Research, Society and Development, v. 11, n. 11, e393111133841, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i11.33841

Cytotoxicity and mutagenicity of female contraceptives in *Allium cepa* L.

Citotoxicidade e mutagenicidade de contraceptivos femininos em *Allium cepa* L.

Citotoxicidad y mutagenicidad de anticonceptivos femeninos en *Allium cepa* L.

Received: 08/08/2022 | Reviewed: 08/17/2022 | Accept: 08/18/2022 | Published: 08/26/2022

Jaqueline Klem Bohrer
ORCID: https://orcid.org/0000-0002-4224-1043
Federal Technological University of Paraná, Brazil
E-mail: jaquelinekbohrer@gmail.com
Adernanda Paula Dos Santos
ORCID: https://orcid.org/0000-0002-6303-3796
Federal Technological University of Paraná, Brazil
E-mail: adernandamarques@hotmail.com
Aline Perszel
ORCID: https://orcid.org/0000-0002-1017-6830
Federal Technological University of Paraná, Brazil
E-mail: alineperszel@alunos.utfpr.edu.br
Eduardo Michel Vieira Gomes
ORCID: https://orcid.org/0000-0002-4341-0021
Federal Technological University of Paraná, Brazil
E-mail eduardogomes@utfpr.edu.br
Alessandra Paim Berti
ORCID: https://orcid.org/0000-0002-8150-6569
State University of Mato Grosso do Sul, Brazil
E-mail: alessandrabiologa@hotmail.com
Elisângela Düsman
ORCID: https://orcid.org/0000-0002-4483-5638
Federal Technological University of Paraná, Brazil
E-mail: edusman@utfpr.edu.br

Abstract
Currently, a large part of the female population uses contraceptive methods due to the benefits provided by this medication. These drugs can be found in oral or injectable form and are basic compounds for progestogen and estrogen. However, despite the benefits, these contraceptives can cause a number of harmful effects as women, due to continuous and prolonged use, in addition to the problem of these compounds being excreted in the sewers, daily, causing problems to the population and the animals exposed. In this context, the present study aimed to evaluate the cytotoxic and mutagenic potential of different contraceptives, using *Allium cepa* L. as a eukaryotic test system. Four contraceptives were used: indicated for lactating mothers (*A1*), the following day pill (*A2*), a drospirenone (*A3*) and an injectable (*A4*). Based on the results, conclude what is the *A1* contraceptive, which is indicated for women in the lactation phase, shows cytotoxic effect, with a decrease in the mitotic index. However, none of the contraceptives studied were mutagenic for the eukaryotic cells studied. Thus, the data of the present study indicate that chronic studies must be carried out in order to protect humans and other organisms that may be exposed to these substances in the environment.
Keywords: Eukaryotic test system; Emergency contraception; Genotoxicity; Lactating; Drospirenone; Injectable contraceptive.

Resumo
Atualmente, grande parte da população feminina utiliza de métodos contraceptivos devido aos benefícios proporcionados por este medicamento. Estes medicamentos podem ser encontrados na forma oral ou injetável, e são compostos basicamente por progestogênio e estrogênio. Entretanto, apesar dos benefícios, esses contraceptivos podem causar uma série de efeitos colaterais as mulheres devido ao uso contínuo e prolongado, além da problemática destes compostos serem excretados nos esgotos, diariamente, causando problemas a população e aos animais expostos. Neste contexto, o presente trabalho teve como objetivo avaliar o potencial citotóxico e mutagênico de diferentes contraceptivos, usando a *Allium cepa* L. como um sistema de teste eucariótico. Foram utilizados quatro contraceptivos, sendo eles um indicado para lactantes (*A1*), pílula do dia seguinte (*A2*), um contendo drospirenona (*A3*) e um injetável (*A4*). Com base nos resultados, conclui-se que o contraceptivo *A1*, que é indicado para mulheres em fase de lactação, apresentou efeito citotóxico, com diminuição do índice mitótico. Entretanto, nenhum dos contraceptivos estudados foi mutagênico para as células eucariontes estudadas. Assim, os dados do presente estudo

indicam que estudos crônicos devem ser realizados, a fim de proteger o homem e os demais organismos que possam se expor à estas substâncias no meio ambiente.
Palavras-chave: Sistema de teste eucariótico; Contracepção de emergência; Genotoxicidade; Lactação; Drospirenona; Anticoncepcional injetável.

Resumen

Actualmente, gran parte de la población femenina utiliza métodos anticonceptivos debido a los beneficios que brinda este fármaco. Estos fármacos se pueden encontrar en forma oral o inyectable, y están compuestos básicamente por progestágenos y estrógenos. Sin embargo, a pesar de los beneficios, estos anticonceptivos pueden causar una serie de efectos secundarios a las mujeres debido al uso continuo y prolongado, además del problema de que estos compuestos se excretan en las alcantarillas, diariamente, causando problemas a la población y animales expuestos. En este contexto, el presente trabajo tuvo como objetivo evaluar el potencial citotóxico y mutagénico de diferentes anticonceptivos, utilizando *Allium cepa* L. como sistema de prueba eucariótico. Se utilizaron cuatro anticonceptivos, uno indicado para mujeres lactantes (*A1*), la píldora del día después (*A2*), uno con drospirenona (*A3*) y uno inyectable (*A4*). Con base en los resultados, se concluye que el anticonceptivo *A1*, que está indicado para mujeres lactantes, tuvo un efecto citotóxico, con disminución del índice mitótico. Sin embargo, ninguno de los anticonceptivos estudiados fue mutagénico para las células eucariotas estudiadas. Así, los datos del presente estudio indican que se deben realizar estudios crónicos para proteger a los humanos y otros organismos que puedan estar expuestos a estas sustancias en el ambiente.
Palabras clave: Sistema de prueba de eucariotas; Anticoncepción de emergencia; Genotoxicidad; Lactancia; Drospirenona; Anticonceptivo inyectable.

