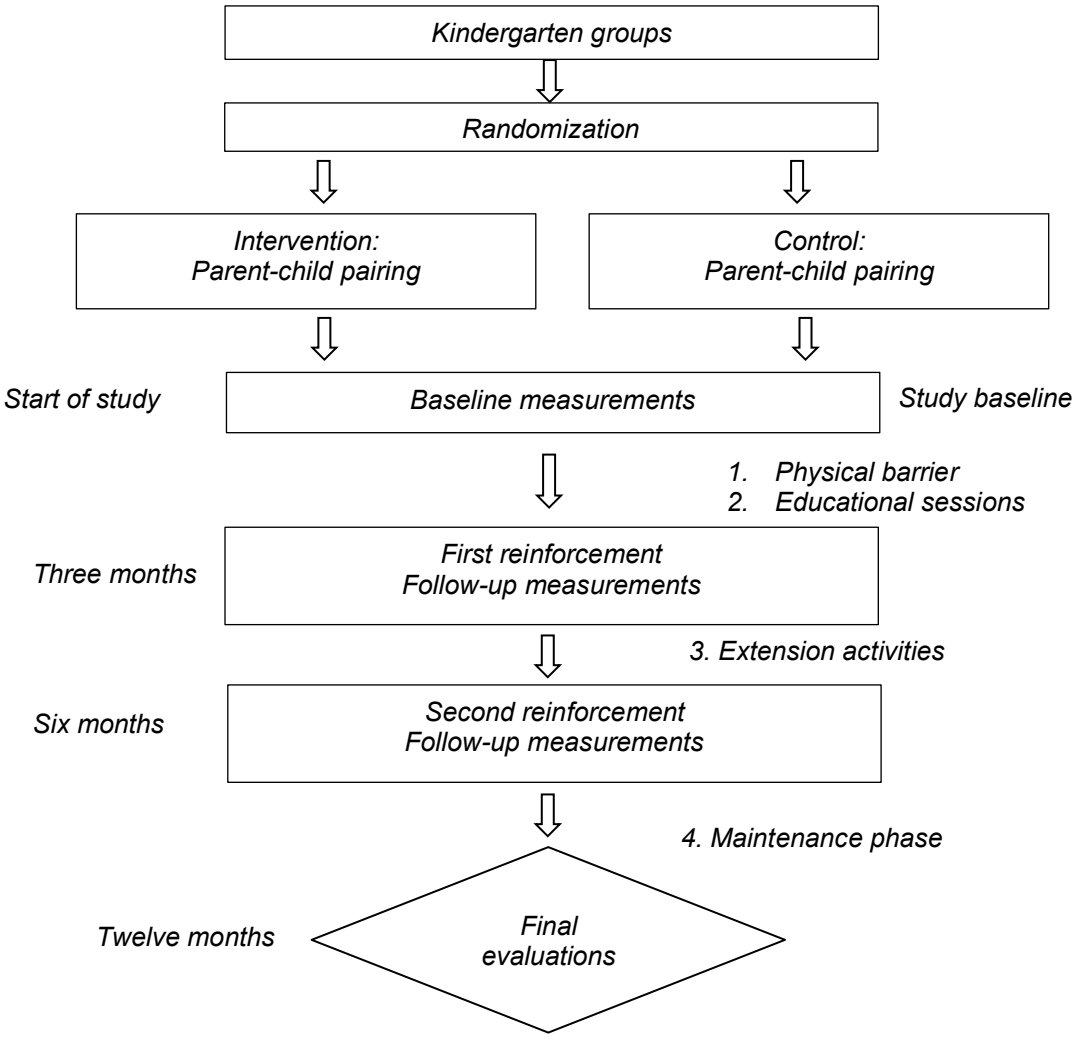

Fig. 1. Study design outline to be applied in both municipalities.

3. *Educational sessions for children:* consists of two sessions to be held over two weeks. It is designed with a playful-pedagogical approach, adapted to the cognitive level and development of early childhood. It includes question and answer games, a practical experiment on the right amount of toothpaste, an educational board game, role-playing about visits to the dentist, and supervised toothbrushing practice. Each educational session lasts 30 minutes and will be conducted simultaneously with module 3 for parents, in order to synchronize the content between parents and children and facilitate reinforcement of learning at home (Table 3)

