

# Patterns of oral contraceptives consumption and estimated concentrations of 17 $\alpha$ -ethinylestradiol in water bodies from the municipality of Santa Maria Madalena, Southeastern Brazil

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## Abstract

Organic contaminants with estrogenic activity have generated increasing concerns in the scientific community since they are capable of triggering adverse effects in the endocrine system of several organisms. 17 $\alpha$ -ethinylestradiol (EE2), a synthetic estrogen used in oral contraceptives, has been identified as one of the main responsible for causing such changes. Thereby the objective of this study was to generate information on EE2 consumption and estimate its possible impact on the water bodies of Santa Maria Madalena. Firstly, a survey was carried out about EE2 consumption through interviews with 325 women aged 15 to 59 residing in the study area, and then estimated the EE2 release in the water bodies. About 30% of the women interviewed reported using oral contraceptives. The EE2 average daily intake for each woman was 20  $\mu\text{g day}^{-1}$ . Extrapolating this intake to 30% of the population and adopting an excretion rate of 57%, it was calculated a total discharge of 4.8  $\text{mg day}^{-1}$  of this estrogen into the local water bodies. It diluted by the small flow of these streams leads to an estimated environmental concentration of 0.3  $\text{ng L}^{-1}$ , which according to ecotoxicological studies is already enough to trigger adverse effects to the aquatic biota.

**Keywords:** ecotoxicology; ethinylestradiol; oral contraceptives; water contamination.

## INTRODUCTION

The consumption of pharmaceuticals in the world has gradually increased in last decades. Brazil is noteworthy in this context, since it is among the ten largest consumers in the world, with anti-inflammatories, analgesics, and oral contraceptives being the most consumed pharmaceutical classes (CNS, 2005). This consumption has been generated concern in the scientific community, mainly among ecotoxicologists because once ingested, these pharmaceuticals are excreted in urine and feces as free compounds and/or metabolites, and subsequently may enter

the aquatic environment and cause adverse effects on biota. This occurs because certain cell receptors of the animal may resemble those found in humans, increasing the chances of triggering responses (Fent *et al.*, 2006).

In Brazil, about 30% of women aged 15 to 44 use oral contraception as a contraceptive method (Ministério da Saúde, 2009). The main active principle in these widely marketed pharmaceuticals is the synthetic estrogen 17 $\alpha$ -ethinylestradiol (EE2), which has high estrogenic activity. In addition to oral contraceptives, other hormonal contraceptives of different pharmaceutical forms (transdermal patch, injectable, vaginal ring, etc.), as well

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as pharmaceuticals used in female hormone replacement therapy (HRT), may also contain EE2 as active principle.

The EE2 estrogen is classified as an endocrine disruptor and can cause adverse effects in several organisms due to its potent estrogenic activity, even in extremely low concentrations (Cunha *et al.*, 2016). According to Sumpter and Johnson (2005) and Runnalls *et al.* (2010) estrogens, such as EE2, can induce adverse effects on fish reproduction in concentrations below 1 ng L<sup>-1</sup>. One of the most observed effects is the induction of vitellogenin synthesis in male fish, which can compromise the balance of the affected species. The continuous exposure to EE2, documented in the literature, in laboratory and field studies, has been broadly associated with changes in mating behavior and development, biochemical, morphological and histopathological alterations, spawning delays and decreases in fertilization rates, and others effects (Volkova *et al.*, 2015; Garmshausen *et al.*, 2015; Bhandari *et al.*, 2015; Luna *et al.*, 2015; Giusti *et al.*, 2014; Hoffmann & Kloas, 2012; Salierno & Kane, 2009; Jaser *et al.*, 2003). Among these adverse EE2 exposure effects, the most alarming is feminization, which causes part of the exposed male population to become intersex, exhibiting female sexual characteristics including female reproductive anatomy (Gilbert, 2012; Salierno & Kane, 2009; Zha *et al.*, 2007; Van Aerle *et al.*, 2002; Metcalfe *et al.*, 2001).

Although there are a number of studies that demonstrate the ecotoxicological effects of the estrogens, research quantifying their presence in aquatic matrices, as well as indicating improvements in wastewater treatment technologies and in the regulatory framework, both in Brazil and worldwide, are still incipient. In this context, the present study generates information regarding the consumption of the synthetic estrogen EE2 and estimates its possible impact on water bodies of the municipality of Santa Maria Madalena, Rio de Janeiro, Brazil.