1. Introduction

The presence of emerging contaminants in the environment, including pharmaceutical chemicals, cosmetics, agrochemicals, among others, is one of the most relevant causes of human pressure on ecosystems (Snow et al., 2019; Ramírez-Malule; Quiñones-Murillo; Manotas-Duque, 2020). These compounds are introduced into the aquatic environment through different routes, which include the release of treated or untreated sewage, hospital effluents and pharmaceutical industries (Patel et al., 2019), leachate from landfills (Li et al., 2016) and runoff from urban or agricultural areas (Burns et al., 2018).

In recent years, the use of pharmaceutical products has shown a significant increase (Malik et al., 2021). In particular, hormonal contraceptives are currently one of the most prescribed drug classes in the world (Porcu; Serra; Concas, 2019). Its widespread use is related to its benefits, such as being a highly effective and reversible form of contraception, regularizing the menstrual cycle, reducing the rate of endometriosis, ovarian and endometrial cancer, in addition to being widely used in the treatment of diseases dermatological (Burrows; Basha; Goldstein, 2012; Porcu; Serra; Concas, 2019). A study carried out by Hall and Trussell (2012) indicated that 63% of compromised women of childbearing age worldwide were using some contraceptive method, with the oral contraceptive pill being the most preferred. In Brazil, Trindade et al. (2021) reported that more than 80% of the women who responded to the survey use some contraceptive method, where again, the oral contraceptive was the most used (34.2%).

Hormonal contraceptive methods are based on steroids used alone or in combination. They can be classified as combined between an estrogen and a progestin, or basically a progestin alone (Brasil, 2010). Ethinylestradiol is the most used estrogen (Sech and Mishell, 2015; Apter et al., 2017), when combined, the most used progestins are dienogest, desogestrel, drospirenone, gestodene, levonorgestrel, norethindrone, norgestimate and norgestrel (Sech and Mishell, 2015; Scarsi et al., 2016). Progestogen-only contraceptives offer similar efficacy to combined contraceptives, and have been preferred for their limited vascular effects, in addition to being a good option for contraception in the puerperium, not interfering with the quality or quantity of lactation (World Health Organization, 2015; Regidor, 2018). There is also emergency contraception, such as the morning-after pill, which consists of a single administration of a higher dose of progestogen, commonly levonorgestrel or

ulipristal acetate (Regidor, 2018), being indicated in cases where it is desired to avoid pregnancy after unprotected sexual intercourse or when there is a failure in the contraceptive method used (Brasil, 2011; Mouro and Gonçalves, 2021).

In addition to pills, there are a variety of contraceptive methods available, including injections, vaginal rings and intrauterine devices (Levin; Vitek; Hammes, 2017). Different contraceptive methods act mainly by inhibiting ovulation, in addition to causing changes in the physical and chemical characteristics of the endometrium and cervical mucus (Brasil, 2010; Rome and Issac, 2017). Despite the great utility of contraceptives, they can have side effects, such as an increased risk of venous thromboembolism in users who smoke or with other predispositions and an increased risk of breast cancer (Porcu; Serra; Concas, 2019; Barbosa and Chaves, 2021). According to Wu et al. (2013) users of contraceptives containing drospirenone are at approximately a threefold increased risk of venous thromboembolism compared with levonorgestrel.

After use, these substances can be absorbed, partially metabolized and excreted in feces and urine, destined for sewage (Liu; Kanjo; Mizutani, 2009). Many of these compounds and their metabolites are only partially removed by conventional treatment in wastewater treatment plants (WTPs) (Škrbić; Kadokami; Antić, 2018; Schmid et al., 2020), where they are often detected in sewage after treatment in concentrations ranging from ng L^{-1} a μg L^{-1} (Tran; Reinhard; Gin, 2018). Thus, many of these compounds can end up in rivers, lakes, streams and groundwater, and can even be detected in drinking water in trace concentrations (ppb ou ppt) (Schmidt and Redshaw, 2015; Su et al., 2020). In fact, the release of treated or untreated sewage is one of the main sources of hormones for the environment and consequently for human beings (Álvarez-Ruiz et al., 2020).

These compounds, classified as endocrine disruptors, have received attention due to their occurrence, persistence and potential toxic effect on exposed organisms, including estrogenicity, mutagenicity, cytotoxicity and genotoxicity (Valdés et al., 2016; Jia et al., 2019). Several studies have demonstrated the impacts of this exposure on humans and animals, such as endocrine changes (Bila and Dezotti, 2007; Orozco-Hernández et al., 2018), neuroendocrine changes (Porcu; Serra; Concas, 2019), disorders of reproductive functions (Sweeney et al., 2015; Gallo et al., 2016; Sheikh et al., 2017), increase in metabolic disorders (Heindel et al., 2017), sex reversal (Laurenson et al., 2014; Gogoi et al., 2018), negative impacts on female and male fertility (Kurowska et al., 2022), decreased production of eggs and sperm (Yan et al., 2012), growth retardation (Pillon et al., 2012; Zheng et al., 2019), vitellogenin in males (Laurenson et al., 2014), development of cancer and tumors (Sifakis et al., 2017).

These compounds are barely regulated by environmental policies around the world, and most worryingly, their toxic effects are still not well known (Peña-Guzmán et al., 2019; Adams et al., 2021). The toxicological analyzes aim to analyze the mechanisms of action of toxic agents capable of causing specific interactions with nucleic acids, which can result in genetic damage, in addition to point mutations, errors during the replication mechanism of deoxyribonucleic acid (DNA), as well as mitotic changes (Matsumoto and Marin-Morales, 2005).

In this context, the use of *Allium cepa* L. (onion) has been recommended in several studies, due to the fact that it has a high sensitivity, a relatively low cost, speed and simplicity in handling, being able to determine the increase or reduction of the mitotic index in an effective way, as well as the formation of chromosomal aberrations (Leme and Marin-Morales, 2009). In addition to good consolidation with other tests used with prokaryotic and eukaryotic cells, being an indicator of risk to human health or the environment (Düsman et al., 2011; Fatma et al., 2018).

Therefore, considering the constant and increasing use of hormonal contraceptives and the dispersion of these hormones in the environment, the objective of this study was to evaluate the cytotoxic and mutagenic potential of different female contraceptive methods available on the market, using *A. cepa* as a test system.

