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Fluoridation of public water supply: bioethical, legal and political approach

Cléa Adas Saliba Garbin¹, Luis Felipe Pupim dos Santos², Artênio José Isper Garbin³, Suzely Adas Saliba Moimaz⁴, Orlando Saliba⁵

Abstract

This article aims to analyze the bioethical aspects and legal and political issues of water fluoridation, creating a discussion based on established studies and new research, in order to contribute to an ethical and impartial perspective on the subject. A bibliographic review study was performed, based on a survey of specialized literature on “fluoridation”, “bioethics” and “fluoride poisoning”. Water fluoridation is an important public health measure for the prevention of dental caries, and its efficacy has been demonstrated in several studies. As it is mandatory under Brazilian law, bioethical dilemmas arise due to the absence of individual choice on whether to consume or not consume fluoridated water. There appears to be no solution to this moral dilemma, as even if the measure were free from any risk, its compulsory application would still violate the principle of autonomy.

Keywords: Fluoridation. Bioethics. Fluoride poisoning.

Resumo

Fluoretação da água de abastecimento público: abordagem bioética, legal e política

Este artigo tem como objetivo analisar aspectos bioéticos e questões legais e políticas da fluoretação de águas de abastecimento público, criando discussões baseadas em estudos consagrados e em novas pesquisas, a fim de contribuir para abordagem imparcial do tema. Trata-se de revisão bibliográfica realizada após levantamento de literatura especializada sobre “fluoretação”, “bioética” e “intoxicação por flúor”. Foram selecionados estudos que possibilitaram discussão plural, relevantes para o debate do tema. A fluoretação das águas de abastecimento público é importante medida de saúde pública para prevenir a cárie dentária, tendo sua eficácia comprovada em vários estudos. Sua obrigatoriedade prevista por lei federal gera dilemas bioéticos, pois exclui a possibilidade de escolha individual de consumir ou não água fluoretada. Não parece haver saída para esse dilema moral, mesmo se a medida fosse livre de qualquer risco, pois ainda assim violaria o princípio da autonomia.

Palavras-chave: Fluoretação. Bioética. Intoxicação por flúor.

Resumen

La fluoración del agua de abastecimiento público: abordaje bioético, legal y político

El objetivo de este artículo es analizar los aspectos bioéticos y asuntos legales y políticos de la fluoración del agua de abastecimiento público, al crear discusiones en base a estudios consagrados y a nuevas investigaciones, con el fin de contribuir con un enfoque imparcial del tema. Se trata de la revisión bibliográfica realizada después de estudiar la literatura especializada sobre la “fluoración”, “bioética” e “intoxicación por flúor”. Se seleccionaron los estudios que permitieron la discusión plural, lo cual fue relevante para el debate del tema. La fluoración de agua de abastecimiento público es una medida de salud pública importante para prevenir la caries dental y su efectividad se comprobó en varios estudios. Su obligatoriedad prevista por la Ley Federal genera dilemas bioéticos, ya que elimina la posibilidad de la elección individual de consumir o no el agua fluorada. No parece haber una salida para este dilema moral, incluso si la medida estuviese libre de cualquier riesgo, ya que aun así estaría violando el principio de autonomía.

Palabras clave: Fluoruración. Bioética. Intoxicación por flúor.

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Declararam não haver conflito de interesse.

Fluoridation of public water supply is the most important method of fluoride use in public health for dental caries prevention. This is because it is safe, low cost and covers a large part of the population¹⁻⁷. The United States Center for Disease Control and Prevention judges the measure as one of the ten most relevant public health achievements of the twentieth century. Many health and science organizations, such as the World Health Organization (WHO), the International Association for Dental Research and the Pan American Health Organization advocate the method.⁹

When the preventive properties of fluoride were discovered, its beneficial effects were believed to occur by the ability of the ion to form fluorapatite instead of hydroxyapatite in the dental enamel phases. This would make the tooth more resistant to the acidic environment caused by the metabolism of the bacteria and their substrates.¹⁰ This current considered that the preventive properties of fluoride would be permanent for the exposed individual during the process of development of the teeth¹¹. However, this hypothesis has been proven to be faulty.