Santa Maria Madalena is located in the state of Rio de Janeiro, Southeastern Brazil, the urban area of its 1<sup>st</sup> district, was selected for this case study since this area does not present pharmaceutical industries, animal breeding sites or other potential sources of EE2. Therefore, the occurrence of this estrogen in the local water can be directly related to hormonal contraceptives intake. Another important point is that this area also has no potential sources of this compound upstream of the researched area. Therefore, the consumption of pharmaceuticals containing EE2, inside the studied area can indicate the occurrence of this estrogen in its water bodies.

## MATERIAL AND METHODS

### *Study Area*

Situated in the mountain region of Rio de Janeiro State, the municipality of Santa Maria Madalena has 815 km<sup>2</sup> and is distributed throughout six districts (Figure 1). Its population is of 10,321 inhabitants, of which 5,932 are urban area residents (mostly concentrated in the 1<sup>st</sup> district) and 4,389 are rural area residents (IBGE, 2010).

The water bodies that drain the urban area of the 1<sup>st</sup> district are the Ribeirão Santíssimo and the São Domingos streams (Figure 1), both the Rio Grande River tributaries.

The sewage treatment system is currently operated by the municipality itself. There are two sewage treatment plants (STPs) in the urban area of the 1<sup>st</sup> district with a biological treatment system at a secondary level (PMSB, 2012). STP I is installed near the São Domingos stream, receiving only the contribution of the Cidade Alta neighborhood, while STP II is located near the Ribeirão Santíssimo stream and receives inputs from the Largo do Machado and Arranchadouro neighborhoods. The central area, Itaporanga, and Jardim Nova Madalena neighborhoods are not served by any treatment plants. However, even with two STPs, a good part of the sanitary effluents does not receive any treatment before being released water bodies, due to some factors: (1) not the whole area is covered by a sewage network; (2) in some cases, where there is the collection network a part of the households is not connected to it; and (3) there are areas with collection network, but it is not linked to the treatment stations. These releases of *in natura* sewage to the local water bodies can cause a number of drawbacks, from unpleasant water appearance and smell to the spread of infectious waterborne diseases.

### *Survey and Estimated Concentrations of EE2 in the Water*

A cross-sectional epidemiological study (survey) was conducted with women aged 15 to 59 living in the urban area of the 1<sup>st</sup> district of Santa Maria Madalena, from September to December 2014. This age range was established using population-based studies conducted in Brazil as reference, which indicates that the average age of the first sexual intercourse in young people is 15.7 years old and the maximum age of occurrence of natural menopause is 58 years old (Hugo *et al.*, 2011; Pedro *et al.*, 2003).

A simple random sampling model with a sample error of 5% and a confidence level of 95% was conducted for a universe of 2,054 women aged 15 to 59, according to the 2010 census (IBGE, 2010). Based on this methodology, the minimum number of women to be interviewed to meet the statistical criteria of this procedure was 325. A visiting of households was done until meeting the minimum aimed number of women, in a proportional amount by the census zones, in order to reach an equiprobabilistic sample of this universe.

Data was collected through a questionnaire containing open and closed questions on the following general items: identification, socio-environmental characteristics and consumption of pharmaceuticals (Table 1). The validation of this question sheet was conducted by means of a pre-test performed before the survey to verify the data collection instruments and to confirm the feasibility of the investigation.

Participating women were all the residents present in the selected households, except women in the gestational period or in cases of refusal or inability to participate in the survey. These women were interviewed individually in a reserved place, in order to guarantee their privacy. In the first contact