2. Methodology

2.1 Treatment Solutions

The following female contraceptives were analyzed:

A1 - Oral contraceptive indicated for lactating women: this contraceptive contains 0.075 mg of the hormone desogestrel, for this reason it is called a pill with isolated progestogen or mini-pill. Most mini pills act primarily by preventing sperm from entering the uterus. Unlike the combined pills, this formulation can be used by women who are tolerant to estrogen or who are breast-feeding.

A2 - The morning after pill: The morning after pill is an emergency contraceptive method, so it should be used only in cases of suspected failure in the normally used contraceptive method, in cases of unprotected sex or in cases of rape. This medicine contains 1.5 mg of levonorgestrel. Its mechanism of action can vary depending on the phase of the menstrual cycle, which can occur due to the inhibition or delay of ovulation, for making it difficult for the sperm to enter the uterus, for altering the passage of the egg or sperm through the uterine tube. After fertilization, the medication will not prevent the pregnancy from progressing.

A3 - Oral contraceptive containing drospirenone: this combined oral contraceptive contains a combination of two hormones: 3 mg of drospirenone (progestogen) and 0.03 mg of ethinyl estradiol (estrogen). These hormones prevent pregnancy through several mechanisms, the most important of which are inhibition of ovulation and changes in cervical secretion (in the cervix).

A4 - Injectable contraceptive: this combined injectable hormonal contraceptive is formulated as a depot preparation. Within the body the active ingredients (50 mg of norethisterone enanthate (progestogen) and 5 mg of estradiol valerate (estrogen)) are released slowly, so that a monthly injection is sufficient. It should always be administered monthly through a deep intramuscular route.

2.2 Solutions Preparation

To estimate the amount of each contraceptive needed to prepare the treatment solutions, a woman with an average mass equal to 70 Kg and an onion with an average mass 0.060 Kg was considered.

For solid contraceptives, considering that one tablet is taken daily, each length was weighed and according to Equation 1, the ideal amount proportional to the onion mass was obtained, resulting in: *A1*: 0.056 mg (1.870 mg L^{-1}); *A2*: 0.170 mg (1,130 mg L^{-1}); *A3*: 0.089 mg (0.590 mg L^{-1}).

$$Mi = \frac{(60 \times Mc)}{70{,}000} \qquad (1)$$

Where:

M_i= Ideal mass of the solid contraceptive (g);

60= Average mass of onion (g);

M_c= Mass of a tablet (g);

70,000= Average mass of the woman (g).

As for the injectable contraceptive that is liquid, the same ratio of female average mass and onion was used, however the ideal volume was obtained through Equation 2, being I: 0.000857 mL (0.029 mL L^{-1}).

$$Vi = \frac{(60 \times Vc)}{70{,}000} \qquad (2)$$

Where:

V_i= Optimal volume of liquid contraceptive (mL);

60= Average mass of onion (g);

V_c= Volume of a dose of contraceptive (mL);

70,000= Average mass of the woman (g).

These quantities were diluted in 50 mL of filtered water to be in contact with each onion. The final solutions showed a hormonal concentration equal to 1.870 µg mL^{-1} (*A1*), 1.130 µg mL^{-1} (*A2*), 0.590 µg mL^{-1} (*A3*) and 0.029 µL mL^{-1} (*A4*).

2.3 Cytotoxicity and mutagenicity test with *A. cepa*

The cytotoxicity and mutagenicity of the samples were evaluated using the meristematic cells of *A. cepa* (onion) root, prepared by the Feulgen reaction and stained with the Schiff's reagent (Fiskesjö, 1985).

The onion bulbs were placed to root in flasks with filtered water at room temperature, aerated and in the dark. Before each treatment, three roots were collected and fixed (3 methanol: 1 acetic acid) to serve as control of the bulb itself (Co 0h). Then, the roots of these bulbs were placed in contact with the treatment solutions, for 24 hours.

After the treatment time, three roots were removed from each onion and fixed (Tr 24h). The remaining roots were washed and the bulbs again placed in filtered water, to recover any damage that occurred, for 24 hours. Afterwards, three roots were removed and fixed (Re 48h).

In the negative control group, the onions remained for the entire sampling time in filtered water (CO-), and in the positive control the roots were exposed to a paracetamol solution (CO +) (0.8 g L^{-1}) (as indicated in the work of Schutz et al. (2021)) in order to compare the different water samples with their respective controls, in the different periods.

The roots remained in the fixative for at least 24 hours under refrigeration, then they were washed with distilled water and underwent hydrolysis with 5 mL of 1N hydrochloric acid at 60° C, for 10 minutes, in an oven at 60° C. After washing, the roots were stained with 5 mL of Schiff's reagent for 45 minutes. For the preparation of the slides, the meristematic region of the roots was used, which was macerated with acetic orcein and covered with a coverslip.

The slides were analyzed in light microscopes with a 40x objective. One thousand cells were counted per repetition, totaling 5,000 cells from each control or treated group, differentiating them according to the phases of the mitotic cell cycle (Interphase, Prophase, Metaphase, Anaphase, Telophase).

In each phase of the cell cycle, cells with structural chromosomal changes were also evaluated, such as: colchicine or disorganized metaphases, multipolar anaphases or with loose chromosomes, micronuclei and others.

To determine the cytotoxicity of the samples, the percentage of the Mitotic Index (MI%) (Equation 3) was calculated. And, to assess the mutagenic potential, the Mutagenic Index (MUTI%) was calculated (Equation 4). The data on mitotic and mutagenic indexes were analyzed using the Kruskal Wallis test (α= 0.05, $p < 0.05$, n= 5).