Even though fluoride apatite is formed during tooth mineralization, fluoride provides greater resistance to the surface of the enamel by means of its continuous presence. This is because periodic demineralization processes (caused by the drop in pH from the formation of acids from dietary carbohydrates) and surface re-mineralization are triggered. The fluoride-containing tooth surface has lower acid solubility compared to the original surface of the enamel¹². The beneficial effects of fluoride are due to its continuous presence in small quantities throughout the life of the individual¹³.

There is research has showing that the amount of caries in children increases in places where water fluoridation has been stopped or interrupted¹⁴⁻¹⁸. The method, however, requires some care regarding the maintenance and monitoring of adequate levels of fluoride contained in the supply water to which the population has access. In a quantity below that recommended, the ion does not have the desired benefit for caries prevention^{6,19}, while high levels increase the risk of developing dental fluorosis, which is the first clinical sign of the toxic effects of fluorine²⁰. This pathology occurs due to the exposure of the dental germ during its formation period to high concentrations of fluorine.

Some requirements must be analyzed for water fluoridation. The DMFT index (decayed,

missing and filled teeth) of the local population should be surveyed; information about the water distribution network must be collected; the recommended fluoride content to be added must be established; the choice of product and equipment; and the method of analysis and sampling must be defined. Fluosilicic acid has been the most widely used product to fluoride water today because of its favorable cost-effectiveness. Basically, the equipment used consist in dosing pumps, constant level feeders, saturation cone and saturation cylinders.

The point of fluorine application should be established taking into account the product to be used and the characteristics of the local water distribution network. In most cases, the outlet of the filters, the contact reservoir or distribution reservoir are application points of the compounds that will release fluorine²¹. When there is a water treatment plant in the city, it becomes responsible for the addition and monitoring of waters intended for the population. Otherwise, water quality control should be the responsibility of local health and surveillance services.

Dental fluorosis becomes more frequent in the permanent dentition, and the age intervals corresponding to the first and second childhood are more susceptible to its harmful effects, caused by the systemic intake of fluoride. Clinically, fluorosis causes opaque spots on the enamel, and in more severe cases, it can damage the normal mineral structure of the dental element, leading to yellowish or brown regions²². This disorder may become a public health problem, as it does not only affect the aesthetics of the individual, but also causes functional alterations that can interfere in issues of self-esteem, besides being a factor that can make difficult the insertion of those affected in the labor market. Dental treatment for the situation can be highly complex, depending on the severity of the case²³.

Investigation of possible harms caused by systemic exposure to fluoride are not limited to dental fluorosis. There are studies in the scientific literature that correlate fluoride intoxication with neurotoxic effects, loss of normal motor activity, increased insulin resistance, hypothyroidism, bone alterations such as osteosclerosis, osteoporosis, among other pathologies^{24,25}.

These studies present results that prove that fluoride is harmful to the human health. However, the fluoride concentrations used in its methods far exceed the levels recommended for water fluoridation, that is, none of these studies can

conclude that the method can cause the mentioned pathologies.

In 1974, the Law 6.050 was passed, determining the obligation of the water fluoridation method in localities with water treatment plants²⁶. Ordinance 2.914, of 2011, by the Ministry of Health, establishes acceptable parameters for the drinkability of public supply water, thus controlling its quality for human consumption. According to the Ordinance, The maximum permitted content for the fluorine ion is 1.5 milligrams per liter of water (mgF/L)²⁷. Bioethics is an area that deals with ethical problems related to the beginning and end of human life²⁸, and can also be defined as *the systematic study of human conduct in the area of life sciences and health care, insofar as this conduct is examined in the light of moral values and principles*²⁹.

