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Radiolytic degradation of levonorgestrel and gestodene: Performance and bioassays

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ABSTRACT

This study reports the feasibility of ionizing sources (^{60}Co source and electron beam radiation) to degrade the progestins hormones levonorgestrel (LNG) and gestodene (GES) in synthetic solutions and real pharmaceutical wastewater (RPW). Doses of 0.5–100 kGy and dose rates of 2.5 and 10 kGy h^{-1} were applied. LNG was shown to be more recalcitrant than GES, with 90% removals achieved at doses around 7.7 kGy (LNG) and 1.6 kGy (GES) in model systems, with LNG showing greater reactivity with reducing species in γ-radiolysis, unlike GES. Furthermore, LNG removal remained around 60% in RPW at low doses, while more than 60% GES removal was observed for all doses. LNG and GES toxicities to *Daphnia similis* were absorbed dose-dependent, with low doses resulting in toxicity reductions of around 32% (LNG) and 42% (GES); in turn, high doses promoted a fourfold increase in toxicity. γ-radiolysis reduced the cytotoxic character of LNG to NIH-3T3-L1 cells, while non-irradiated or irradiated GES solutions did not exhibit any cytotoxic effect. Finally, the estrogenic activity, evaluated by the YES assay, was dose-dependent for both progestins, which may be related to the evolution of transformation products formed by water radiolysis in each case, decreasing for high doses.

1. Introduction

The fate of active pharmaceutical ingredients (APIs) as hormones in water bodies poses a challenge to water quality and wastewater technologies, making it an emerging topic worldwide. The environmental concern regarding these compounds is based on the large variety and amounts of drugs consumed yearly, their regular discharge from anthropogenic activities, and hazardous classification (Desbiolles et al., 2018). In 2019, more than 150 million women in the world had chosen the pill as the usual contraceptive method, among which 29.7 million were Brazilian (United Nations 2019). Among these contraceptives, levonorgestrel (LNG) and gestodene (GES) are classified as progestins, commonly used for hormonal control and pregnancy prevention (Fent, 2015). Contraceptive pills are a combined medication, containing both an estrogen and a progestin, such as ethinylestradiol (EE2) and LNG, respectively, while emergency contraceptives contain LNG as essentially the only active ingredient with a usual dosage of 1.50 mg (King et al., 2016). After consumption, active principles are not completely metabolized, being 53%, 77%, and 10% of the ingested EE2, LNG, and GES eliminated in their active form via urine, respectively (King et al., 2016; Besse and Garric, 2009).

Anthropogenic activities are responsible for releasing hormones into the environment, being wastewater treatment plants (WWTPs) the most important source (Heberer, 2002). Besides, pharmaceutical manufacture/formulation facilities are considered an important source of pharmaceutical wastes (Table S1). These are classified within the “red category” owing to the high load of pharmaceuticals and chemical compounds manipulated to produce medicines, generating complex and hazardous wastewater (Gadipelly et al., 2014).

However, established treatment processes, such as coagulation, flocculation, and biological process are unable to completely remove these emerging contaminants (Pal, 2018; Yu et al., 2019), which may

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undergo chemical transformations in WWTPs, resulting in new bioactive metabolites and by-products which may still affect ecosystems (Quintana et al., 2005; Farré et al. 2008). For example, an investigation conducted in China highlights LNG detection frequencies above 75% in influent and effluent samples from 21 investigated WWTPs across the country (Yu et al., 2019). According to the authors, LNG showed a recalcitrant character with removal efficiencies below 50%, thus confirming the priority concern in controlling the release of the hormone into water bodies.

In this context, advanced wastewater treatments have been evaluated in combination with these processes, aiming to mineralize recalcitrant and toxic chemicals in pharmaceutical wastewater, moving towards a sustainable strategy (Pal, 2018). Advanced oxidation processes (AOPs) are considered an alternative for treating pharmaceutical wastewater, due to their capability to generate highly reactive species, mainly but not exclusively, hydroxyl radicals ($HO^{\bullet}$). The application of AOPs to LNG and GES removal has been investigated in the last ten years, for example, with the use of ozone (Broséus et al., 2009; Rokhina et al., 2012), ultrasound (Fu et al., 2007), photocatalysis (Nasuhoglu et al., 2012), UV photolysis (Eckert et al., 2012), electrochemical technologies (Nájera-Aguila et al., 2016; AlQaim et al., 2018), wet oxidation (Sirinukulwatana et al., 2017) and homogeneous photocatalysis with persulfate (Narváez et al., 2019). However, the use of real pharmaceutical wastewater has been reported in only three publications, in which LNG concentrations varied in the range 0.025–50 mg L^{-1}(Nasuhoglu et al., 2012; Eckert et al., 2012; Sirinukulwatana et al., 2017), while GES has not been evaluated in real pharmaceutical matrices. In addition, the use of radiolysis-based processes has not yet been described in the literature for degrading these compounds.

Ionizing radiation can be applied to wastewater treatment aiming to generate reactive species, essentially from water radiolysis (Eq. 1). The bracketed values in Eq. 1 denote the radiation chemical yields (G-values) of the primary reactive species in μmol J^{-1}, namely hydroxyl radicals ($HO^{\bullet}$), hydrogen atoms ($H^{\bullet}$) and aqueous electrons (e_{aq}^{-}), the latter two being reductive species (Khan et al., 2019; Trojanowicz et al., 2017; Trojanowicz, 2020).

$$H_2O \rightarrow [0{\cdot}28]\ HO^{\bullet} + [0{\cdot}27]\ e_{aq}^{-} + [0{\cdot}06]\ H^{\bullet} + [0{\cdot}07]\ H_2O_2 + [0{\cdot}27]\ H_3O^{+} + [0{\cdot}05]\ H_2 \quad (1)$$

One of the advantages of treatment processes based on water radiolysis compared with other AOPs is the ability to promote the generation of reducing reactive species besides $HO^{\bullet}$ radicals, without the addition of auxiliary oxidants. Hydrated electrons participate in nucleophilic reactions, especially with aromatic hydrocarbons, halogenated hydrocarbons, and compounds containing carboxylic groups, and reactions involving hydrogen atoms can be observed in the direct reduction of metals and organic compounds (Tang, 2004; Cooper et al., 2004; Trojanowicz, 2020). Chen et al. (2019) investigated the use of gamma radiolysis to degrade cephalosporin C, using N_2 or synthetic air, with and without tert-BuOH, to identify the role of oxidizing and reducing species. The antibiotic degradation was improved in solutions saturated with nitrogen, in which $HO^{\bullet}$, e_{aq}^{-} and $H^{\bullet}$ were the major reactive species. However, the observed degradation rate was higher in the presence of t-BuOH, a well-known $HO^{\bullet}$ quencher. The authors concluded that antibiotic oxidation occurred, although reducing species also played a significant role in its degradation.

Water radiolysis can be achieved by using γ-rays (generated from radionuclides, such as ^{60}Co and ^{137}Cs) or electron beam irradiation (EBI). Although these sources exhibit different properties and operational conditions, they show similar results regarding the generation of reactive species (Khan et al., 2019; Trojanowicz et al., 2017;Trojanowicz, 2020). The feasibility of this process has been investigated for the degradation of different pharmaceutical contaminants (Wang and Chu, 2016), e.g., 17β-estradiol (Ren et al., 2011), nitroimidazoles (Sánchez-Polo et al., 2009), chloramphenicol (Csay et al., 2012), aspirin and fluoxetine (Tominaga et al., 2021), and clofibric acid (Shi et al., 2019). The ^{60}Co radionuclide-based technology is suitable for small-scale batch application, while EB is more appropriate for large-scale continuous flow operation (Cooper et al., 2004; Khan et al., 2019; Changotra et al., 2019, 2020).