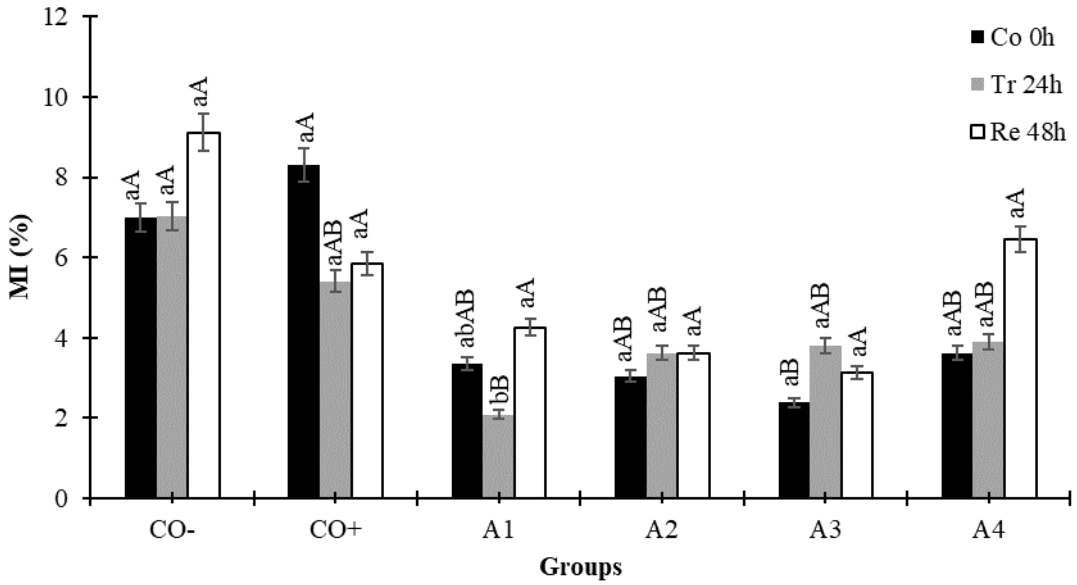
$$MI\% = \left(\frac{number\ of\ cells\ in\ division}{total\ number\ of\ cells\ analyzed}\right) \times 100 \qquad (3)$$

$$MUTI\% = \left(\frac{number\ of\ cells\ changed}{total\ number\ of\ cells\ analysed}\right) \times 100 \qquad (4)$$

3. Results and Discussion

The data in Figure 1 show the average percentages of mitotic indexes obtained for the control and treated groups, at 0, 24 and 48 hours. Statistical analysis showed that contraceptives *A2*, *A3* and *A4* exhibited mitotic index, in the treatment time (24 h), equal to the exposure time of the negative control (CO- 24 h), the control 0 h (Co 0h) and the recovery time (Re 48 h) of the bulb itself, indicating their absence of cytotoxic effect.

Figure 1. Average percentages of Mitotic Indexes (MI) and standard deviations for the negative (CO-) and positive (CO+) control groups and treated with different oral contraceptives.



A1: suitable for lactating women. *A2*: morning after pill. *A3*: oral contraceptive containing drospirenone. *A4*: injectable contraceptive. Equal lowercase letters indicate statistically equal means within the same group for each time (0h, 24h and 48h) and equal uppercase letters indicate statistically equal means between different groups within each treatment time, using the Kruskal Wallis test at the 5% level of significance (n= 5). Source: Authors.

A similar result was observed by Garcia, Golveia and Santiago (2014), in which *A. cepa* treated with a mixture of the hormones ethinyl estradiol, gestodene, cyproterone acetate and levonorgestrel, also showed no changes in mitotic indices and, thus, cytotoxicity, after 24 and 72 hours of treatment. In the study by Shyama, Abdul and Vijayalaxmi (1991) with the contraceptive Anovlar 21, which contains the combination of estrogen ethinyl estradiol and progesterone norethisterone acetate (similar to the *A3* contraceptive in the present study, which contains an estrogen and a progestogen in its composition), using bone marrow cells from mice in vivo, there were also no statistically significant differences in mitotic indices in any of the tested concentrations. Viega, Rocha and Düsman (2020), evaluating gel containing estradiol, present in the composition of *A4*, also using cytotoxicity test with *A. cepa*, also did not identify changes in mitotic indices after 24 hours of treatment.

However, the *A1* contraceptive, indicated for lactating women and containing the progestogen hormone desogestrel in its composition, showed a mitotic index (*A1* Tr 24h) statistically lower than that of the negative control (CO- Tr 24h), with a decrease of 70.38%. These data indicate the cytotoxic effect of this contraceptive for eukaryotic cells used in the present study. If compared to the mitotic index of the control of the bulb itself (*A1* Co 0h), the decrease in cell divisions was 37.72%, but it was not statistically significant.

This cytotoxic effect of the *A1* contraceptive is ratified by the results found by Ahmad et al. (2001), in which the norgestrel progestin, at the highest concentrations, strongly inhibited the proliferation of human lymphocytes both in the

presence and in the absence of metabolic activation system. This result was justified with the hypothesis that the inhibition of cell growth occurred due to a prolonged G2 phase. Liang et al. (2015) demonstrate that norgestrel at concentrations ≥ 5 ng L^{-1} can alter the transcription levels of some target genes during the early development of zebrafish, and this change was directly related to the concentration and the exposure time.

According to the instructions for this contraceptive (*A1*), small amounts of the active substance in desogestrel can pass into breast milk. Therefore, the cytotoxicity data of this contraceptive is very worrying, since the child in the breastfeeding phase, sometimes up to 2 years or more, is in a constant growth stage and, possible interferences in his cell divisions can result in serious future losses.

A study by Wagner (2006), for example, indicated that the cerebral cortex may be sensitive to exposure of the hormone progesterone during its formation, demonstrating that steroid hormones can influence fundamental development processes in some regions of the brain. In addition, progesterone can facilitate, inhibit or imitate the actions of testosterone in male behavior in different species, from lizards to humans, suggesting that this hormone can cause changes in the sexual differentiation of the brain, as well as in male behavior.

On the other hand, in a review by Phillips et al. (2016) showed no effects on the child exposed to progestogen through lactation with respect to growth, development and health in the first years of life. However, the author points out that no data has been verified regarding the long-term effects. Garbett et al. (2020), for example, shows that exposure to synthetic hormones can induce changes in germ cells, resulting in an altered neurodevelopment in the next generation, requiring the use of complementary tests to assess this potential.