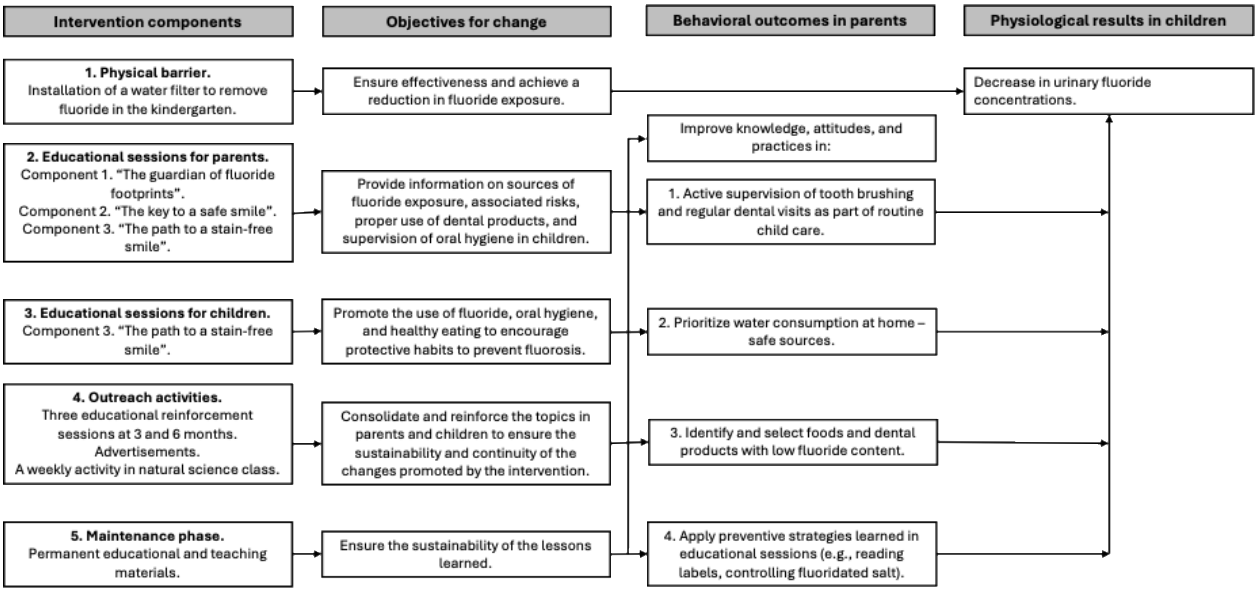

Fig. 2. Logical model of the intervention.

4. *Outreach activities:* To ensure the sustainability of the intervention, kindergarten teachers will be trained. The goal is for them to integrate oral health content into their planning, replicating the lessons weekly with the children over a period of six months. The research team will conduct three reinforcement sessions three and six months after implementation. In addition, posters and brochures with key messages will be used to reinforce preventive practices in the school environment and maintain the visibility of the program (Table 1).

5. *Maintenance phase: (Table 1) The population will have free access to educational resources through the "Camaleón Sonriente cuida tus dientes" (Smiling Chameleon Takes Care of Your Teeth) platform, via the official link:* https://sites.google.com/uan.edu.co/camalensonrientecuidatusdiente/p%C3%A1gina-principal?authuser=0

Strategies for monitoring and maintaining adherence to the intervention.

To ensure the reliability of the intervention in each of the components of the educational intervention, the entire team will be trained in advance for the implementation of the intervention, technical assistance will be provided to the team, and the intervention will be supervised by the intervention coordinator (Table 4).

Control conditions.

The control group intervention will include training on the cariogenic diet, flossing, and handwashing, provided to the parent-child pair. The training will be monitored, but the progress of the implementation will not be monitored.

Table 1. Components, implementation, and monitoring of the multicomponent intervention to reduce the risk of fluoride exposure in children.

Components of the multicomponent intervention.	Thematic module of the multicomponent intervention to be implemented.	Follow-up (baseline, 3, 6, and 12 months) to the intervention.
1. Physical barrier.	Filter installation, use, care, and maintenance.	Measurement of fluoride concentrations in water.
2. Educational sessions for parents.	Thematic module 1. “The guardian of fluoride footprints.” Thematic module 2. “The key to a risk-free smile.” Thematic module 3. “The path to a stain-free smile.”	Evidence of the activities carried out in each session will be collected. Survey application.
3. Educational sessions for children.	Thematic module 3. The path to a stain-free smile. Educational session 4: directions of the fluoride mission and session 5: walking safe paths.	Measurement of fluoride concentrations in urine.
4. Outreach activities.	Three reinforcement sessions will be held at three and six months. Advertisements. A weekly activity with the teacher in the classroom.	Survey application. Activity checklist. Technical assistance.
5. Maintenance phase.	Engaging participants in the “Smiling Chameleon Takes Care of Your Teeth” educational model	Final survey. Activity checklis.