According to considerations of this field, the fluoridation of water becomes a pertinent theme for the following reasons: 1) it is known that, in the dental area, the already consecrated method, has contributed to the control of caries of the world population; 2) some research suggests that the consumption of fluoride causes harmful effects on health, causing pathologies in the nervous system, bone tissue, endocrine system, loss of normal motor functions, dental fluorosis, among several other complications; 3) currently, the fluoridation of the public water supply is a federal law and covers a large part of the population; 4) some researchers discuss the issue of the autonomy of individuals, stating that the population should have the chance to choose whether to consume fluoridated water^{30,31}.

This study aimed to analyze bioethical aspects of the fluoridation of public water supply and to explore the points that lead to the bioethical issues involved in the theme. We problematize its scientific duality through discussions based on consecrated studies and new research, in order to contribute to an ethical and impartial approach to the theme. The questions that will guide the discussion in this study involve scientific research on the benefits and harmful effects of fluorine, taken from bioethical milestones, in order to promote understanding of the problem and stimulate conduct in harmony with ethics.

Methods

This is a bibliographic review study carried out after a survey of the specialized literature. To be included in this paper, publications should include

the topics “water fluoridation”, “bioethics”, “fluoride poisoning”. We excluded articles that did not include the topics cited or did not fit with the descriptors, in addition to those that did not contain bibliographical references. We also sought studies on laws and policies that govern fluoridation in Brazil, in order to highlight one of the points regarding the bioethical issues involved in the method, which is its obligation set forth in the Federal Constitution, restricting the possibility of individual choice.

The SciELO, PubMed, Virtual Health Library (VHL) and Google Scholar databases were used. The selected sources date from 1950 to 2016, totaling 68 scientific articles, collaborating so that consecrated studies and current research are involved in the complexity of the theme over the years. After the analysis of the material, the article started to be elaborated - in addition to the final considerations, the text presents three topics that will be addressed separately: legal and political aspects, bioethical issues and harms. For each of these subdivisions, studies were included that would allow plural discussion, being relevant to the multifaceted debate of the theme.

Legal and political aspects

Water fluoridation was discussed at three national oral health conferences in 1986, 1993 and 2004³². The method is one of the priorities of the Diretrizes da Política Nacional de Saúde Bucal (National Oral Health Policy Guidelines), which includes a specific topic for the topic:

*It is understood that access to treated and fluoridated water is critical to the health conditions of the population. Thus, to make public policies that guarantee the implementation of fluoridation of water, extension of the program to municipalities with treatment systems is the most comprehensive and socially fair way of access to fluoride. In this sense, developing intersectoral actions to expand water fluoridation in Brazil is a government priority, ensuring continuity and adequate levels under the law 6,050 and complementary standards, with the creation and / or development of compatible surveillance systems. The organization of such systems is the responsibility of the management bodies of Sistema Único de Saúde – SUS (Brazilian Unified Health System)*³³.

Baixo Guandú, in the State of Espírito Santo, was the first Brazilian city to fluoride the water

supply in 1953, and the Serviço Especial de Saúde Pública, SESP (Special Public Health Service) was responsible for the implementing the method³³. However, the Federal Law 6.050, already mentioned, was only passed in 1974²⁶. The Pesquisa Nacional de Saneamento Básico (National Survey of Basic Sanitation), conducted in 2008, showed that 60.6% of Brazilian municipalities add fluoride in adequate quantities to the waters supplied to the population, but there is no public system for consultations on ion concentration.

Cesa, Abegg and Aerts³⁴ assessed the monitoring of fluoride in public water supply in the Brazilian capitals and found that in most of them fluoride levels in the waters were not properly monitored. Therefore, there is a need for greater intersectoral commitment to improve the fluoridation of water in the country. According to the British Fluoridation Society, Brazil is the country with the second largest coverage of fluoride in water, second only to the USA³⁵.

Bioethical issues

The areas of bioethics and public health, despite being distinct fields, often present points of intersection related to measures or technologies that put in discussion the decisions taken by public policy-makers³⁶. Faced with collective health problems, strategies and actions in health are designed to protect the population of a particular location. Since these measures are often mandatory, the choice of individuals is withdrawn, which in some cases does not have the pretension or even the need for access to the benefit.