In the present study, it can be observed that the mitotic index of recovery time (*A1* Re 48 h) was statistically different from the treatment time (*A1* Tr 24 h), showing that the cells were able to recover after removing contact with this contraceptive. A similar result was also observed by Garcia, Golveia and Santiago (2014), in which after exposure the mixture of the hormones ethinyl estradiol, gestodene, cyproterone acetate and levonorgestrel, within 48 hours of recovery, it was proved that the bulbs still had the rooting power, and the presence of the hormones did not damage the growth of the onions.

The data in Table 1 show the results of mutagenic indices and the types of chromosomal changes identified. According to the statistical analysis, the contraceptive samples evaluated did not show statistical differences when compared to the control, at the same exposure time, nor did it show differences in the comparison of the different exposure times within the same group, not indicating a mutagenic effect for onions at this time of evaluation. The genotoxicity of ethinylestradiol (substance that makes up the drug *A3*) was investigated by Siddique, Beg and Afzal (2005), in three different concentrations, and there was no genotoxicity in the absence of metabolic activation (S9 mix) and with metabolic activation without NADP (nicotinamide adenine dinucleotide phosphate), as found in the present study. Estradiol (present in *A4*) also showed negative results for mutagenicity by the chromosomal aberration test with *A. cepa* performed by Viega, Rocha and Düsman (2020).

Table 1. Types, numbers, total changes and mutagenic index (%) obtained for the negative (CO-) and positive (CO+) control groups and the groups treated with different female contraceptives, at times 0, 24 and 48 hours.

Treatment		Phases of Mitosis											Total Changes	Mutagenic Index (%)
		Interphase	Metaphase				Anaphase							
		AN	CM	MA	D	LC	SM	AB	MA	D	LC	MN		
CO-	0h	0	0	0	3	0	0	0	0	0	0	0	03	0.06 aA
	24h	0	0	0	2	0	1	1	3	0	0	0	07	0.14 aA
	48h	0	3	0	8	1	0	2	0	1	0	0	15	0.30 aA
CO+	0h	0	3	0	5	0	0	1	1	1	1	0	12	0.24 aA
	24h	0	1	0	1	2	0	0	0	0	0	0	04	0.08 aA
	48h	0	3	0	6	3	0	3	0	1	0	0	16	0.32 aA
A1	0h	0	3	0	2	0	0	0	1	0	0	0	06	0.12 aA
	24h	0	0	0	2	2	0	0	0	1	0	0	05	0.10 aA
	48h	0	1	0	6	1	0	0	0	1	2	0	11	0.22 aA
A2	0h	1	0	0	3	3	0	0	0	0	3	0	10	0.20 aA
	24h	0	1	0	3	3	1	2	0	2	0	0	12	0.24 aA
	48h	2	2	0	4	3	0	0	0	0	0	0	11	0.22 aA
A3	0h	0	0	0	3	2	0	0	0	0	0	0	05	0.10 aA
	24h	2	0	0	0	4	0	0	0	1	0	0	07	0.14 aA
	48h	1	0	0	4	2	0	0	0	1	1	0	09	0.18 aA
A4	0h	1	0	1	4	2	0	0	2	1	1	0	12	0.24 aA
	24h	1	0	1	2	1	0	1	0	0	0	1	07	0.14 aA
	48h	1	1	0	6	1	0	4	0	0	0	0	13	0.26 aA

AN: altered nucleus; CM: colchicine metaphase; MA: metaphase with adherence; D: disorganized; LC: loose chromosome; SM: sticky metaphase; AB: anaphase with bridge; MA: multipolar anaphase; MN: Micronucleus. *A1*: suitable for lactating women. *A2*: morning after pill. *A3*: oral contraceptive containing drospirenone. *A4*: injectable contraceptive. Averages followed by the same lowercase letter do not differ in the comparison between the three sampling times of the same control or treated group, and the same uppercase letters do not differ in comparison between the different sampling times of all groups among themselves, by the test Kruskal Wallis ($p<0.05$, $n= 5$). Source: Authors.

In a search conducted by Naz et al. (2016), for assessing DNA damage to blood lymphocytes, the authors indicated that the longer the period of use of oral contraceptives, the greater the damage found. This hypothesis can be verified according to the reports of Ahmad et al. (2001), who confirmed the genotoxic potential of synthetic progestins, used as contraceptives, and the influence of time and dose in the experiments was also observed. Therefore, it may be possible that if the exposure time of the present study was longer, higher percentages of mutagenicity could have been detected for *A1*, *A2*, *A3* and *A4*.

4. Conclusion

Thus, knowing the importance of the use of contraceptive methods today to prevent unwanted pregnancies, in the control of irregular menstrual cycles, as well as in the treatment of dermatological diseases, it is essential to know the reactions of these compounds. Based on the results of this research, it was found that the *A1* contraceptive, which is recommended for lactating women, showed a decrease in the mitotic index, demonstrating a cytotoxic effect to *A. cepa*. Despite this, none of the contraceptives studied were mutagenic for this indicator.

Therefore, it becomes increasingly necessary to develop more effective processes of sewage treatment, since they are one of the main routes of entry of hormones into ecosystems, as well as the application of cytotoxicity and mutagenicity tests as a tool to monitor the efficiency of these treatments. In addition, chronic studies should be carried out, mainly due to the results found for *A1*, in order to protect humans and other organisms that may be exposed to these substances in the environment.

References

Adams, E., Neves, B. B., Prola, L. D., de Liz, M. V., Martins, L. R., Ramsdorf, W. A., & de Freitas, A. M. (2021). Ecotoxicity and genotoxicity assessment of losartan after UV/H2O2 and UVC/photolysis treatments. *Environmental Science and Pollution Research*, *28*(19), 23812-23821. https://doi.org/10.1007/s11356-020-11420-9

Ahmad, E., Shadab, G. G. H. A., Azfer, A., & Afzal, M. (2001). Evaluation of genotoxic potential of synthetic progestins-norethindrone and norgestrel in human lymphocytes in vitro. *Mutation Research*, *494*(1-2), 13-20. https://doi.org/10.1016/S1383-5718(01)00164-4.