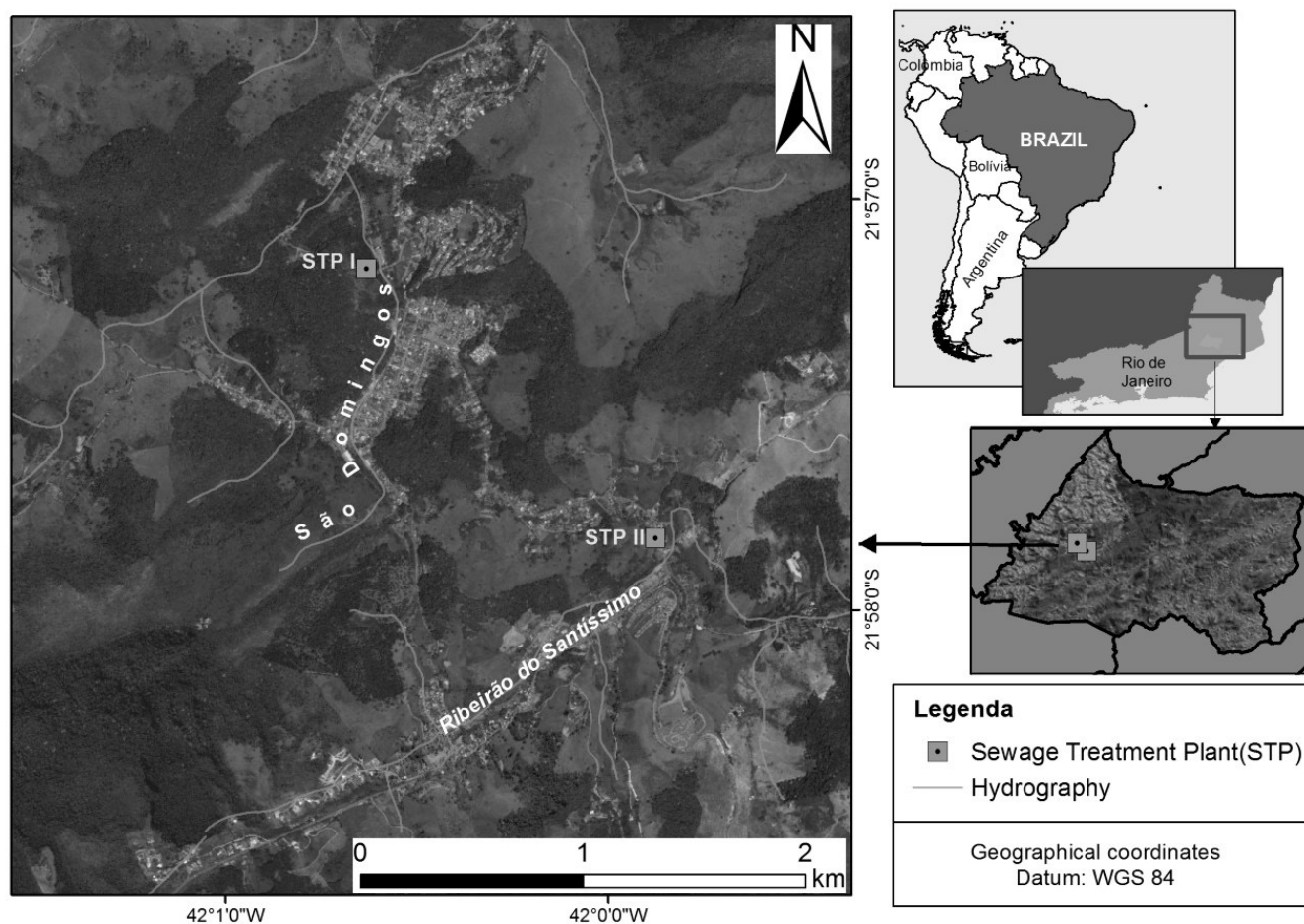


Figure 1: Location and hydrography of the study area.

with each interviewee was explained to them about the objectives of the study and the questions contained in the questionnaire, also emphasizing that personal information would be kept confidential and anonymous. The women-only participated in the study after signing the Free and Informed Consent Form, in accordance with the principles established in Resolution 196/1996 of the National Health Council (CNS, 1996).

Data organization obeyed ethical criteria, preserving the anonymity of each interviewee. Only the researchers ahead of the survey had access to interviewee identifications. A second free access database, containing the data collected during the interviews, was made using a numerical code identification system.

To complement the study, data was also collected at health centers (Basileu Estrela Hospital, Almeida Santos Colombian Family Health Program, Doutor Manoel Verbicário Central Health Unit) and all local drugstores.

The research protocol was submitted and approved by the Research Ethics Committee of the National School of Public Health/Oswaldo Cruz Foundation (CAAE 17134213.6.0000.5240). The collected data was stored in a database (Epi Info version 6.04) and its analysis was performed using the statistical software SPSS, version 17.0.

In order to evaluate the environmental impact of oral contraceptives into the local water bodies, it was used the methodology proposed by Johnson and Williams (2004). Therefore, based on the rates of metabolism and excretion of the EE2, the release of this estrogen to the water bodies, was calculated through the average administered EE2 dosage (information extracted from the interview data) and then its possible impact on the water environment was estimated, considering the dilution capacity of the streams that drain the region.

## RESULTS AND DISCUSSION

### *EE2 Consumption Pattern*

The following data presentation considers all interviews ( $n = 325$ ), since no losses or refusals have occurred. The general characteristics of the sampled population according to age, education, occupation, water supply, and sewage treatment are shown in Table 2.