Ecotoxicological assays are an important tool to evaluate the risk assessment of pharmaceuticals in water matrices. Desbiolles et al. (2018) made an inventory of 43 pharmaceuticals compounds and their ecotoxicological risks already established in the literature. Among the APIs selected, some hormones were included, such as EE2 and 17β-estradiol, which was considered the most toxic among the four hormones evaluated. The authors highlight that in the 25 years until 2018, only 18 papers were published aiming to investigate the ecotoxicity of hormones, an issue not yet addressed for progestins and androgens.

Likewise, results of cytotoxic assays, which can be used to improve the risk assessment of progestins in water bodies, are still lacking in the literature. The same applies to the evaluation of the residual estrogenic activity of treated solutions containing progestins after water radiolysis, which can be assessed by in vitro recombinant reporter gene assays, such as Yeast Estrogen Screen (YES) (Routledge and Sumpter, 1996). This assay uses the yeast *Saccharomyces cerevisiae* modified with a human estrogen receptor (hER), which expresses a lac-Z gene receptor in the presence of estrogenic compounds. This induces the cells to release β-galactosidase, which converts the added chromogenic substrate chlorophenol red-β-D-galactopyranoside (CPRG) to chlorophenol red (CPR), turning the color of the medium from yellow to reddish (Argolo et al., 2021).

In this context, the present study aimed to evaluate the radiolytic degradation of the progestins LNG and GES in aqueous solutions submitted to gamma-rays and electron beam radiation. The progestins concentrations simulate those found in a real pharmaceutical wastewater sample, which had been previously collected and analyzed, finding (4.00 ± 0.31) mg L^{-1} and (0.66 ± 0.63) mg L^{-1} of LNG and GES, respectively. These values were used as a reference for selecting the initial concentrations of the progestins in this work. Doses of 0.5–100 kGy, and dose rates of 2.5 and 10.0 kGy h^{-1}, were applied. The main objectives were (i) to investigate LNG and GES degradation in synthetic solutions; (ii) to assess the effects of initial progestin concentrations, scavengers, G-values, and water matrix; (iii) to evaluate the radiolytic removal process through acute toxicity tests with *Daphnia similis*; (iv) to investigate the initial and final progestin cytotoxicity using NIH-3T3 mouse cells; and (v) to assess the estrogenicity of progestin samples, before and after γ-radiolysis using the Yeast Estrogen Screen (YES) assay.

2. Methodology

2.1. Reagents

Levonorgestrel (LNG, $C_{21}H_{28}O_2$, ≥ 98.0%) and gestodene (GES, $C_{21}H_{26}O_2$, ≥ 98.0) (Table S2) were purchased from Zhejiang Xianju Pharmaceutical Co. Ltd.17 β-estradiol (E2, $C_{18}H_{24}O_2$, ≥ 98%, CAS 50–28–2) was purchased from Sigma-Aldrich and chlorophenol red-β-D-galactopyranoside (CPRG) from Merck. LNG and GES were used as a standard in chromatographic analysis and all the experiments. HPLC grade methanol (MeOH), *tert*-butanol (t-BuOH) (≥ 99.0%), phenol (C_6H_5OH) (≥ 99.0%), perchloric acid ($HClO_4$) (70%), and acetic acid (CH_3CO_2H) were purchased from Sigma-Aldrich and used without further purification. Oxygen (O_2) with a purity of 99.5% was used. Ultra-pure water with a resistivity of 18.2 MΩ cm from a Milli-Q® system (Millipore) was used for preparing the synthetic solutions used in the present study.

Table 1
Concentrations of levonorgestrel (LNG) and gestodene (GES) in the matrices studied and irradiation treatments (γ-radiolysis, electron beam irradiation-EBI).

Matrix	$[progestin]_0$ mg L^{-1}	Irradiation treatment
PURE I-LNG	0.080 ± 0.001	γ-radiolysis
	0.50 ± 0.01	γ-radiolysis/EBI
	1.70 ± 0.08	γ-radiolysis
PURE I-GES	0.60 ± 0.03	γ-radiolysis/EBI
	2.460 ± 0.002	γ-radiolysis
	4.54 ± 0.01	γ-radiolysis
	8.06 ± 0.01	γ-radiolysis
PURE II-LNG	1.70 ± 0.08	γ-radiolysis
PURE II-GES	8.02 ± 0.04	γ-radiolysis
MIX	LNG = 1.70 ± 0.01	γ-radiolysis
	GES = 0.52 ± 0.01	γ-radiolysis
RPW	LNG = 4.00 ± 0.31	γ-radiolysis
	GES = 0.66 ± 0.63	γ-radiolysis

2.2. Progestins solutions and types of matrices

The degradation of the target progestins was studied in four matrices: (i) aqueous solutions of the pure contaminants (PURE I-LNG and PURE I-GES); (ii) solutions of the pure contaminants in water in the presence of scavengers (PURE II-LNG and PURE II-GES); (iii) aqueous solutions of LNG and GES (MIX); (iv) real pharmaceutical wastewater (RPW). The concentrations of LNG and GES in the matrices studied are given in Table 1 and were selected based on progestin concentrations found in RPW and their water solubility limits.

PURE II systems were selected in this study to investigate the scavenging effect of methanol, O_2, *tert*-butanol, and phenol on γ-radiolysis (Shan et al., 2014; Nisar et al., 2016). Oxygen was sparged into the solution, before the irradiation process, for 20 min. The organic solvents were added to the PURE II solutions at the following concentrations: [MeOH] = 10.0 mol L^{-1}; [t-BuOH] = 6 mol L^{-1} (pH = 3.0, corrected with 5-mol L^{-1} $HClO_4$ solution); [phenol] = 1.0 mol L^{-1}.

The real wastewater (RPW) was sampled in a pharmaceutical industry located in Goiânia (Brazil), before biological treatment; the sample was immediately characterized (Table S3) (APHA (2005). Visual observations indicated that the RPW sample contained a considerable amount of material in suspension; thus, LNG and GES can be found in solution and associated with suspended solids, due to their low solubility. Total LNG and GES concentrations were determined by UFLC analysis, for which RPW samples were previously syringe-filtered with 0.22-μm membranes and concentrated according to the SPE protocol described in Section 2.4.1 *Solid-phase extraction (SPE)*.

2.3. Irradiation treatment

The irradiation experiments were conducted on a batch scale using two ionizing sources, a Dynamitron® electron beam accelerator at 37.5 kW and 1.4 MeV, and a high-level ^{60}Co source (Cobalt-60 Multipurpose Radiator). Both sources are located at the Radiation Technology Center (Nuclear and Energy Research Institute-IPEN-CNEN/SP, São Paulo, Brazil). Absorbed doses were measured using a Perspex Harwell Red Batch KZ-4034 dosimeter with less than 5% variation. For γ-radiolysis, progestin solutions were placed into 40-mL glass vials in triplicate and irradiated at doses from 0.5 to100 kGy, and dose rates of 2.5 and 10 kGy h^{-1}. For the electron beam irradiation (EBI) experiments, solutions of the progestins were placed in Petri dishes; sample volumes of 50 mL were used, considering the maximum exposed liquid thickness of 4 mm (Tominaga et al., 2021). The doses applied to the electron beam process ranged from 1 to 10 kGy. An automated conveyor at 6.72 m min^{-1} was used to move the samples under the electron beam, twice in a row. The irradiation treatment to which each sample was submitted is indicated in Table 1.