Expected results.

The multicomponent intervention is expected to be more effective than the conventional strategy in reducing fluoride exposure in children.

Primary outcomes.

Decrease in urinary fluoride concentrations in children in the intervention group.

Secondary outcomes.

Positive changes in knowledge, attitudes, and practices (KAP) to reduce the risk of fluoride exposure in children.

Reduction in fluoride concentrations in water.

Table 2. Educational sessions for parents in the multicomponent intervention to reduce the risk of fluoride exposure in children and learning objectives.

Sessions	Learning objectives
Thematic module 1. "The guardian of fluoride footprints."	
Session 1: The journey of fluoride in water.	- Explore water as the primary natural source of fluoride exposure. - Learn about the presence of fluoride in the environment. - Learn about dental fluorosis and skeletal fluorosis as effects of chronic fluoride exposure.
Thematic module 2. "The key to a risk-free smile."	
Session 2: The mystery of fluoride.	- Learn about the sources of risks associated with fluoride exposure. - Define the presence of fluoride in salt. - Identify the presence of fluoride in frequently consumed foods. - Recognize the presence of fluoride in dental products.
Session 3: The secret of fluoride flavors.	- Identify fluoride intake from water. - List fluoride intake from table salt. - Indicate fluoride intake from food. - Learn about fluoride intake from dental products. - Label fluoride content in salt, food, and dental products.
Thematic module 3. "The path to a stain-free smile."	
Session 4: Directions for the fluoride mission.	- Organize the importance and role of fluoride in oral health. - Review the proper use of fluoride in oral health.
Session 5: Walking safe paths.	- Distinguishing between appropriate doses of fluoride in oral health care. - Recognizing healthy oral hygiene habits for controlling fluoride intake. - Recognizing the importance of adult supervision in the oral hygiene process. - Recognizing the importance of regular visits to the dentist.

Participants and schedule

Sample size.

The sample size (parent-child pair) will be estimated based on one of the outcome variables: urinary fluoride concentration. This will use the formula for the difference in means as follows: $n = 4f(\alpha, \beta)\left(\frac{s^2}{d^2}\right)$ therefore:

$f(\alpha, \beta)$: α, β (Type I error) 0,05 y f (Power) 0,90. According to the table of critical values for the confidence level and power, this corresponds to 8,56.

s^2: Variability in urinary fluoride concentrations is 2,42 mg/L, taken from a measurement of urinary fluoride concentrations in children from El Juncal previously.

d^2: Clinically significant difference, urinary fluoride concentrations 1.5 mg/L taken from a measurement of urinary fluoride concentrations in children from El Juncal.

$$n = 4f(\alpha, \beta)\left(\frac{s^2}{d^2}\right)$$

$$n = 4(8{,}56)\left(\frac{2{,}42^2}{1{,}5^2}\right)$$

$$n = \frac{34{,}24x5{,}8564}{2{,}25}$$
$$n = \frac{34{,}24x5{,}8564}{2{,}25}$$
$$n = \frac{200{,}523136}{2{,}25}$$
$$n = 89{,}1213 \cong 90$$

The calculated sample size corresponds to 90 pairs, 45 for the intervention group and 45 for the control group.
For the community outreach component, all teachers and staff working in the kindergartens attended by eligible children will be invited to implement the training component. Those who voluntarily agree to participate in the study will be eligible.

Table 3. Sessions for children in the multicomponent intervention to reduce the risk of fluoride exposure in children and learning objectives.

Sessions	Learning objectives
Thematic module 3. "The path to a stain-free smile."	
Session 4: Directions for the Fluoride Mission	- Understand what fluorides are and why they are important for oral health. - Learn some important precautions when using dental products with fluoride.
Session 5: Walking safe paths.	- Distinguishing between appropriate doses of fluoride in oral health care. - Recognizing healthy oral hygiene habits for controlling fluoride intake. - Recognizing the importance of regular visits to the dentist.

Recruitment of kindergartens and parent-child pairs.
The unit of analysis will be the kindergartens. The participant recruitment process will begin with the sending of a letter requesting voluntary participation in the study. Subsequently, a meeting will be held with the administrators and teachers of the selected kindergartens to present the objectives and procedures of the study. For the recruitment of the parent-child dyad, parents will be invited to participate through the institution's official communication channels. The intervention will be presented in detail to the parents/caregivers, and they will be asked to sign the informed consent form for both parents and children, thus ensuring a structured and transparent process.