Almost 30% of the interviewed women reported using oral contraceptives. This result is similar to data of the national pattern of consumption. According to a study conducted by the National Demography and Health Survey

Table 1: Questionnaire on the consumption pattern of 17 $\alpha$ -ethinylestradiol in Santa Maria Madalena - RJ / Brazil.

Number Code: \_\_\_\_\_ Date: \_\_\_\_ / \_\_\_\_ / \_\_\_\_

Part I - Identification and socio-demography

Name: \_\_\_\_\_

Age: \_\_\_\_ years old Birth date: \_\_\_\_ / \_\_\_\_ / \_\_\_\_

Address: \_\_\_\_\_

Scholarity: \_\_\_\_\_

Are you currently working?

Yes  No

If yes, what is your occupation? \_\_\_\_\_

Water supply system:

Public  Artesian well  Groundwater well  Surface source/spring  Does not know

Sewage system:

Septic tank  Rudimentary cesspit  Public network with treatment

Public network without treatment  *in natura* discharge  Does not know

Part II - Consumption of pharmaceuticals

Do you use hormonal contraceptives?

Yes  No

Are you in Hormonal Replacement Treatment (HRT)?

Yes  No

If yes (for one of the two previous questions), what its commercial name? \_\_\_\_\_

In which laboratory was it produced? \_\_\_\_\_

What is(are) the active principle(s) and dosage? \_\_\_\_\_

\_\_\_\_\_

What is its pharmaceutical form?

Pill  Injectable  Transdermal patch  Vaginal ring  Capsule  Dragee  Tablet

For how long (in years) have you been taking it? \_\_\_\_\_

At what time do you usually administrate it? \_\_\_\_\_

Was it obtained in Santa Maria Madalena?

Yes  No

If yes, in which establishment?

Health Center  Drugstore  Hospital  Other municipalities

Reserved to the researcher:

Did the interviewee present proof of the pharmaceuticals used?

Yes  No

If yes, what kind of proof?

Packing  Blister  Bula  Medical prescription

(*Pesquisa Nacional de Demografia e Saúde* - PNDS), the use of these pharmaceuticals in Brazil in women aged 15-44 was of 23.1% in 1996, increasing to 27.4% in 2006 (Ministério da Saúde, 2009).

Information on the consumption of other hormonal contraceptives was also collected, such as transdermal

contraceptive patch, vaginal ring and injectable, as well as pharmaceuticals used in the female HRT (Table 2). Only 5.2% of the interviewees were doing female HRT, and these pharmaceuticals were composed of natural estrogens (17 $\beta$ -estradiol, estrone, and estriol) or phytoestrogens. For the other hormonal contraceptives mentioned above, a small



Table 2: General characterization of the study population (n = 325).

Variables	Sample	
	N	%
Use of oral contraceptives	95	29.2
Use of others hormonal contraceptive	6	1.9
Female HTR	17	5.2
Does not use any of these methods	207	63.7
Age group (years)		
15 to 29	109	33.5
30 to 44	136	41.8
45 to 59	80	24.6
Scholarity (study years)		
≥ 12	87	26.8
9 to 11	164	50.5
≤ 8	74	22.8
Employed		
Yes	262	80.6
No	63	19.4
Water supply system		
Public	252	77.5
Artesian well	19	5.8
Groundwater well	10	3.1
Surface source/spring	44	13.6
Does not know	-	-
Sewage treatment system		
Septic tank	14	4.3
Rudimentary cesspit	-	-
Public collection network with treatment	117	36
Public collection network without treatment	2	0.6
<i>in natura</i> release	185	56.9
Does not know	7	2.2

percentage (1.9%) of the women used injectable contraceptives. And these also had no EE2 as the active principle, only progesterone. Therefore, this discussion primarily just focuses on those numbers related to oral contraceptives, which present the EE2 in their formulations.

The overall mean age of the women interviewed was 35.5 years (SD ± 11.7), with a slightly higher frequency of women between the ages of 30 and 34 (14.8%). It is noteworthy that about 70% of women reported having at least completed high school education and that the proportion of working women was about 80%.