Some authors point out that the legitimacy of sanitary actions and restrictions to individual autonomy are necessary, being characteristic of protective acts, then health justice prevails over the autonomy of the citizen^{37,38}. Health policy-makers often face dilemmas as both the principle of protection and the precautionary principle come into conflict. The principle of protection is linked to scientific evidence of the necessity and effectiveness of the method, while that of precaution is associated with the risks or damages arising from a particular measure. In the health area, dilemma situations are those in which two approaches are possible and justifiable scientifically and technically³⁷.

It is also possible to relate fluoridation to the principle of non-maleficence, which can be defined as the *principle according to which we should not*

*inflict harm or damage to others*³⁹. With current knowledge evidenced by scientific research, is there concrete confirmation that water fluoridation can harm the population? The plurality of results and opinions in the articles on the subject can make the answer to this questioning at least controversial. The potential harms of fluoride poisoning will be more broadly specified in the next topic of this study.

For some authors, certain points should be evaluated in the target population when considering the use of certain public health technologies, such as socioeconomic and cultural factors, as well as the moral and social conflicts that may arise. Moral problems are related to the constraints of individual freedom that may arise in the quest for collective well-being. These questions also arise when the objectives of the measure are not reached, either by errors of execution or conflict of interests, by blocking the access of some parts of the population to the public health strategy in question⁴⁰.

In general, fluorides can be found in various products, such as dentifrices and even processed foods, making the availability of the ion larger and more diversified. However, bioethical dilemmas are restricted to the fluoride contained in the water supply, precisely because the population does not have the option of not consuming it, as in the case of the products.

Harms

To better understand the possible negative consequences of fluoride consumption, we present a brief understanding of its kinetics: the fluorine ion can be absorbed by the oral mucosa, but most of the absorption occurs in the gastrointestinal tract. The reduction in pH accelerates this process, and thus, the acidity of the stomach contributes to absorption and toxic effects. After being absorbed, the ion goes into the bloodstream: part of it accumulates in the bones and the rest is excreted by urine, feces, sweat and breast milk.

As for the acute toxicity of fluoride, that is, when a very high dose is ingested at one time, there are reports of death in the literature. In the fatal cases reported, doses ranged from 4 to 30 mgF/kg⁴¹. Signs and symptoms of acute fluoride poisoning include vomiting, diarrhea, ventricular fibrillation, bronchospasm, hemoptysis, dilated pupils, cramps, cardiac collapse, hypocalcemia, hypokalemia, impaired renal function, and other complications⁴².

Adverse effects of fluoride at the level of water fluoridation, that is, low doses ingested over a long period, characterize chronic intoxication, which generates the most common condition in this category of toxicity, dental fluorosis, and, in more severe cases, bone pathologies, such as osteosclerosis. Fluorine stimulates the precipitation of calcium, an essential element to various physiological functions, such as the muscles and nervous system. This fact would explain the disorders related to loss of normal motor activities and neurotoxic effects of fluoride intoxication. The ion, in its affinity with calcium, forms calcium fluoride, which is poorly soluble. Thus, bone and dental tissues - which have a high amount of calcium and phosphate, with which fluorine also has affinity, forming fluorapatite - may suffer toxic effects.

These effects cause dental changes, such as fluorosis, and bone changes, such as hypercalcification, called osteosclerosis or osteopetrosis, which makes bones brittle²⁴. Another bone change that can be caused is osteoporosis, which leaves the bones porous and friable. Although the two bone tissue pathologies (osteoporosis and osteosclerosis) have different characteristics, both are associated with the same causal factor: disturbance of the phosphocalcic metabolism²⁴.

Bone fluorosis is endemic in some regions of the globe, especially in the Asian subcontinent. This disease causes joint pain and can be diagnosed as rheumatoid arthritis or ankylosing spondylitis⁴³.

The possible neurotoxicity caused by chronic fluoride poisoning has been the basis of several studies. The blood-brain barrier is relatively impermeable to fluorine; However, when consumed in large quantities or when in high concentrations in the body, fluoride can transpose it²⁵.