Schedule: Parents and children will be assessed at four points during the study: baseline (T0, start of the study, before implementing the intervention), three months (T1), six months (T2), and twelve months (T3) at the end of the maintenance phase (Table 5).

Table 4. Monitoring the fidelity of implementation of the multicomponent intervention to reduce the risk of fluoride exposure in children.

Intervention actions.	**Intervention components that should be implemented.**	**Inadequate reliability is triggered.**	**Follow-up and actions to address it.**
Physical barrier.	Installation of filters in kindergartens prior to implementation of the intervention.	Understanding how to use the filter. Use of less than 70% of the filter.	Follow-up visits and checklists.
Educational sessions for parents.	The five educational sessions are held twice a week. Parents participate in 80% of the sessions and activities.	Fewer than five sessions held. Team staff will report if there is a low level of understanding of the materials used in the session. Team staff will report if participant attendance is less than 70%.	Weekly meetings with the team to verify information about the educational sessions held during the week. Direct observation by the work team. Checklists for participation in educational sessions.
Educational sessions for children.	The two educational sessions are held over the course of a week. Children participate in 80% of the sessions and activities.	Team staff will report if there is a low level of understanding of the materials used in the session. Team staff will report if participant attendance is less than 70%.	Technical assistance.
Outreach activities.	Reinforcement sessions: Three months: Three educational sessions. One per week. Six months: Three educational sessions. One per week. Monthly follow-up visits: advertisements, two advertisements	Fewer than 3 sessions taught. Team staff will report if there is a low level of understanding of the materials used in the session. Team staff will report less than 70% attendance by participants. One out of every two advertisements was not completed within	Monthly meetings with the team to verify information about the educational sessions applied in the reinforcements. Checklist for participation in educational sessions. Direct observation.

	are derived from each session. Material sent home for reference and practice. Activities in natural science class. Two classroom activities derived from each session.	the established time frame. One out of every two classroom activities was not completed.	Activity checklist. Technical assistance.
Maintenance phase	Visits to educational model activities.	Less than 70% of the population visiting educational model activities.	Direct observation. Technical assistance.

Method for assigning the intervention.

Randomization and blinding

Participants will be randomly assigned to the intervention in the kindergartens. To do this, the list of parent-child pairs from each kindergarten who have agreed to participate in the study will be used. Randomization will be performed using SPSS V.25 software, using the transformation module to generate a table of random numbers according to the calculated sample size.

Data collection personnel will remain blinded and will not know which group (intervention or control) participants belong to. Similarly, parent-child pairs will not know which research group they belong to.

Method for data collection.

Fluoride concentration in water.

To calculate fluoride concentrations in water, liquid will be collected from underground wells in the municipalities of El Juncal and Riofrío in 250 ml polyethylene bottles. They will be pre-treated with 10% HNO3 for 2 hours, rinsed with distilled and deionized water, and dried (to avoid contamination). The containers will also be rinsed three times with the same sampling water before sample collection. After sample collection, the containers will be refrigerated at three degrees Celsius and will be transferred and stored in the laboratory of Antonio Nariño University until analysis.

Table 5. Schedule of study measures and deadlines for implementing the multicomponent intervention to reduce the risk of fluoride exposure in children.

Procedure - Activity	Registration of participants	T0 – Baseline (before implementation of the intervention)	Implementation of the intervention (first three months)	T1 - Follow-up measurement	Implementation of the intervention (six months)	T2 — Follow-up measurement (six months)	Implementation of the intervention (maintenance phase)	T3 - Final measurement (after one year)
Kindergarten assignment	X							
Signed consent form	X							
Verify participant eligibility criteria	X							
Implementation of the intervention			X		X		X	
Fluoride concentrations in water		X		X		X		X
Fluoride concentrations in urine		X		X		X		X
CAP survey for parents		X		X		X		X
Monitor fidelity of intention		X		X		X		X

Fluoride concentration in urine.
To estimate fluoride levels in the urine of participating children, parents will be trained to collect samples. The first urine of the morning will be collected, transferred to sterile 50 ml Falcon tubes, and a standard volume of 20 ml will be taken for analysis. After sample collection, the containers will be refrigerated (three degrees Celsius), transported, and stored in the laboratory until analysis. This sampling will be carried out at four points in time: before the intervention is implemented and after it at three, six, and twelve months.