Regarding sanitation characteristics, 77.5% of the interviewees stated that the water supply of their households comes from the public network. According to data from the 2010 census (IBGE, 2010), about 80% of the households in the urban area of the municipality of Santa Maria Madalena are served by public supplier, which corroborates the results

found during the survey conducted by the present study. Regarding sewage treatment, 56.9% of the interviewees stated that their sewage is released directly into the local water bodies (*in natura*). When comparing this data with those from the 2010 census (IBGE, 2010), it can be seen that the classification utilized in the present study regarding the type of sewage treatment system was somewhat different. This is due to the fact that the present survey has sought a classification that better reflects the conditions of this service in the study area. It should be noted that the IBGE classification does not apply well to the study area, and it masks the actual sewage collection and treatment status in this locality since it has as one of its classes as “general sewage or rainwater network”. This classification presents two basic problems: 1) it does not discriminate if the sewage carried by this network is carried to a treatment station, or if it is released *in natura*; and 2) the rainwater network can only be considered as part of the sewage system in the few cases, where it is utilized a type of united network system. Notwithstanding, in this study site the rainwater network was not designed to conduct sewage, and in cases where it does (unduly), this sewage is released directly into the local water bodies.

Table 3 shows the group of interviewees who uses oral contraceptives (n = 95). In addition to general data, data on the consumption of these pharmaceuticals is also presented.

A proportional distribution between two age groups was observed among the interviewees who use oral contraceptives: 50.5% were between 15 and 29 years old and 48.4% were between 30 and 44 years old, leaving only 1.1% of women aged over 45. According to Tavares (2006), older women are less likely to choose hormonal methods, since they believe they are at lower risks for pregnancy, so usually going for other methods, which can also be seen in this study results.

Table 3 also presents data on the schooling of those interviewed who use oral contraception, where approximately 73% of them report having at least high school education. It is noted that the educational level of women who use oral contraceptives (n = 95) is similar to the pattern observed for the whole sample (n = 325).

Regarding the households of the interviewed who use oral contraceptives, 76.8% of the interviewees stated that their households have a public water supply system and 58.9% stated that their sewers are released *in natura* in water bodies draining the region of study. This percentage of non-treatment of sewage is another indicator of the relevance of the study of emerging micropollutants in the waters of these water bodies.

Among the consumed oral contraceptives, 95.8% have synthetic estrogen EE2 as the main active principle (Table 3). Those which do not present EE2 as the active principle, containing only progesterone and/or the natural estrogen 17β-estradiol.

Figure 3 shows the results regarding the pattern of consumption of oral contraceptives most consumed by the women in the studied area. It was observed that, of the most consumed oral contraceptives, almost 50% have a dosage of 0.03 mg EE2.

Table 3: General characterization of oral contraceptives users (n = 95) and consumption prevalence according to the studied variables.

Variables	Sample	
	N	%
Oral contraceptives formulation		
Contains EE2	91	95.8
Does not contain EE2	4	4.2
Oral contraceptives consumption (years)		
< 1	15	15.8
1 to 3	26	27.4
4 to 7	27	28.4
> 7	27	28.4
Age group (years)		
15 to 29	48	50.5
30 to 44	46	48.4
45 to 59	1	1.1
Scholarity (study years)		
≥ 12	19	20.0
9 to 11	46	48.4
≤ 8	30	31.6
Employed		
Yes	83	87.4
No	12	12.6
Location where oral contraceptive was obtained		
In Santa Maria Madalena	83	87.4
Drugstore	76	80
Health Center	7	7.4
Other municipalities	12	12.6

It should be clarified that, while the oral contraceptives presented here are classified by their EE2 dosage, their commercial names were also acquired. And when analyzing these data, one important feature is the fact that the four most consumed oral contraceptives, responsible for approximately 50% of the total, are those with more affordable prices. In view of this behavior, it can be concluded that price is of great relevance regarding the choice of the contraceptive.

Regarding where the oral contraceptives were obtained, local drugstores stand out as being responsible for 80% of the supply of these pharmaceuticals in the studied area. To complement the survey, data on the distribution of oral contraceptives were also collected in the two local health centers (Almeida Santos Colombian Family Health Program and Doutor Manoel Verbicário Central Health Unit) and in the all five drugstores located in the study area, where monthly sales records were accessed, as shown in Figure 3.

Similar to the results shown in Figure 2, Figure 3 indicates that about 50% of the oral contraceptives supplied by the

surveyed health centers and drugstores also contain a 0.03 mg EE2 dosage. However, higher values of dispensation were found in relation to oral contraceptives that do not present EE2 in their formulation.