Álvarez-Ruiz, R., Picó, Y., Alfarhan, A. H., El-Sheikh, M. A., Alshahrani, H. O., & Barceló, D. (2020). Dataset of pesticides, pharmaceuticals and personal care products occurrence in wetlands of Saudi Arabia. *Data in brief*, *31*, 105776. https://doi.org/10.1016/j.dib.2020.105776

Apter, D., Zimmerman, Y., Beekman, L., Mawet, M., Maillard, C., Foidart, J. M., & Coelingh Bennink, H. J. (2017). Estetrol combined with drospirenone: an oral contraceptive with high acceptability, user satisfaction, well-being and favourable body weight control. *The European Journal of Contraception & Reproductive Health Care*, *22*(4), 260-267. https://doi.org/10.1080/13625187.2017.1336532

Barbosa, A. S., & Chaves, C. T. de O. P. . (2021). Consequences of continuous use of contraceptive: an alert to women. *Research, Society and Development*, *10*(15), e349101522949. https://doi.org/10.33448/rsd-v10i15.22949

Bila, D. M., & Dezotti, M. (2007). Desreguladores endócrinos no meio ambiente: efeitos e consequências. *Química nova*, *30*(3), 651-666. https://doi.org/10.1590/S0100-40422007000300027.

Brasil. Ministério da Saúde (MS), Secretaria de Atenção à Saúde. Departamento de Ações Programáticas Estratégicas. (2011). *Anticoncepção de emergência: perguntas e respostas para profissionais de saúde 2 ed.* (p. 7-8). Brasília: Editora do Ministério da Saúde.

Brasil. Ministério da Saúde (MS). Secretaria de Atenção à Saúde. Departamento de Atenção Básica. (2010). *Saúde sexual e saúde reprodutiva* (p. 173-174). Brasília: Editora do Ministério da Saúde.

Burns, E. E., Carter, L. J., Kolpin, D. W., Thomas-Oates, J., & Boxall, A. B. (2018). Temporal and spatial variation in pharmaceutical concentrations in an urban river system. *Water research*, *137*, 72-85. https://doi.org/10.1016/j.watres.2018.02.066

Burrows, L. J., Basha, M., & Goldstein, A. T. (2012). The effects of hormonal contraceptives on female sexuality: a review. *The journal of sexual medicine*, *9*(9), 2213-2223. https://doi.org/10.1111/j.1743-6109.2012.02848.x

Düsman, E., Faria, J.S., Toledo, F., Mazeti, C.M., Gonçalves, M.E.K., Vicentini, V.E.P. (2011). Vegetal test-system investigation on cytotoxicity of water from urban streams located in the northeastern region of Maringá, Paraná State, Brazil. *Acta Scientiarum. Biological Sciences*, *33*, 71-77. https://doi.org/10.4025/actascibiolsci.v33i1.4924.

Fatma, F., Verma S., Kamal, A., & Srivastava, A. (2018). Monitoring of morphotoxic, cytotoxic and genotoxic potential of mancozeb using *Allium* assay. *Chemosphere*, *195*, 864-870. https://doi.org/10.1016/j.chemosphere.2017.12.052.

Fiskesjö, G. (1985). The *Allium* test as a standard in environmental monitoring. *Hereditas*, *102*(1), 99-112. https://doi.org/10.1111/j.1601-5223.1985.tb00471.x.

Gallo, M. V., Ravenscroft, J., Carpenter, D. O., Frye, C., Cook, B., Schell, L. M., & Akwesasne Task Force on the Environment. (2016). Endocrine disrupting chemicals and ovulation: Is there a relationship?. *Environmental Research*, *151*, 410-418. https://doi.org/10.1016/j.envres.2016.08.007

Garbett, K. A., Tianbing, D., John, A., Carrie, A. G., Brad, A. G., Kevin, G. O., … Sweatt, J. D. (2020). Synthetic female gonadal hormones alter neurodevelopmental programming and behavior in F1 offspring. *Hormones and Behavior*, *126*, 104848. https://doi.org/10.1016/j.yhbeh.2020.104848.

Garcia, L. F., Golveia, J. C. S., & Santiago, M. F. (2014). Avaliação do potencial toxicológico de hormônios sexuais sintéticos utilizando bioensaios. *Revista Intertox de Toxicologia*, *7*(2), 58-75. https://doi.org/10.22280/revintervol7ed2.173.

Gogoi, A., Mazumder, P., Tyagi, V. K., Chaminda, G. T., An, A. K., & Kumar, M. (2018). Occurrence and fate of emerging contaminants in water environment: a review. *Groundwater for Sustainable Development*, *6*, 169-180. https://doi.org/10.1016/j.gsd.2017.12.009

Hall, K. S., & Trussell, J. (2012). Types of combined oral contraceptives used by US women. *Contraception*, *86*(6), 659-665. https://doi.org/10.1016/j.contraception.2012.05.017

Heindel, J. J., Blumberg, B., Cave, M., Machtinger, R., Mantovani, A., Mendez, M. A., ... & Vom Saal, F. (2017). Metabolism disrupting chemicals and metabolic disorders. *Reproductive toxicology*, *68*, 3-33. https://doi.org/10.1016/j.reprotox.2016.10.001

Jia, Y., Hammers-Wirtz, M., Crawford, S. E., Chen, Q., Seiler, T. B., Schäffer, A., & Hollert, H. (2019). Effect-based and chemical analyses of agonistic and antagonistic endocrine disruptors in multiple matrices of eutrophic freshwaters. *Science of the Total Environment*, *651*, 1096-1104. https://doi.org/10.1016/j.scitotenv.2018.09.199

Kurowska, P., Mlyczyńska, E., Dawid, M., Respekta, N., Pich, K., Serra, L., ... & Rak, A. (2022). Endocrine disruptor chemicals, adipokines and reproductive functions. *Endocrine*, 1-14. https://doi.org/10.1007/s12020-022-03061-4

Laurenson, J. P., Bloom, R. A., Page, S., & Sadrieh, N. (2014). Ethinyl estradiol and other human pharmaceutical estrogens in the aquatic environment: a review of recent risk assessment data. *The AAPS journal*, *16*(2), 299-310. https://doi.org/10.1208/s12248-014-9561-3

Leme, D. M., & Marin-Morales, M. A. (2009). *Allium cepa* test in environmental monitoring: a review on its application. *Mutation Research*, *682*(1), 71-81. https://doi.org/10.1016/j.mrrev.2009.06.002.