A meta-analysis⁴⁴ on the neurotoxic effects of fluoride conducted in 2012 aimed to compare intelligence quotient (IQ) scores and measures of cognitive function in children exposed to high levels of fluoride in water and children living in areas with low levels.

The authors selected 27 studies to be included in the meta-analysis and found a statistically significant difference between the groups: populations exposed to higher levels of fluoride obtained lower scores than those living in areas with lower levels. The conclusions of this study warned of the possibility of an adverse effect of high exposure to fluoride in children's neurodevelopment⁴⁴. However, this research presented some methodological biases

pointed out by other authors: lack of information at the individual level and high probability of confusion, since covariables were not adjusted.

In addition, the results were also challenged, since the IQ difference between the groups is clinically insignificant^{45,46}, although statistically significant. The mean IQ difference between populations exposed to high levels of fluoride and those exposed to low levels was -0.4 (95% confidence interval: -0.5, -0.3). In general, clinical evidence has priority over statistical findings. The sample size and/or the mean difference and standard deviation of the variable in the study population may change the p-value (used for comparisons between groups) from significant to non-significant.

About this, Sabour and Ghorbani considered the conclusions of the ecological fallacy review, which *may lead to errors of interpretation of results*⁴⁷. They added that statistics can not provide a simple subsidy for clinical judgment. Another problem of the meta-analysis was the heterogeneity of fluorine levels, categorized only as "high levels" and "low levels". In the first group, the maximum and minimum values obtained according to the criteria of the 27 papers evaluated were, respectively, 11.5 mgF/L and 0.57 mgF/L. In the category referring to the low contents or in the groups used as reference, the highest value was 1.2 mgF/L, and 0.18 mgF/L was the lowest.

In 2015, a cross-sectional study was conducted in England using secondary data to develop binary logistic regression models of predictive factors for hypothyroidism prevalence using data from 2012 on fluoride levels in drinking water. In regions with fluoridation, there were twice as many cases of hypothyroidism as reported in regions with non-fluoridated water⁴⁸.

Another study aimed to examine the relationship between exposure to fluoridated water and prevalence of hyperactivity with attention deficit in children and adolescents in the USA. It was carried out using a method similar to the previous research, and concluded that there were higher rates of hyperactivity and attention deficit in children from states where the majority of the population receives fluoridated water⁴⁹.

It can be noted from the studies presented in the annex that the concentrations of fluorine involved in their methods as well as in most similar studies are much higher than the recommended levels for public water supply. In Brazil, the Ministry of Health admits 1.5 mg/L as the maximum allowed

value, but research groups suggest that this value be revised for each region.

Studies on water fluoridation usually involve municipalities or regions where there are universities. Commonly, those who analyze and control the fluoride levels of the public supply are the very organs responsible for the addition of the ion. That is, it is not a question of heterocontrol (surveillance carried out by a distinct institution, other than that responsible for fluoridation). As mentioned previously in this paper, water fluoridation is a federal law; however, states may issue their own regulations, specifying or adapting recommended fluorine levels taking into account local variables.

Final considerations

Water consumption by the population may vary according to the temperatures of each locality, because consumption tends to be higher in places with higher temperatures and vice versa. Therefore, some authors state that, for each region, the fluoride contents in the supply should be recommended taking this variable into account⁵⁰.

In 2011, the Centro Colaborador do Ministério da Saúde em Vigilância da Saúde Bucal da Faculdade de Saúde Pública da Universidade de São Paulo (Collaborating Center of the Ministry of Health in Oral Health Surveillance of the School of Public Health of the University of São Paulo) issued a document that represents a technical consensus on the classification of fluoride levels in public water supply based on the maximum benefit of prevention of dental caries and minimal risk of development of fluorosis⁵¹.

According to the document, the values considered optimum vary according to the temperatures of each locality. Thus, considering the local variables, not only the temperature, but the socioeconomic condition of the local population, ease of access to other fluoridated products, their dietary habits, among other factors, reduces the risks of the method. The Ministry of Health recommends, as stated above, the top value of 1.5 mgF/L not to be exceeded, but state ordinances already limit this content. For the state of São Paulo, for example, Resolution SS 250/1995 establishes values from 0.6 to 0.7 mgF/L⁵².