2.4. Analytical techniques

GES and LNG concentrations were monitored by ultra-fast liquid chromatography (UFLC) using Shimadzu equipment (LC 20AD) equipped with a UV–visible detector (SPD 20A) and a C18 column (ACE, 250 mm × 4.60 mm, 5 μm). The isocratic method, adapted from Nájera-Aguila et al. (2016), was applied by using 70% methanol and 30% water containing 1% v/v of acetic acid as the mobile phase. Both hormones were detected at 244 nm. The sample injection volume, oven temperature, and flow rate were 50 μL, 40 °C, and 1.0 mL min^{-1}, respectively. Under these conditions, the retention time of LNG and GES were 8.0 and 10.0 min, respectively. Due to the low water solubility of LNG (Table S2), stock solutions of LNG and GES (10 mg L^{-1}) were prepared in methanol and used to formulate progestin standards. Two calibration curves were obtained by the dilution of stock solutions prepared to obtain LNG and GES standards of 0.05–10.0 mg L^{-1}. Table S4 presents the validation parameters of the calibration curves.

2.4.1. Solid-phase extraction (SPE)

Synthetic and RPW samples were concentrated using C18 cartridges (SPE Strata 200 mg/3 mL). The SPE protocol consisted of the following steps: (i) cartridges conditioning with 10 mL of methanol and 10 mL of pure water; (ii) percolation of 10 mL of the progestin samples at 4 mL min^{-1}; (iii) rinse with 10 mL of an aqueous solution of methanol (2% v/v); and (iv) elution of LNG and GES analytes with 2 mL methanol. LNG and GES extraction recoveries were (91.60 ± 0.04)% and (84.90 ± 2.47)%, respectively (Table S4).

2.5. Acute toxicity assays

Ecotoxicity assays were performed with the microcrustacean *Daphnia similis* according to the Brazilian Standard ABNT NBR 12713/2016 (ABNT, 2016). The assays were based on the evaluation of the effects of GES and LNG solutions to the test organisms, before and after gamma-radiolysis treatment; immobility of *D. similis* after 48 h was the endpoint measured for this assay. Daphnids were cultivated at the Laboratory of Biological and Environmental Assays (Nuclear and Energy Research Institute-IPEN-CNEN/SP, São Paulo, Brazil). For this purpose, neonates between 6 h and 24 h of age were exposed to several dilutions of irradiated and non-irradiated progestins solutions for 48 h. LNG and GES concentrations of non-irradiated solutions used in the toxicity assays were 0.33 and 1.10 mg L^{-1}, respectively. The initial pH of these solutions was about 6.0–6.5 and varied to 4.5 ± 1.1 and 6.2 ± 1.4, respectively, following irradiation. In both cases, the pH was previously adjusted to 7.0 before the toxicity assays. The acute toxicity is expressed in toxicity units (TU = 100/EC50%) and corresponds to the average effect concentrations that promoted 50% immobility of exposed living organisms (EC_{50}-48 h, expressed in % v/v).

2.6. Cytotoxicity assays

NIH-3T3-L1 cells were seeded onto 96-mm plates at the density of 1.0 × 10^4 cells per well in 100 μL of culture medium (RPMI 1640 supplemented with 10% of fetal bovine serum and antibiotics). The plates were incubated at 37 °C and 5% CO_2 for 24 h. The residual medium was removed one day later, and the cells were exposed to several dilutions of irradiated and non-irradiated LNG and GES solutions diluted in the medium. The cytotoxicity was also monitored in γ-irradiated progestins solutions and real pharmaceutical wastewater; for this, 100 μL of treated solutions were diluted in 900 μL of RPMI medium and exposed to NIH-3T3-L1 cells over 24 h of

incubation. In addition, solutions containing 5% v/v NaCl or 5% v/v dimethyl sulfoxide (DMSO) were applied as the negative and positive control, respectively. The plate was incubated again for a period of 24 h under the same conditions. After the contact period, the wells were washed with 100 µL of PBS buffer solution. Then, 120 µL per well of CellTiter 96® AQueous MTS solution (Promega) and 0.9% of phenazine methosulfate (PMS, Sigma-Aldrich, CAS 299–11–6) diluted in RPMI were added and the plate was incubated for 2 h. Finally, the plate was subjected to spectrophotometric analysis at 490 nm using Multiskan EX® equipment. Cytotoxic results are expressed in terms of percentage of cell viability (CV), which can be indicated as CV50 or CV90, that is, the concentration of hormone responsible for rendering 50% and 90% of exposed cells unviable, respectively.

2.7. Yeast Estrogen Screen (YES) assays

The estrogenicity of progestin samples was evaluated for selected samples, before and after γ-radiolysis, through YES (Yeast Estrogen Screen), an in vitro recombinant reporter gene assay (Routledge and Sumpter, 1996). Before the assay, progestin samples were concentrated using C18 cartridges (SPE Strata 200 mg/3 mL), following the procedure described in Section 2.4.1 *Solid-phase extraction (SPE)*.

The bioassay was performed in 96-well microplates with serial dilution of sample extracts in ethanol (Argolo et al., 2021). 17β-estradiol (E2) was used as a positive control and to generate the standard curve in the range from 1.33 to 2724 ng L^{-1}, while ethanol was used as a negative control (blanks). 10 µL of each sample dilution was transferred to a test plate and allowed to evaporate. 200 µL of culture medium containing yeast and chlorophenol red-β-D-galactopyranoside (CPRG) were then added. After incubation for 72 h at 30 °C, absorbances at 575 and 620 nm were measured using a VersaMax microplate reader (Molecular Devices).

2.7.1. Data analysis

Eq. 2 was used to correct the measured absorbances in order to discount the turbidity effect from the estrogenic response:

$$Abs_{corr\ \ (sample)} = Abs_{575\ \ (sample)} - (Abs_{620\ \ (sample)} - Abs_{620\ \ (blanks)}) \quad (2)$$

The resulting sigmoidal curves generated from plotting the corrected absorbance and concentration values were fitted to a symmetric logistic function using the software Origin 2020 (OriginLab). Estradiol equivalent results (E2-EQ, ng L^{-1}) were obtained by interpolation of the standard E2 dose-response curve (Fig. S1) and sample data through the log-logistic model given by Eq. 3:

$$y = \frac{A_1 - A_2}{1 + (x_0/x)^p} + A_2 \quad (3)$$

In which the parameters A_1 and A_2 refer to the maximum and minimum β-galactosidase induction in corrected absorbance, x_0 corresponds to the median effect concentration for E2 (EC_{50}, ng L^{-1}), p is the slope of the sigmoidal curve, and (x, y) refers the ordered pair related to a sample concentration and its corrected absorbance response. The lowest x that elucidated an agonist response, divided by the final sample enrichment factor in the assay was used to calculate the E2-EQ (Argolo et al., 2021)."