Levin, E.R., & Vitek W.S., & Hammes S.R. (2017). Estrogens, progestins, and the female reproductive tract. Brunton L.L., & Hilal-Dandan R, & Knollmann B.C.(Eds.), *Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 13e*. McGraw Hill. https://accessmedicine.mhmedical.com/content.aspx?bookid=2189&sectionid=172482097

Li, Q., Wang, P., Chen, L., Gao, H., & Wu, L. (2016). Acute toxicity and histopathological effects of naproxen in zebrafish (*Danio rerio*) early life stages. *Environmental Science and Pollution Research*, *23*(18), 18832-18841. https://doi.org/10.1007/s11356-016-7092-4

Liang, Y. Q., Huang, G. Y., Ying, G. G., Liu, S. S., Jiang, Y. X., & Liu, S. (2015). Progesterone and norgestrel alter transcriptional expression of genes along the hypothalamic–pituitary–thyroid axis in zebrafish embryos-larvae. *Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology*, *167*, 101-107. https://doi.org/10.1016/j.cbpc.2014.09.007.

Liu, Z. H., Kanjo, Y., & Mizutani, S. (2009). Urinary excretion rates of natural estrogens and androgens from humans, and their occurrence and fate in the environment: a review. *Science of the Total Environment*, *407*(18), 4975-4985. https://doi.org/10.1016/j.scitotenv.2009.06.001

Malik, M. Z., Musharavati, F., Khanmohammadi, S., Khanmohammadi, S., & Nguyen, D. D. (2021). Solar still desalination system equipped with paraffin as phase change material: exergoeconomic analysis and multi-objective optimization. *Environmental Science and Pollution Research*, *28*(1), 220-234. https://doi.org/10.1007/s11356-020-10335-9

Matsumoto, S. T., & Marin-Morales, M. A. (2005). Toxic and genotoxic effects of trivalent and hexavalent chromium-a review. *Revista Brasileira de Toxicologia*, 77-85. http://hdl.handle.net/11449/68282

Mouro, L. B., & Gonçalves, K. A. M. . (2021). The immoderate use of emergency contraception by young women. *Research, Society and Development*, *10*(15), e366101522857. https://doi.org/10.33448/rsd-v10i15.22857

Naz, F., Jyoti, S., Rahul, Akhtar, N., & Siddique, Y. H. (2016). Effect of oral contraceptive pills on the blood serum enzymes and DNA damage in lymphocytes among users. *Indian Journal of Clinical Biochemistry*, *31*(3), 294-301. https://doi.org/10.1007/s12291-015-0533-x.

Orozco-Hernández, L., Gutiérrez-Gómez, A. A., SanJuan-Reyes, N., Islas-Flores, H., García-Medina, S., Galar-Martínez, M., ... & Gómez-Oliván, L. M. (2018). 17β-Estradiol induces cyto-genotoxicity on blood cells of common carp (Cyprinus carpio). *Chemosphere*, *191*, 118-127. https://doi.org/10.1016/j.chemosphere.2017.10.030

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman Jr, C. U., & Mohan, D. (2019). Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods. *Chemical reviews*, *119*(6), 3510-3673. https://doi.org/10.1021/acs.chemrev.8b00299

Peña-Guzmán, C., Ulloa-Sánchez, S., Mora, K., Helena-Bustos, R., Lopez-Barrera, E., Alvarez, J., & Rodriguez-Pinzón, M. (2019). Emerging pollutants in the urban water cycle in Latin America: a review of the current literature. *Journal of environmental management*, *237*, 408-423. https://doi.org/10.1016/j.jenvman.2019.02.100

Phillips, S. J., Tepper, N. K., Kapp, N., Nanda, K., Temmerman, M., & Curtis, K. M. (2016). Progestogen-only contraceptive use among breastfeeding women: a systematic review. *Contraception*, *82*(1), 17-37. https://doi.org/10.1016/j.contraception.2010.02.002.

Pillon, D., Cadiou, V., Angulo, L., & Duittoz, A. H. (2012). Maternal exposure to 17-alpha-ethinylestradiol alters embryonic development of GnRH-1 neurons in mouse. *Brain research*, *1433*, 29-37. https://doi.org/10.1016/j.brainres.2011.11.030

Porcu, P., Serra, M., & Concas, A. (2019). The brain as a target of hormonal contraceptives: Evidence from animal studies. *Frontiers in Neuroendocrinology*, *55*, 100799. https://doi.org/10.1016/j.yfrne.2019.100799

Ramírez-Malule, H., Quiñones-Murillo, D. H., & Manotas-Duque, D. (2020). Emerging contaminants as global environmental hazards. A bibliometric analysis. *Emerging contaminants*, *6*, 179-193. https://doi.org/10.1016/j.emcon.2020.05.001

Regidor, P. A. (2018). The clinical relevance of progestogens in hormonal contraception: Present status and future developments. *Oncotarget*, *9*(77), 34628. https://doi.org/10.18632/oncotarget.26015

Rome, E. S., & Issac, V. (2017). Sometimes You Do Get a Second Chance: Emergency Contraception for Adolescents. *Pediatric Clinics*, *64*(2), 371-380. https://doi.org/10.1016/j.pcl.2016.11.006

Scarsi, K. K., Darin, K. M., Chappell, C. A., Nitz, S. M., & Lamorde, M. (2016). Drug–drug interactions, effectiveness, and safety of hormonal contraceptives in women living with HIV. *Drug safety*, *39*(11), 1053-1072. https://doi.org/10.1007/s40264-016-0452-7

Schmid, S., Willi, R. A., Salgueiro-González, N., & Fent, K. (2020). Effects of new generation progestins, including as mixtures and in combination with other classes of steroid hormones, on zebrafish early life stages. *Science of The Total Environment*, *709*, 136262. https://doi.org/10.1016/j.scitotenv.2019.136262

Schmidt, W., & Redshaw, C. H. (2015). Evaluation of biological endpoints in crop plants after exposure to non-steroidal anti-inflammatory drugs (NSAIDs): Implications for phytotoxicological assessment of novel contaminants. *Ecotoxicology and environmental safety*, *112*, 212-222. https://doi.org/10.1016/j.ecoenv.2014.11.008