The available technical and academic literature indicates that still there are not many studies regarding the characterization of pharmaceuticals consumption by the Brazilian population. According to the National Health Council (CNS), medicines more easily purchased at drugstore counters are among the most widely used pharmaceuticals in Brazil and include contraceptives, analgesics, and anti-inflammatories (CNS, 2005). Regarding contraceptive methods, studies indicate that oral contraception is the most used method, followed by female sterilization (tubal ligation), condoms, intrauterine devices (IUD) and the diaphragm.

The Ministry of Health PNDS carried out a study that approached 8,707 married women (15-44 years old) and found that, in general, the prevalence of contraception in Brazil is notably high (81% used some contraceptive method) being the most utilized methods the female/male sterilization (31%) and the oral contraception (27%), followed by the use of condoms (13%) and other methods (10%) (Ministério da Saúde, 2009).

In Pelotas city, located in the state of Rio Grande do Sul, a cross-sectional study was carried out on the use of contraceptive methods on women aged 20 to 49 residing in the urban area. The study concluded that the use of oral contraceptives was the most applied method among women, followed by tubal ligation and condoms (Dias-da-Costa *et al.*, 2002). This profile was also observed in a study conducted with women between 20 and 60, with active sex lives, in the urban area of São Leopoldo, also in the state of Rio Grande do Sul, and in another study carried out in the state of Paraná, on people of the public health aged 35 to 49 enrolled in the Maringá Family Health Program (Carreno *et al.*, 2006; Souza *et al.*, 2006).

At Tubarão, located in the state of Santa Catarina, the contraceptive method most used by undergraduate medical students with ages between 18 and 41 was also oral contraception, followed by combinations of methods (oral contraception plus condoms) and condoms (Neumann *et al.*, 2011). Other studies, such as the one performed by Molina *et al.* (2015) in Cuiabá, located in the state of Mato Grosso, analyzing contraceptive methods and their uses in adolescents, and also demonstrated that combinations of methods (oral contraceptive plus condoms) were the most frequent.

Studies carried out in southeastern Brazil, in the São Paulo city with on women aged 10 to 49, in Campinas city with on women aged 30 to 49 and in three units of the Unified Health System in the State of Rio de Janeiro with women aged 18 to 39, also verified a prevalence of oral contraception methods and condoms (Heilborn *et al.*, 2009; Espejo *et al.*, 2003; Schor *et al.*, 2000).

At the global scale, the United Nations Department of Economic and Social Affairs in 2012 compiled data on contraceptive methods used in several countries. Portugal and

France stand out among the countries with higher rates of oral contraception use, of 60% and 41.5%, respectively. Comparing Brazil to other countries, with regard to oral contraceptives consumption as a contraception method, it presents similar levels as the United Kingdom, Norway, Australia, and Chile, with values of the order of 30% (United Nations, 2012).

It should be noted that in the present study no data were sought on all contraceptive methods such as: condom use, tubal ligation, IUD use, emergency pill, among others. The quantitative data here presented focus withal on the use of oral contraceptives, and it is probable that a significant number of the surveyed women who do not use this method use some other method of contraception, such as condoms or sterilization (tubal ligation). On the other hand, although the objective of this study is to analyze the pattern of EE2 consumption (found in formulations of a large part of oral contraceptives), given the scarcity of epidemiological information on this subject, the presented results can serve as a basis for elaboration of new studies.

**Estimated Concentrations of EE2 in the Water Bodies and Possible Impacts**

In view of the data presented herein and the pharmacological characteristics of EE2, it is possible to establish an approximate value of the release of this synthetic estrogen into the water bodies that drain the study area.

Based on Table 3, which presents the dosage of the active principle of interest (EE2) in the oral contraceptive formulations consumed by these women, the average daily consumption of 27 µg day<sup>-1</sup> can be calculated for the urban area of the 1<sup>st</sup> district of Santa Maria Madalena. It should be noted that, in general, the active oral contraceptives that contain EE2 are administered only during 3 of the 4 weeks of the menstrual cycle. Therefore, the average daily consumption of 20 µg day<sup>-1</sup> was considered for the study region.

As presented in the study done by Johnson and Williams (2004), it is important to consider the excretion rate of this estrogen in relation to the total ingested content. This rate is variable according to a series of factors, such as age, race, diet, and other interferences of the human metabolism. Even so, it is possible to utilize average values in order to facilitate studies like the one conducted herein. Thus, 40% was used as the excretion rate of EE2 in its free form or estrogenically active conjugate, being 23% in feces and 17% in urine (Figure 4). Therefore, it was possible to estimate that each woman using oral contraception releases an average load of 8 µg day<sup>-1</sup> of EE2 in sewage, which is then released to the local water bodies.