3. Results and discussion

3.1. Radiolytic degradation of progestins in PURE I AND PURE II solutions

3.1.1. Selection of the irradiation source

The first step in the study of radiolytic degradation of progestins consisted of evaluating the ionizing source, considering high removal efficiency, cost, degradation time and equipment availability, using pure contaminant solutions (PURE I-LNG and PURE I-GES) with $[LNG]_0$ = 0.50 ± 0.01 mg L^{-1} and $[GES]_0$ = 0.60 ± 0.03 mg L^{-1}, respectively; previous tests revealed that these concentrations were adequate for this comparative study, also taking into account the water solubility of the hormones Fig. 1 compares the reductions in relative progestin concentrations as a function of the absorbed dose for both ionizing sources (γ-rays and EBI). Progestin removals of 65.4% and 96.0% were obtained for LNG and GES, respectively, when the solutions were irradiated at 1 kGy by the Co^{60} source. LNG showed a distinct recalcitrant behavior under gamma radiolysis, with maximum removal of 84% at 10 kGy and a dose rate of 10 kGy h^{-1}. In contrast, EBI showed greater hormones removals (94% and 98% for LNG and GES, respectively) for the same dose, due to the high energy provided by this source and operational conditions, as the lowest dose rate (2.23 kGy s^{-1}); in addition, EBI was also studied at shorter irradiation times (0.5 s), as also conducted by Reinholds et al. (2017).

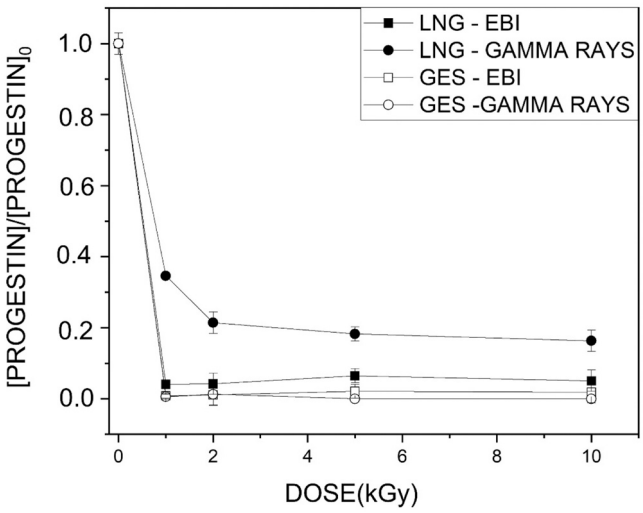

Fig. 1. Relative progestin concentrations as a function of absorbed dose for γ-radiolysis and electron beam irradiation. Conditions: $[LNG]_0$ = 0.50 ± 0.01 mg L^{-1} and $[GES]_0$ = 0.60 ± 0.03 mg L^{-1}; dose rates: 10 kGy h^{-1} (γ-radiolysis) and 2.23–10 kGy s^{-1} (EBI). Error bars correspond to n = 3 replicates.

Regardless of the ionizing source, the radiolytic degradation of progestins showed a direct relationship between the increase in the absorbed dose (1.0–2.0 kGy) and removal efficiency. Certainly, the use of high doses (5.0–10 kGy) increases the efficiency of the process, due to the maximization of reactive species generation during radiolysis.

To elucidate the distinct effect of the ionizing source on progestins solutions, chemical radiation yields (G-values) were calculated, which represent the efficiency of radiolytic degradation under different conditions. The G-value is defined as the concentration of a compound consumed or produced by the absorption of 100 eV of radiation energy and is calculated by Eq. 4 (Wang et al., 2017).

$$G = \frac{\Delta R N_A}{6.24 \times 10^{19} D} \quad (4)$$

Where ΔR is the variation of progestins concentration ($[progestin]_{initial} - [progestin]_{final}$, in mol L^{-1}); N_A is the Avogadro number; D is the absorbed dose (kGy), and 6.24×10^{19} corresponds to the conversion factor from kGy to 100 eV L^{-1}. G-values are expressed in µmol J^{-1}, considering 1 molecule $(100\ eV)^{-1}$ = 0.10364 µmol J^{-1} (Wang et al., 2017).

Table 2 presents the relationship between removal efficiency, chemical radiation yield, and pseudo-first-order specific degradation rate (k_{obs}) for LNG and GES, as also noted by Sánchez-Polo et al.

Table 2
Comparison of LNG and GES removals by water radiolysis by γ-rays and electron beam irradiation. Dose rates: 10 kGy h^{-1} (γ-radiolysis) and 2.23–10 kGy s^{-1} (EBI); $[LNG]_0$ = 0.50 ± 0.01 mg L^{-1}; $[GES]_0$ = 0.60 ± 0.03 mg L^{-1}. Experiments run in triplicate.

	LNG							
	GAMMA-RAYS				**ELECTRON BEAM**			
Absorbed dose (kGy)	Removal Efficiency (%)	G-value (μmol J^{-1})	$-k_{obs}$ (kGy^{-1})	R^2	Removal Efficiency (%)	G-value (μmol J^{-1})	$-k_{obs}$ (kGy^{-1})	R^2
1.0	65.4 ± 0.1	0.010 ± 0.0001	0.473 ± 0.010	0.994	96.0 ± 0.2	0.015 ± 0.0001	0.510 ± 0.030	0.646
2.0	77.7 ± 0.2	0.007 ± 0.0001			95.8 ± 0.3	0.008 ± 0.0001		
5.0	81.7 ± 0.1	0.003 ± 0.0001			93.3 ± 0.2	0.003 ± 0.0001		
GES								
	GAMMA-RAYS				**ELECTRON BEAM**			
Absorbed dose (kGy)	Removal Efficiency (%)	G-value (μmol J^{-1})	$-k_{obs}$ (kGy^{-1})	R^2	Removal Efficiency (%)	G-value (μmol J^{-1})	$-k_{obs}$ (kGy^{-1})	R^2
1.0	99.4 ± 0.1	0.273 ± 0.0001	2.082 ± 0.109	0.964	76.8 ± 0.1	0.064 ± 0.0000001	1.036 ± 0.010	0.883
2.0	98.7 ± 0.1	0.182 ± 0.0001			78.7 ± 0.1	0.033 ± 0.000001		
5.0	100.0[a]	0.074			94.1 ± 0.1	0.016 ± 0.000001		

[a] GES concentration below the LOD (Table S4).

(2009), who investigated the use of gamma-rays in the mineralization of three antibiotics of the nitroimidazoles class in natural waters. The authors observed an increase in percent removals of target compounds and a decrease in G-values with increasing absorbed doses, indicating that the efficiency of the radiolytic process decreased with increasing exposure time. These results were associated with two assumptions: (i) competition between nitroimidazoles and reactive radicals; and (ii) competition between the parent compound and by-products for active species (Sánchez-Polo et al., 2009). In our study, GES exhibited higher G-values and removal percentages than LNG, whose recalcitrant character, even at high doses, was not affected by the ionizing source. Another parameter that indicates the ease of GES degradation by γ-radiolysis is the dose constant obtained for GES, 2.082 kGy^{-1}, twice as high as that observed for LNG.

As presented by Fig. 1 and Table 2, the use of gamma-rays to induce progestin degradation was the most effective alternative. For this reason, it was selected as the standard ionizing source to investigate the effects of the initial concentration of progestins, dose rate, scavengers, and water matrix in the degradation of the target compounds, in addition to acute toxicity and cytotoxic studies.