Quantification of fluoride in water and urine.
The quantification of fluoride ions in groundwater and urine samples will be performed using a potentiometer equipped with a fluoride ion-selective electrode, specifically model HI 5315. Samples will be analyzed in duplicate, after adding TISAB II buffer with CDTA to stabilize the pH and compensate for interference from other ions. To ensure the accuracy and validity of the results, certified reference materials, such as the NIST-USA 1984 standard (SRM 3183), will be used. In the case of urine samples, fluoride concentrations will be adjusted by dilution to control for variations in fluid balance, specifically through the determination of urinary creatinine. The latter will be quantified using a Genesys 10S UV-VIS spectrophotometer (Thermo Scientific, USA). Finally, urinary fluoride levels will be expressed in milligrams of fluoride per gram of creatinine, allowing for a more representative and standardized analysis of fluoride exposure in the individuals studied.

Knowledge, Attitudes, and Practices Survey.
To assess changes in parents' knowledge and attitudes, the Knowledge, Attitudes, and Practices (KAP) survey will be administered, after validation. The instrument was designed for this purpose, taking into account the components of the intervention. The knowledge section consists of 12 items and explores parents' understanding of natural and non-natural sources of fluoride and its effects on oral and systemic health. The attitudes section consists of 10 items and identifies perceptions, beliefs, motivations, and willingness to act in response to fluoride risk, water quality, willingness to change habits, and dental follow-ups. The practices section, with 9 items, addresses information on current behaviors related to consulting fluoride content, modifying water sources, dental care, and supervision in the use of dental products. Each section of the instrument includes ordinal scale variables and provides an overall score that allows each section to be classified as good, fair, or poor.

Participant retention.
To facilitate retention with high levels of parent-child participation, this intervention aims to promote the bond between parents and children and the research team. Initially, awareness will be raised by emphasizing the general health risks to the community and specifically to children. In addition, it will be fun for children, attractive to parents/caregivers, and culturally sensitive.

Data analysis.

The analysis seeks to compare changes between the intervention and control groups over time. The information will be recorded in a digital database designed in Microsoft Excel (2021), each dyad will be coded, the data will be stored securely with limited access, and data quality assurance will be ensured through data validation.

To this end, an analysis will be performed for each component of the intervention to determine the effectiveness of each one. First, comparisons will be made between the intervention group and the control group at the beginning of the study using the Chi-square (χ^2) test for categorical variables. For continuous variables, such as age and baseline urinary fluoride concentrations, Student's t-test will be used if they have a normal distribution or the Mann-Whitney U test if the distribution is non-parametric.

For longitudinal measurements, Generalized Estimating Equations (GEE) models will be used to compare the means of fluoride concentrations in water and urine between the two groups over time (baseline measurements, at 3, 6, and 12 months). For this analysis, these parameters will be used to model the relationship between fluoride concentrations and the model variables, and an autoregressive correlation will be implemented to model the relationship between repeated measurements in each individual.

The main analysis will focus on the difference-in-differences (DID) method to estimate the net effect of the intervention. Under the assumption of parallel trends, this method will allow us to compare changes in the intervention group with those in the control group over time. A linear regression model will be used to implement the DID, where the interaction coefficient between the group (intervention vs. control) and time (before vs. after) will represent the actual effect of the intervention. If the distributions are not normal, variable transformations or the use of generalized linear models will be considered.

Data on parents' knowledge, attitudes, and practices will be analyzed as ordinal variables. Comparisons within each group before and after the intervention will be evaluated using the Wilcoxon test for paired data or the Student's t-test for paired samples, as appropriate. For comparisons between groups (intervention vs. control), the Mann-Whitney U test or Student's t-test for independent samples will be used.

A significance level of $p < 0.05$ will be established to determine whether the differences are statistically significant.

Analysis to handle missing data: Data from parent-child dyads who drop out of the intervention before completion will be managed separately. A Student's t-test analysis will be performed to compare the baseline mean urinary fluoride concentrations of children who dropped out of the study during the one-year follow-up period with those who remained in the study. If they dropped out of the study after T1 or T2, the parents' CAPs will also be compared with those who remained.

INFORMED CONSENT TO PARTICIPATE IN THIS RESEARCH STUDY.

PRINCIPAL INVESTIGATOR: Claudia Lorena García Rojas.
WHERE THE STUDY IS BEING CONDUCTED: Department of Huila..