Employing the 95 women amongst the 325 interviewees as being statistically representative of the urban area of study, it is coherent to extrapolate the release value (8 µg day<sup>-1</sup>) to 602 women (29.3 % of the total population of women aged 15-59 residing in the study area, totaling 2,054 women). Thus, it resulted in a total release value of EE2 in the study area of 4.8 mg day<sup>-1</sup>.

When considering the characteristics of local sanitation (*in natura* sewer release or treatment technology not efficient in

the removal of EE2), it is estimated that the most, even all, of this compound go into the local water bodies. Then assuming that the sum of the flow of the two streams that drain the study area is of a relatively small order ( $\cong 280 \text{ L s}^{-1}$ ) - which limits their dilution capacity - the relevance of this release into these water bodies is increased. Considering that the estimated EE2 daily release (4.8 mg day<sup>-1</sup>) is done in a period of about 16 hours (discounting 8 hours of sleep), it was calculated for the area of study an environmental concentration of approximately 0.3 ng L<sup>-1</sup> EE2.

The literature has shown that very low concentrations (< 1 ng L<sup>-1</sup>), close to what was estimated for the water bodies of the study region, may already trigger adverse effects on species. According to Hoffmann and Kloas (2012) male amphibians of the species *Xenopus laevis* exposed to 0.3 ng L<sup>-1</sup> of EE2 had their mating behavior affected and became less attractive for females selection. Dang *et al.* (2017) showed that acute exposure to EE2 at low concentrations in the order of 0.5 to 5 ng L<sup>-1</sup> altered the behavior of the male mosquito

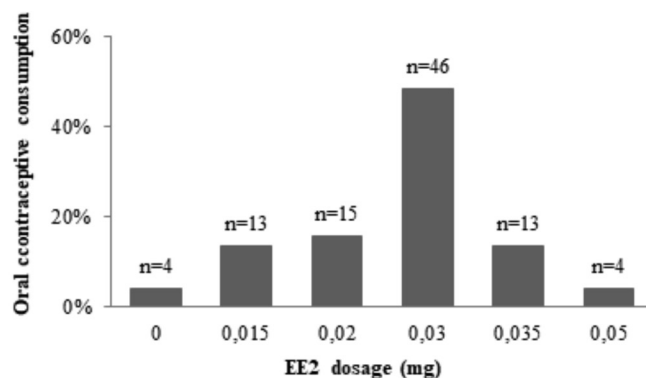


Figure 2: Consumption pattern of oral contraceptives (n = 95) in the study area. In oral contraceptives with different synthetic estrogen dosages, i.e., bi or three-phase formulations, the most prevalent dosage was considered.

For example, in biphasic formulas containing seven 0.04 mg EE2 pills and twenty-two 0.03 mg EE2 tablets, it was considered the EE2 dosage of 0.03 mg.

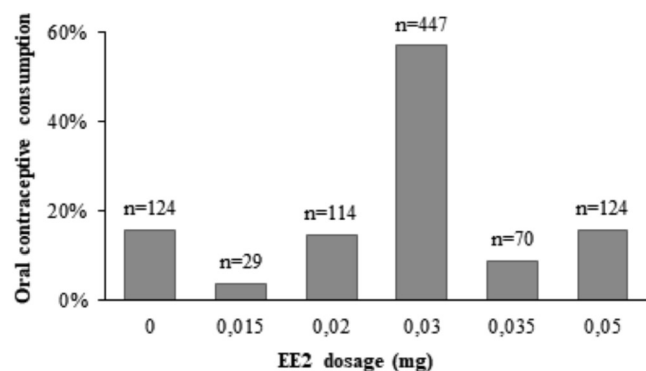


Figure 3: Monthly distribution of oral contraceptives at health centers and drugstores in the urban area of the 1<sup>st</sup> district of Santa Maria Madalena. In oral contraceptives with different synthetic estrogen dosages, i.e., bi or three-phase formulations, the most prevalent dosage was considered. For example, in biphasic formulas containing seven 0.04 mg EE2 pills and twenty-two 0.03 mg EE2 pills, it was considered the EE2 dosage of 0.03 mg.