3.1.2. Effect of initial concentration and dose rate on γ-radiolysis of progestins

The effect of initial concentration and dose rate on the gamma radiolytic degradation of progestins in PURE I solutions was investigated. Fig. 2 shows the LNG degradation profiles for different initial concentrations (0.08, 0.5, and 1.70 mg L^{-1}). The solutions were submitted to two dose rates, 2.5 kGy h^{-1} (Figs. 2a) and 10.0 kGy h^{-1} (Fig. 2b), and absorbed doses of 0.5–100 kGy, without aeration or quenchers. The radiolytic degradation followed *pseudo-first*-order kinetics relative to the absorbed dose, as confirmed by the plot of −ln ($[LNG]/[LNG]_0$) vs. *D* (inset of Fig. 2). The LNG concentration was below the limit of detection (Table S4), for all absorbed doses and both dose rates. For this evaluation, just low doses were applied, due to the high removal efficiency of the γ-rays for doses above 10.0 kGy.

The LNG-PURE I system of 0.50 ± 0.01 mg L^{-1} showed dose constants of 0.8640 and 0.3907 kGy^{-1} for 2.5 and 10.0 kGy h^{-1}, respectively. In contrast, for $[LNG]_0$ = 1.70 mg L^{-1}, k_{obs} values of 0.300 and 0.322 were obtained for 2.5 and 10.0 kGy h^{-1}, respectively Fig. 2 also indicates that at low dose rates, LNG removal was faster, due to the increased radiolysis rate. For example, the application of 10.0 kGy with 2.5 kGy h^{-1} and 10.0 kGy h^{-1} resulted in hormone removals of 98% and 86.5%, respectively. Khan et al. (2015) described the positive influence of low dose rates on ionizing processes, promoting a reduction in recombination reactions between reactive species, with increased pollutants removal.

As observed for the LNG-PURE I solution, the GES removal efficiency also decreased with increasing initial GES concentration

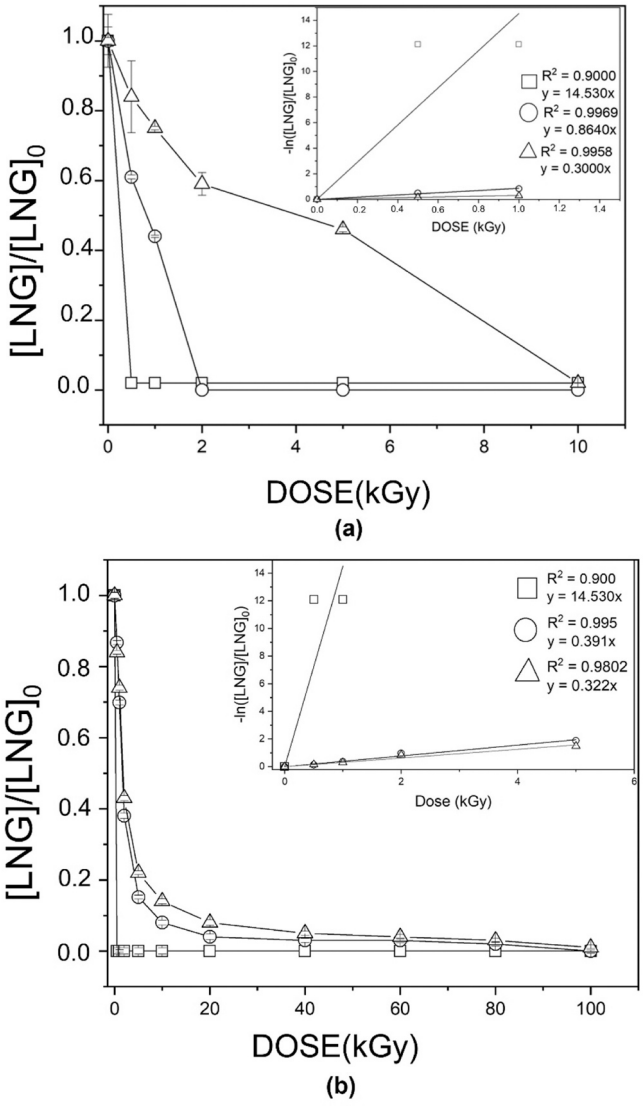

Fig. 2. Dependence of relative levonorgestrel (LNG) concentrations and *pseudo-first-order* fittings (inset) as a function of absorbed dose for dose rates of 2.5 kGy h^{-1} (a) and 10.0 kGy h^{-1} (b) in γ-radiolysis experiments. Conditions: $[LNG]_0$ = 0.080 ± 0.001 mg L^{-1} (□), 0.50 ± 0.01 mg L^{-1} (○) and 1.70 ± 0.08 mg L^{-1} (△). Error bars correspond to *n* = 3 replicates.

(Fig. 3), due to the competition between radical species, intermediates, and the parent compound (Khan et al., 2015). Fig. 3 also presents the normalized decay of GES concentration for GES-PURE I

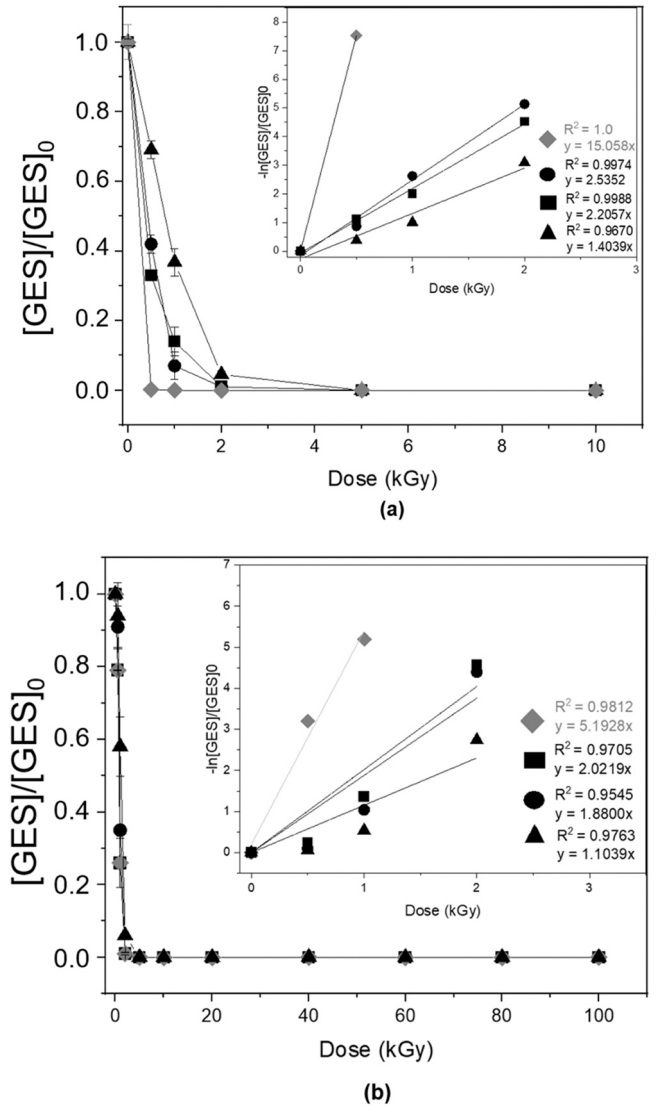

Fig. 3. Dependence of relative gestodene (GES) concentrations and *pseudo-first-order* fittings (inset) as a function of absorbed dose for dose rates of 2.5 kGy h^{-1} (a) and 10.0 kGy h^{-1} (b) in γ-radiolysis experiments. Conditions: $[GES]_0$ = 0.60 ± 0.03 mg L^{-1} (◆), 2.460 ± 0.002 mg L^{-1} (■), 4.54 ± 0.01 mg L^{-1} (•) and 8.06 ± 0.04 mg L^{-1} (▲). Error bars correspond to n = 3 replicates.