You are being invited to participate in this research study. Before deciding whether or not to participate, you should read and understand each of the following sections. This process is known as informed consent. Please feel free to ask any questions you may have to clarify any doubts you may have.

Once you have understood the study and if you wish to participate, you will be asked to sign this consent form, a signed and dated copy of which will be given to you.

General Objective

- *To evaluate the effectiveness of a multicomponent intervention to reduce the risk of fluoride exposure in children in the department of Huila.*

1. Justification for the study.

This study seeks to evaluate the effectiveness of a multicomponent intervention to reduce the risk of exposure to high concentrations of fluoride in children in the department of Huila, in accordance with the guidelines and actions for prevention and control of risks associated with the event. By coordinating the different actors and knowing that educational interventions modify habits in the population, we propose to evaluate the effectiveness of this intervention in the population.

2. Benefits of the study.

As a university with a dentistry faculty in the region, we embrace our social responsibility as a management policy in line with the challenges of the world in this century, whose fundamental objective is to develop a series of actions that strengthen the university's relationship with society. Hence the motivation of the oral health research group at the Antonio Nariño University School of Dentistry to reach out to the population that needs it. This is done after analyzing the needs of the population and the capacity to intervene, evaluating and developing strategies that result in the well-being of society. The goal is to demonstrate behavioral change for the better well-being of the children in the department.

Likewise, another benefit would be related to preventive actions, mainly aimed at reducing the intake of water with high concentrations of fluoride, including during pregnancy and throughout childhood, especially up to the age of eight. The same applies to the consumption of water, whether natural or artificial, which is generally fluoridated, as well as food preparation, the use of fluoridated toothpaste in children, and preferably a diet high in calcium, magnesium, and antioxidants.

3. Study procedures.

An initial phase will be carried out to measure fluoride concentrations in water and urine, diagnose dental fluorosis, and complete a survey. Subsequently, the multi-component intervention will be implemented, followed by a post-implementation evaluation of both the intervention group and the control group.

4. ***Risks associated with the study (according to Resolution 8430 of 1993):***

This study will be conducted to evaluate the effectiveness of a multicomponent intervention for children exposed to fluoride. The risk of the research is minimal, as the effects of natural fluoride exposure on children will be determined through the implementation of the TF Index for diagnosis and to determine the level of fluoride exposure.

5. ***Clarifications:***

Your decision to participate in the study is completely voluntary.

There will be no adverse consequences for you if you do not accept the invitation.

If you decide to participate in the study, you may withdraw at any time, even if the researcher in charge does not ask you to do so, and you may or may not give reasons for your decision, which will be respected in its entirety.

You will not incur any expenses during the study.

You will not receive payment for your participation.

During the course of the study, you may request updated information about it from the principal investigator.

The information obtained in this study, used to identify each patient, will be kept strictly confidential by the research team.

If you have any questions or require clarification, please contact Claudia Lorena García Rojas at 3166917683.

If you believe that there are no doubts or questions regarding your participation, you may, if you wish, sign the informed consent form that is part of this document.

INFORMED CONSENT.

I, _____ ID c.c N° _____ of _____ have read and understood the above information, and my questions have been answered satisfactorily by the researcher who interviewed me. I have been informed and understand that the data obtained in the study may be published or disseminated for scientific purposes. Therefore, I wish to voluntarily participate in the research project.

_____

Participant's First and Last Name

_____

Participant's Signature.

C.C N°

_____

Witness' Name

_____

Witness' Signature.

C.C N°

This section must be completed by the researcher (or their supervisor).

I have explained to Mr./Msal _____ the purpose of the research and the risks and benefits of their

participation. I have answered the questions to the best of my knowledge and asked if they have any questions. I acknowledge that I have read and understand the applicable regulations for conducting research with human subjects and that I abide by them (Resolution 8430 of 1993). Once the question-and-answer session was concluded, this document was signed..

_____ _____

Researcher's Signature. *Date*

_____

_____

INFORMED WITHDRAWAL

I _____ *identified with the CC* _____ *of* _____ *voluntarily participated in the study until the* ____ *day of the month* _____ *of the year* ____*, exercising the right that was mentioned to me from the beginning of the research, which states that I can withdraw at any time, stating the following reasons:*

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

The following sign on the record::

_____

___

Name and Signature of the person participating in the project or legal guardian who withdraws from participating in the study

C.C. No.

_____

Signature of Witness (1)

C.C. No.

_____

Signature of Witness (2)

C.C. No.

_____

Signature of Principal Investigator

C.C. No.