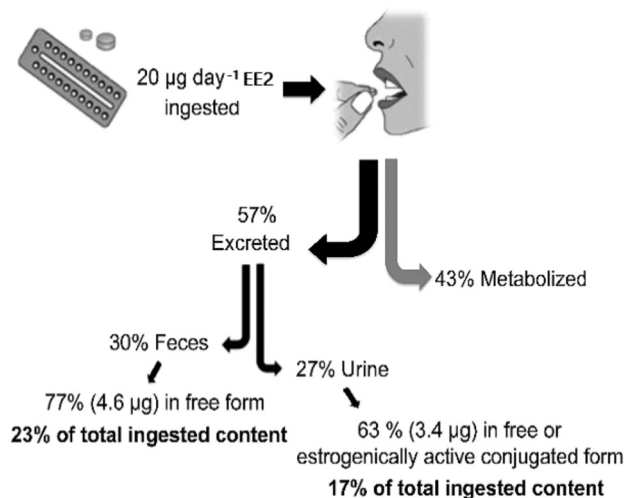


Figure 4: EE2 excretion scheme, using average consumption values in Santa Maria Madalena ( $20 \mu\text{g day}^{-1}$ ) and EE2 metabolism according to Johnson and Williams (2004).

(*Gambusia affinis*), becoming it more aggressive. Studies to assess changes in the immune system catfish (*Sparus aurata L.*) indicate that after 15 days of exposure to EE2 at concentrations between  $0.5$  and  $5 \text{ ng L}^{-1}$  can change the fish's ability to adequately respond to infection (Cabas *et al.* 2012). An assessment of the potential endocrine responses promoted by EE2 in different species led the European Commission to propose in 2012 a limit of this estrogen in surface waters. This study considered the adverse effects on fish associated with different concentrations of EE2. It was concluded that the no observed adverse effect level (NOAEL) is  $0.035 \text{ ng L}^{-1}$  of EE2; between  $0.1$  and  $0.2 \text{ ng L}^{-1}$  it is already possible to observe some adverse effects (LOAEL); at the concentration of  $1 \text{ ng L}^{-1}$  it is possible to induce the vitellogenin in male fish and at  $4 \text{ ng L}^{-1}$  the feminization of these; and values above  $5 \text{ ng L}^{-1}$  can impose population damages (Cunha *et al.*, 2016; Gilbert, 2012).

It should be noted that EE2 is not the only compound released into water bodies that can induce potential endocrine disruption. Moreover, as documented in the literature, EE2 associated with other compounds may trigger even more severe adverse effects, due to additive and synergistic mechanisms (Fent *et al.*, 2006). According to Hua *et al.* 2016, the combined exposure of megestrol acetate (progestogen) and EE2 in zebra fish (*Danio rerio*) caused a further reduction in fertility compared to exposure to EE2 alone, suggesting an additive effect on egg production when EE2 is supplemented with this progestogen. Örn *et al.* (2016) observed that exposure to mixtures of EE2 with  $17\beta$ -trenbolone (androgen) affects the process of gonad differentiation in zebra fish and leads to sexual interruption.

## CONCLUSIONS

In view of the discussion conducted by the present study, it is pertinent to highlight the relevance of the synthetic estrogen EE2 as an important micropollutant and endocrine disruptor,

and which can cause significant potential adverse effects in the aquatic biota.

The area selected for the elaboration of this study, the 1<sup>st</sup> district of Santa Maria Madalena, in Rio de Janeiro, presented itself as a favorable region for the development of this study since it was possible to access STPs, health units, drugstores, and other relevant points of information.

Regarding the survey, the contact with the interviewees can be evaluated as positive, since it introduced a topic of interest for public health and the environment, which brings forward discussions on environmental sanitation.

Based on the information obtained through a survey-type study that addressed a representative sample of women residing in the study area, a similar pattern of oral contraceptive consumption was found compared to that observed in studies with a national scope, in which about 30 % of the interviewees use such pharmaceuticals.

In addition, it was also observed that most of the sanitary effluents produced in this municipality go untreated before being released into the local water bodies. This is because a considerable part of the households is not connected to the sewage network, or because several localities have this network, but it is not linked to any treatment stations. This reality, unfortunately, can be observed throughout the entire country, which constitutes an important challenge for public and environmental health.

From the information obtained it was possible to estimate the occurrence of a considerable release of EE2 into the water bodies of Santa Maria Madalena, which suggests the possible threat to the local aquatic biota.

Due to the risks related to the introduction of these pharmaceuticals in the aquatic environment, studies on the improvement of wastewater treatment technologies have increasingly advanced with the aim of achieving greater efficiency in the removal of estrogens and other contaminants. In addition, some countries in the European Union and the United States have increasingly aimed to extend the regulations and monitoring of new compounds, not previously addressed by legal provisions regarding the control of water pollutants, such as EE2 (Cunha *et al.*, 2016).

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