solutions for different initial concentrations (0.60, 2.46, 4.54, and 8.06 mg L^{-1}), which were subjected to gamma irradiation at 2.5 and 10.0 kGy h^{-1}, and absorbed doses of 0.5–100 kGy (Fig. 3**b**). The effect of γ-radiolysis on the dose rate was analogous to GES, resulting in higher k_{obs} values for low concentrations and high dose rates, as can be seen in the inset of Fig. 3a. As an example, the k_{obs} values and percentage removals for $[GES]_0$ = 2.46 mg L^{-1}, 1.0–3.0 kGy and dose rates of 2.5 and 10.0 kGy h^{-1}, were 2.206 and 2.022 kGy^{-1}, and 86.4% and 74.3%, respectively (inset of Fig. 3a and Fig. 3b).

3.1.3. Effect of scavengers on progestins removal

The radiolysis of PURE II solutions was investigated in the presence of the scavengers MeOH, oxygen, t-BuOH, and phenol, to direct the degradation of progestins towards the reduction or oxidation pathways. Since high dose rates resulted in lower dose constants, 10 kGy h^{-1} was applied in this investigation. The results are shown in Table 3. Analysis of variance (ANOVA, Tables S5 and S6) and Tukey's tests were performed on the data obtained in triplicate for each hormone, using the Origin 2020 software to identify the scavengers that resulted in statistically significant effects on progestins degradation with a 95% confidence level; the results in Table 3 were compared two by two for each response (k_{obs}, removal efficiency, and G-value) (Fig. S2).

As suggested by the results in Table 3, the degradation of LNG was favored under reduction conditions by reactions with hydrated electrons (e_{aq}^-) and hydrogen atoms (H•); in fact, addition of MeOH and t-BuOH (pH 3) resulted in LNG removals of (97.7 ± 0.6)% and (91.6 ± 0.7)%, respectively, with high dose constants, (0.793 ± 0.050) kGy^{-1} and (0.533 ± 0.030) kGy^{-1}, respectively. The highest G-value (0.0340 ± 0.0002 μmol J^{-1}) was also obtained using 10 mol L^{-1} of MeOH as a scavenger, in which case e_{aq}^- (E^0 = −2.87 V SHE) generated with a high G-value from water radiolysis (0.27 μmol J^{-1}, Eq. 1), was the only reducing species available. This species reacts rapidly by one-electron transfer with substrates with more positive reduction potentials, and also with hydroxyl radicals and hydrogen atoms (Oppenländer, 2003). In addition, according to Tang (2004) and Cooper et al. (2004), MeOH scavenges both HO• and H•, with second-order rate constants of 9.7×10^8 L mol^{-1} s^{-1} and 2.6×10^6 L mol^{-1} s^{-1}, respectively. On the other hand, the remaining hydrogen atoms in the system containing t-BuOH, which correspond to the conjugated acid of e_{aq}^- and have a slightly lower reduction potential (E^0 = −2.30 V SHE), react with organic substrates by addition to double bonds or by hydrogen abstraction, yielding carbon-centered radicals (Oppenländer, 2003). The values of k_{obs} and LNG removal efficiency in the presence of MeOH and t-BuOH are higher than those obtained in the absence of scavengers, i.e., (77.8 ± 0.5)% and (0.322 ± 0.040) kGy^{-1}, when both reductive and oxidative pathways occur competitively. The addition of phenol to the system reinforces the role played by e_{aq}^- and H• in the degradation of LNG; however, as the dose rate and removal efficiency are statistically equal to those obtained in the absence of scavengers (Fig. S2), we hypothesize that in this case the quencher concentration ([phenol] = 1 mol L^{-1}) was not high enough to completely suppress the hydroxyl radicals generated in the reaction medium.

In aerated PURE II-LNG solutions, hydrogen atoms and hydrated electrons are quenched by oxygen, resulting in superoxide radical anions ($O_2^{\bullet-}$) and their conjugated acid hydroperoxyl radicals ($HO_2^{\bullet}$) (Eqs. 5–7**)** which, together with remaining HO• radicals formed from water radiolysis, can attack LNG molecules. Nevertheless, as discussed above, this reaction pathway in the presence of oxidative species only seems to contribute less to the degradation of LNG, resulting in low removal efficiency, dose constant and G-value, i.e., (64.4 ± 0.5)%, (0.179 ± 0.020) kGy^{-1} and (0.0090 ± 0.0001) μmol J^{-1}, respectively (Table 3).

$$H^{\bullet} + O_2 \rightarrow HO_2^{\bullet} \quad k = 2{\cdot}1\times10^{10}\ \text{L mol}^{-1}\ \text{s}^{-1} \tag{5}$$

$$e_{aq}^- + O_2 \rightarrow O_2^{\bullet-} \quad k = 1{\cdot}9\times10^{10}\ \text{L mol}^{-1}\ \text{s}^{-1} \tag{6}$$

$$HO_2^{\bullet} \leftrightarrows H^+ + O_2^{\bullet-} \quad pk_a = 4{\cdot}8 \tag{7}$$

For GES, the results in Table 3 indicate that the fastest removal was achieved in the absence of scavengers, resulting in a dose constant and G-value of (1.104 ± 0.070) kGy^{-1} and (0.2400 ± 0.0001) μmol J^{-1}, respectively; high removal efficiencies ranging from 92.2% to 99.5% were obtained, with some treatments being statistically equivalent when compared two-by-two (Table 3 and Fig. S2). The lowest values of removal efficiency, dose constant, and G-value were obtained in the presence of phenol, with (68.0 ± 0.3)%, (0.251 ± 0.010) kGy^{-1} and (0.0340 ± 0.0001) μmol J^{-1}, respectively. In this case, phenol reacts with HO• with a high bimolecular rate constant ($k = 1.8 \times 10^{10}$ L mol^{-1} s^{-1}), resulting in e_{aq}^- and H• as the remaining reactive species. This result suggests a more important role of hydroxyl radicals in the degradation of GES, whose molecules exhibit an additional double bond, favoring electrophilic attack. Furthermore, the k_{obs} values obtained in the presence of MeOH and

Table 3
Effect of scavengers on the quenching of active species, k_{obs}, G-values, and removal efficiency of LNG and GES under different conditions. $[LNG]_0$ = 1.70 ± 0.08 mg L^{-1}; $[GES]_0$ = 8.06 ± 0.04 mg L^{-1}; [MeOH] = 10.0 mol L^{-1}; [t-BuOH] = 6 mol L^{-1}; [phenol] = 1.0 mol L^{-1}. Irradiation conditions: dose rate of 10 kGy h^{-1} at 5 kGy. Experiments run in triplicate.