The DMFT index (decayed, missing and filled teeth) is used in dental epidemiological studies to record dental elements that are or have been

affected by caries. According to the National Oral Health Survey conducted in Brazil in 2010 (Projeto SB Brasil 2010), DMFT at age 12 was 2.07 (approximately two teeth affected by caries), corresponding to a reduction of 26.2% in seven years⁵³. This means the exit from the average prevalence condition (DMFT between 2.7 and 4.4) in 2003 to a mild prevalence condition (DMFT between 1.2 and 2.6), according to the WHO⁵⁴.

However, the national survey showed that there are significant differences between DMFT indices at 12 years among the macro-regions of the country: in the North, the index was 3.16, and in the Southeast, 1.72, these being the region with the highest and lowest DMFT indices, respectively. China has been conducting a number of studies aimed at demonstrating the neurotoxic properties of chronic fluoride intoxication from areas where fluoride is typically found naturally in the water supply and its concentration depends on local geological characteristics. In the country, there are areas where the population is exposed to high levels of the ion, mainly in rural areas⁵⁵, where, in many occasions, levels substantially exceed the value of 1 mgF / L⁴⁴.

Another concern regarding the consumption of fluoride is its presence in several industrialized products and foods, which could result in cumulative intoxication. The fluoride content in cooked foods is low, but this value may be higher in foodstuffs where bone tissue is included or processed⁵⁶. High levels of fluoride were found in barley and rice (approximately 2 mg/kg); in meats, however, fluoride contents tend to be low (0.2 to 1.0 mg/kg). Nevertheless, even if we assume that some foods may be important sources of fluoride, the highest daily intake comes from water⁵⁸.

However, in some cases, water may not be considered the major contributor, since dietary changes and high fluoride concentrations in the air may imply greater exposures to the ion⁵⁷. Just as fluorine is present in the soil, it is also found in the air, coming from the dust of soils rich in fluoride.

It can also be found in locations that house certain industries, such as aluminum smelting, production of mineral fertilizer, glassmaking and ceramics, or industries that use large coal-based incinerators, among others.⁵⁸. Sites near these facilities present levels of 1.4 mgF/m³ in the air. In non-industrial regions, this is generally insignificant⁵⁸.

The lack of laboratory and technical infrastructure for the periodic measurement of

fluoride levels in the public water supply is a problem for small or medium-sized localities. To aggravate the situation, most of the time it is in these regions that the population needs access to fluoride the most, since precarious social conditions are related to weakened oral hygiene. Likewise, without periodic analysis, the population of certain localities where there is excess fluoride in the public supply may be exposed to the method's risks.

Some developed countries, especially those with high HDI in Europe, are withdrawing the fluoridation system from their supply. This served as justification for certain authors, and even for laymen, to consider the method outdated and dangerous to the population.

With regard to oral health, the Brazilian reality can not be compared to the conditions in developed countries. The great disparity between the regions of Brazil as to the value of DMFT indices shows that the fluoridation method is still very necessary in the country, especially in less developed localities, where the population will hardly have access to varnishes, gels or other products with fluoride.

Dental fluorosis is one of the major concerns related to chronic fluoride poisoning, as discussed in this study. However, according to current studies and reviews, this condition, when present, occurs mainly in its mild or very mild form, and is therefore not a public health problem^{59,60}. Ecological studies that correlate areas where fluoridation exists and areas of occurrence of neuropathologies may demonstrate positive results. However, in many cases, this type of work does not consider individual and other covariates, or even the average level of fluoride contained in water.

Laboratory research on animals have also shown the negative properties of fluoride.