Schutz, D. L., de Marco, I. G., Alves, G. L., Vincoski, J. V. A., Ishikawa, S., de Oliveira Schmitz, A. P., ... & Düsman, E. (2021). Biomonitoring of surface water quality in the Chopim River within the Conservation Unit Campos de Palmas Wildlife Refuge, southern Brazil. *Environmental Monitoring and Assessment*, *193*(11), 1-18. https://doi.org/10.1007/s10661-021-09464-6

Sech, L. A., & Mishell Jr, D. R. (2015). Oral steroid contraception. *Women's Health*, *11*(6), 743-748. https://doi.org/10.2217/whe.15.82

Sheikh, I. A., Tayubi, I. A., Ahmad, E., Ganaie, M. A., Bajouh, O. S., AlBasri, S. F., ... & Beg, M. A. (2017). Computational insights into the molecular interactions of environmental xenoestrogens 4-tert-octylphenol, 4-nonylphenol, bisphenol A (BPA), and BPA metabolite, 4-methyl-2, 4-bis (4-hydroxyphenyl) pent-1-ene (MBP) with human sex hormone-binding globulin. *Ecotoxicology and Environmental Safety*, *135*, 284-291. https://doi.org/10.1016/j.ecoenv.2016.10.005

Shyama, S. K., Abdul, R. M., & Vijayalaxmi, K. K. (1991). Genotoxic effect of Anovlar 21, an oral contraceptive, on mouse bone marrow. *Mutation Research, 260*(1), 47-53. https://doi.org/10.1016/0165-1218(91)90079-2.

Siddique, Y. H., Beg, T., & Afzal, M. (2005). Genotoxic potential of ethinylestradiol in cultured mammalian cells. *Chemico-Biological Interactions, 151*(2), 133-41. https://doi.org/10.1016/j.cbi.2004.10.008.

Sifakis, S., Androutsopoulos, V. P., Tsatsakis, A. M., & Spandidos, D. A. (2017). Human exposure to endocrine disrupting chemicals: effects on the male and female reproductive systems. *Environmental Toxicology and Pharmacology, 51*, 56-70. https://doi.org/10.1016/j.etap.2017.02.024

Škrbić, B. D., Kadokami, K., & Antić, I. (2018). Survey on the micro-pollutants presence in surface water system of northern Serbia and environmental and health risk assessment. *Environmental research, 166*, 130-140. https://doi.org/10.1016/j.envres.2018.05.034

Snow, D. D., Cassada, D. A., Biswas, S., Malakar, A., D'Alessio, M., Carter, L. J., ... & Sallach, J. B. (2019). Detection, occurrence, and fate of emerging contaminants in agricultural environments. *Water Environment Research, 91*(10), 1103-1113. https://doi.org/10.1002/wer.1204

Su, C., Cui, Y., Liu, D., Zhang, H., & Baninla, Y. (2020). Endocrine disrupting compounds, pharmaceuticals and personal care products in the aquatic environment of China: which chemicals are the prioritized ones?. *Science of The Total Environment*, *720*, 137652. https://doi.org/10.1016/j.scitotenv.2020.137652

Sweeney, M. F., Hasan, N., Soto, A. M., & Sonnenschein, C. (2015). Environmental endocrine disruptors: effects on the human male reproductive system. *Reviews in Endocrine and Metabolic Disorders*, *16*(4), 341-357. https://doi.org/10.1007/s11154-016-9337-4

Tran, N. H., Reinhard, M., & Gin, K. Y. H. (2018). Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. *Water research, 133*, 182-207. https://doi.org/10.1016/j.watres.2017.12.029

Trindade, R. E. D., Siqueira, B. B., Paula, T. F. D., & Felisbino-Mendes, M. S. (2021). Uso de contracepção e desigualdades do planejamento reprodutivo das mulheres brasileiras. *Ciência & Saúde Coletiva, 26*, 3493-3504. https://doi.org/10.1590/1413-81232021269.2.24332019

Valdés, M. E., Huerta, B., Wunderlin, D. A., Bistoni, M. A., Barceló, D., & Rodriguez-Mozaz, S. (2016). Bioaccumulation and bioconcentration of carbamazepine and other pharmaceuticals in fish under field and controlled laboratory experiments. Evidences of carbamazepine metabolization by fish. *Science of The Total Environment, 557*, 58-67. https://doi.org/10.1016/j.scitotenv.2016.03.045

Viega, B. L., Rocha, A. M., & Düsman, E. (2020). Cosmetics with hormonal composition for bioindicators *Artemia salina* L. and *Allium cepa* L. toxic potential. *Environmental Science and Pollution Research, 27*(6), 6659-6666. https://doi.org/10.1007/s11356-019-07392-0.

Wagner, C. K. (2006). The many faces of progesterone: a role in adult and developing male brain. *Frontiers in Neuroendocrinology*, *27*(3), 340-359. https://doi.org/10.1016/j.yfrne.2006.07.003.

World Health Organization (2015). Medical eligibility criteria for contraceptive use. 5 ed. *World Health Organization.*

Wu, C. Q., Grandi, S. M., Filion, K. B., Abenhaim, H. A., Joseph, L., & Eisenberg, M. J. (2013). Drospirenone-containing oral contraceptive pills and the risk of venous and arterial thrombosis: a systematic review. *BJOG: An International Journal of Obstetrics & Gynaecology*, *120*(7), 801-811. https://doi.org/10.1111/1471-0528.12210

Yan, Z., Lu, G., Liu, J., & Jin, S. (2012). An integrated assessment of estrogenic contamination and feminization risk in fish in Taihu Lake, China. *Ecotoxicology and environmental safety*, *84*, 334-340. https://doi.org/10.1016/j.ecoenv.2012.08.010

Zheng, Y., Yuan, J., Meng, S., Chen, J., & Gu, Z. (2019). Testicular transcriptome alterations in zebrafish (*Danio rerio*) exposure to 17β-estradiol. *Chemosphere*, *218*, 14-25. https://doi.org/10.1016/j.chemosphere.2018.11.092