Scavenger	Quenched species	Main remaining species	k_{obs} (kGy^{-1})		Removal efficiency (%)		G-value (μmol J^{-1})	
			LNG	GES	LNG	GES	LNG	GES
None	none	$HO^{\bullet}$, e_{aq}^{-}, $H^{\bullet}$	0.322^{a} ± 0.040	1.104^{b} ± 0.070	77.8^{a} ± 0.5	99.5^{b} ± 0.2	0.0200^{a} ± 0.0010	0.2400^{a} ± 0.0001
MeOH[1,2,3]	$HO^{\bullet}$ and $H^{\bullet}$	e_{aq}^{-}	0.793^{b} ± 0.050	0.558^{a} ± 0.020	97.7^{b} ± 0.6	92.2^{c} ± 0.2	0.0340^{b} ± 0.0002	0.0470^{b} ± 0.0003
O_2[1,2,3]	e_{aq}^{-} and $H^{\bullet}$	$HO^{\bullet}$	0.179^{c} ± 0.020	0.761^{c} ± 0.020	64.4^{c} ± 0.5	95.7^{a} ± 0.6	0.0090^{c} ± 0.0001	0.0480^{c} ± 0.0001
t-BuOH[1,2,3] (pH 3)	$HO^{\bullet}$ and e_{aq}^{-}	$H^{\bullet}$	0.533^{d} ± 0.030	0.637^{a} ± 0.050	91.6^{d} ± 0.7	$96.6^{a,b}$ ± 1.0	0.0130^{d} ± 0.0001	$0.0480^{a,b,c}$ ± 0.0001
Phenol[1,2,3]	$HO^{\bullet}$	e_{aq}^{-}, $H^{\bullet}$	0.354^{a} ± 0.030	0.251^{d} ± 0.010	79.2^{a} ± 0.7	68.0^{d} ± 0.3	0.0110^{e} ± 0.0001	0.0340^{d} ± 0.0001

[1,2,3] Shah et al. 2014; Khan et al. (2015); Nisar et al. (2016).
The same lowercase letters in each column indicate the response variables that do not show statistical difference by Tukey's test at a 95% confidence level ($p < 0.05$).

t-BuOH, which are statistically equal with a 95% confidence level (Fig. S2), also suggest a less important role played by reducing species in GES degradation compared to LNG. The same conclusion can be drawn considering the dose rate obtained for GES in the aerated system, i.e., (0.761 ± 0.020) kGy^{-1} (Table 3). Shah et al. (2014) also studied the addition of phenol in the removal of endosulfan by γ-radiolysis, resulting in 92% removal, while lower contaminant removal was achieved in the presence of t-BuOH.

3.2. Matrix effect

Fig. 4 compares the γ-radiolytic degradation of progestins in different water matrices containing LNG and GES (MIX and RPW solutions), irradiated at 10 kGy h^{-1}. In addition, this dose rate was applied in the case of solutions used in acute toxicity assays.

As shown in Fig. 4a, maximum LNG removals were achieved using high doses (60 and 100 kGy), for both matrices, i.e., 98% (MIX) and 99% (RPW). Noteworthy, doses as low as 0.5 and 2.0 kGy resulted in percent removals of 55.0% and 80.3%, respectively. LNG in the MIX and RPW solutions showed a greater recalcitrant character, compared with GES, mainly at low doses (0.5–5.0 kGy), which can be explained by the complexity of the matrices, whereby the competition between reactive species is higher than in pure model solutions. In contrast, Fig. 4b shows that GES removals in target matrices were greater than 60%, even for low doses.

Nasuhoglu et al. (2012) evaluated the removal of LNG in different matrices (pure water, real pharmaceutical effluent, and simulated wastewater), obtaining maximum removals of 55% and 76% in the real wastewater by photocatalytic and photolytic processes, respectively. In contrast, Olmez-Hanci et al. (2020) investigated the feasibility of ozone and ozone combined with H_2O_2 (peroxone) for treating four different effluents, including pharmaceutical wastewater, achieving 100% and 80% GES removals, respectively, even in a complex matrix containing more than 17 endocrine compounds. Therefore, this corroborates our findings that LNG has a more recalcitrant character than GES, even when different advanced oxidation processes are used.

3.3. Toxicity assessment of gamma-rays on progestins solutions

3.3.1. Effect of γ-radiolysis on acute toxicity

Fig. 5 shows the results of acute toxicity to the test-organism *D. similis* as a function of the absorbed dose, for PURE I-LNG and PURE I GES solutions. The values of EC50% and TU for the non-irradiated LNG solution were 17.9 ± 1.7% v/v and 5.6 ± 1.7%, respectively. For GES, these values were 35.0 ± 1.8% v/v and 2.9 ± 1.8, respectively.

According to the classification proposed by Persoone et al. (2003), the non-irradiated LNG and GES solutions showed acute toxicity to the test organism. Fig. 5a also shows that the radiolytic degradation of LNG at low doses promoted a decrease of about 32% in comparison with the initial toxicity. In contrast, the use of high doses led to residual toxicity reaching levels close to that found in the non-irradiated solution. In the case o GES solution (Fig. 5b), however, irradiation at 5 kGy achieved 41.8% toxicity removal, while for 60 and 100 kGy, a three-fold increase in acute toxicity was observed. For both progestins, the increase in EC50% and TU values for

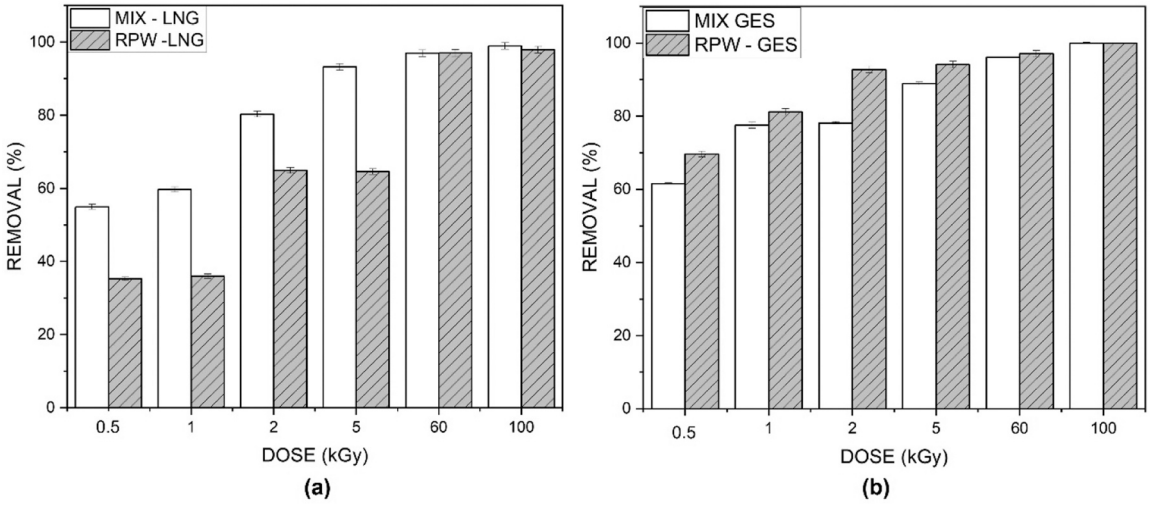

Fig. 4. Percent removals of progestins in complex water matrices submitted to γ-radiolysis at a dose rate of 10 kGy h^{-1} and different doses (run in triplicate). (a) LNG and (b) GES. MIX: aqueous solution containing $[LNG]_0$ = 1.70 ± 0.08 mg L^{-1} and $[GES]_0$ = 0.52 ± 0.03 mg L^{-1}. RPW: real pharmaceutical wastewater containing $[LNG]_0$ = 4.0 ± 0.3 mg L^{-1} and $[GES]_0$ = 0.66 ± 0.63 mg L^{-1}, besides other unknown constituents.