However, they use much higher doses than those recommended for drinking water, and do not serve as a proof of the harmfulness of fluoridation, but they show that fluorine is toxic and dangerous if not used with due caution. For Cohen and Locker⁶¹, there seems to be no way out of this moral dilemma, even if the measure were free from any risk, for it would still violate the principle of autonomy. Since there is no ethical solution, decisions on fluoridation must be taken at the political level, which will address certain interests and oppose others, directly linking public health and democratic values³⁷.

In 2004, the Brazilian government rejected a bill aimed at suspending the fluoridation of water in the country³². The method reaches a large part of the population, being extremely important mainly in regions where residents have little access to other preventive methods.

The effects of mild fluorosis, almost imperceptible aesthetically, can be considered less severe than the pain and suffering generated by dental caries. The measure requires periodic analysis of the water for ion measurement, which can be a problem, since not all Brazilian municipalities have the resources to do so, thus requiring a greater intersectoral commitment for this to be solved.

Fluoridation of the public water supply is a safe, effective and inexpensive method that has been helping humankind to control and prevent decay. Fluoride is toxic at certain concentrations, causing various complications and even death in cases of acute intoxication. However, the levels recommended for fluoridation of the water are very low, not exposing the population to its toxic effects, except for dental fluorosis, in some cases, which usually occurs in its mild form.

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Participation of the authors

Luis Felipe Pupim dos Santos performed the survey and interpretation of the studies used. Cléa Adas Saliba Garbin and Artênio José Ispier Garbin were co-supervisors and participated in the critical review of the article regarding ethical principles. Suzely Adas Saliba Moimaz was co-supervisor and Orlando Saliba was the supervisor, both participated in the critical review of the article regarding the issue of fluoridation of water for public supply.

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Annex

Studies performed with administration of fluoride in different doses and their results

Author/year	Fluoride dose administered	Results
Paul, Ekambaram, Jayakumar (1998) ⁶²	20 or 40 mgF/kg in female rats for 60 days	Dose-dependent reduction on spontaneous motor activity; motor coordination did not present changes; loss of weight gain (dose-dependent); decreased total plasma, liver and skeletal muscle protein concentration; decreased cholinesterase activity in the blood, but acetylcholinesterase activity in the nervous system remained normal
Collins and collaborators (1995) ⁶³	10 to 250 ppm of fluoride in female rats for evaluation of fetal development up to the 20 th day of gestation	There were no differences in relation to the control group; the groups that consumed 250 ppm of fluoride showed a decrease in food and water intake; fluoride showed no teratogenicity
Trabelsi, Guerrazi, Zeghal (2001) ⁶⁴	500 mgF/L in the drinking water of female rats from the 15 th day of gestation until birth. The objective was to evaluate the possible influence of fluoride on the development and functioning of the thyroid gland of the pups, who continued to receive treatment until the 14 th day of life	Animals receiving NaF had a 75% decrease in plasma free T4 (thyroxine); fluoride was able to cause a strong reduction in the hormone thyroxine, and this could be related to the histological changes and apoptosis observed in the cerebellum of these animals
Ekambaram, Paul (2001) ⁶⁵	500 ppm in water. Female adult Wistar rats were treated for 60 days	Animals treated with NaF showed increased plasma concentration, decreased food intake with a consequent reduction in body weight gain, impairment of exploratory motor activity and motor coordination, dental injuries, inhibition of total cholinesterase activity in the blood, brain acetylcholinesterase and hypocalcemia
Mullenix and collaborators (1995) ⁶⁶	75 to 125 ppm of fluoride for six weeks in rats	After a three-week interval, they had plasma levels of 0.059 to 0.640 ppm of fluoride, similar to those reported in humans exposed to 5-10 ppm of fluoride. Animals treated with higher concentrations of NaF also showed a behavioral pattern rupture when exposed to a new environment
Peng, Xu, Zhang (2016) ⁶⁷	50, 100 and 150 mgF/L in rat embryonic cancer cells	Reduction of cell viability and damage to DNA
Chen e colaboradores (2016) ⁶⁸	50 mgF/L in different frog species to asses the effect of fluorine on growth, metamorphosis and skeletal development	Fluoride caused an increase in mortality, inhibition of metamorphosis and delay in skeletal development