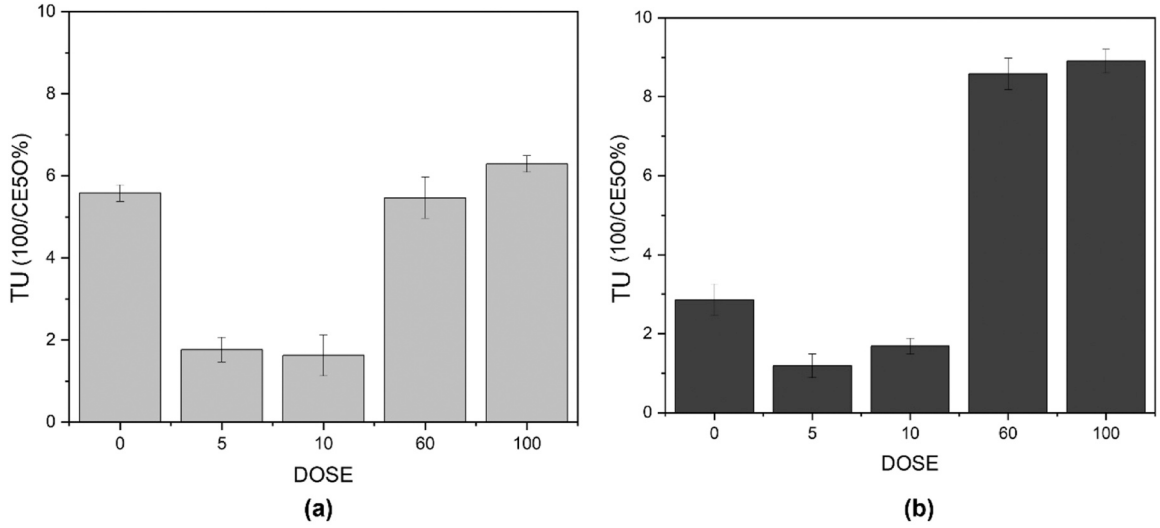

Fig. 5. Results of acute toxicity (in toxic units, TU= 100/EC50%) to *D. similis* before and after γ-radiolysis of progestins solutions irradiated at 5, 10, 60, and 100 kGy, at a dose rate of 10 kGy h^{-1}. (a) $[LNG]_0$ = 1.70 ± 0.08 mg L^{-1}; (b) $[GES]_0$ = 8.06 ± 0.01 mg L^{-1}. Measurements performed with PURE I solutions. The values correspond to an average of ten replicates.

higher doses may be related to the formation of by-products more toxic than the parent compound. To the best of our knowledge, these results were observed for the first time. Tominaga et al., 2021 reported the use of EB to degrade acetylsalicylic acid and fluoxetine in synthetic solutions of 10 mg L^{-1}. The radiation was conducted in batches for 1.0, 2.5, and 5.0 kGy. The radiolytic degradation was evaluated for single solutions and mixed systems. According to the authors, the decrease in acute toxicity to *D. similis* after irradiation for doses of 1.0 and 2.5 kGy was about 54.4% and 57.4% for the mixture of the target compounds. However, the use of 5 kGy promoted a two-fold increase in a mixture solution highlighting that the use of high doses can lead to the formation of toxic by-products, as also observed in the present study.

3.3.2. *Effect of gamma-rays on the cytotoxicity to NIH-3T3-L1 cells*

The cytotoxicity of LNG and GES solutions was investigated before γ-radiolysis, by dissolving progestins in the RPMI medium to reach the concentrations of 0.005-1.0 mg L^{-1} (LNG) and 0.002-8.0 mg L^{-1} (GES). Fig. 6**a** shows the viability of the NIH-3T3 cell as a function of log($[progestin]_0$). From these results, it was found that the LNG concentrations that cause cytotoxic effects to 50 or 90% of the cells were 0.45 mg L^{-1} (CV50) and 0.24 mg L^{-1} (CV90), respectively. In contrast, Fig. 6b indicates no cytotoxic effects of GES solutions to NIH-3T3 cells.

Fig. 7 shows the effect of γ-radiolysis on the cytotoxicity of progestin solutions and real pharmaceutical wastewater to NIH-3T3 cells. According to Fig. 7a, irradiation of LNG solutions at 5–100 kGy resulted in nearly 100% cell viability, indicating the decrease of initial cytotoxicity, and suggesting the formation of non-harmful by-products to the test cells. For all the doses evaluated, Fig. 7b and c suggest that the radiolysis of GES solutions in RPW did not result in the formation of cytotoxic by-products, since cell viability remained close to 100%.

There are no records in the literature on the use of NIH-3T3-L1 cells to assess the cytotoxicity of the progestins under study. Cavalcante et al. (2013) investigated the antineoplastic activity of a pharmaceutical compound (MTX, 1,4-dihydroxy-5–8-bis{[2-[(2-hydroxyethyl) a-mino] ethyl]}amino-9,10-anthraquinone dihydrochloride) using NIH/3T3 mouse fibroblasts cells. The authors evaluated the use of photo-Fenton and UV/H_2O_2 to remove MTX (0.07 mmol L^{-1}) in synthetic solutions. The biological assay allowed the evaluation of MTX in inhibiting the growth of the target cells, which was proportional to the increase in drug concentration, with 87% inhibition at 250 μg mL^{-1}. The inhibitory concentration (IC50)

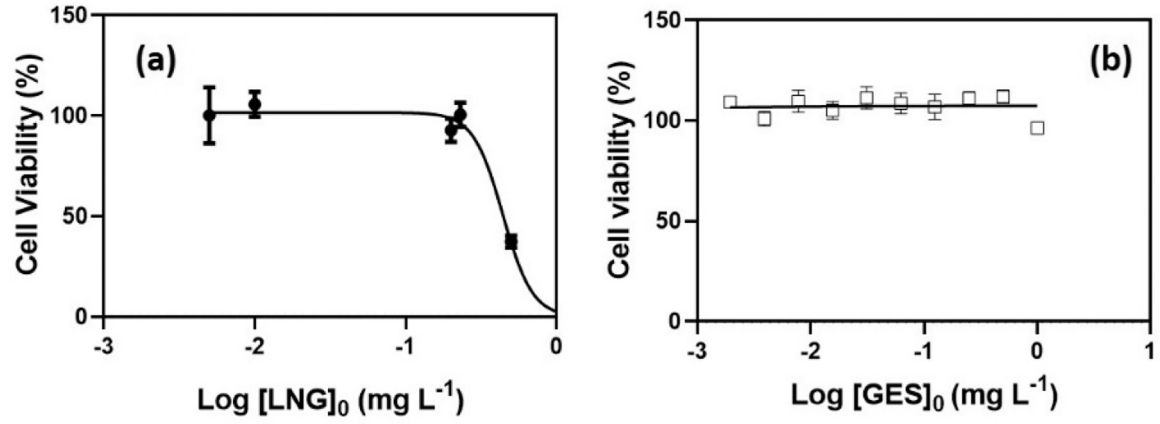

Fig. 6. Effect of LNG (a) and GES (b) concentrations on NIH-3T3 cells viability, expressed as the percentage of cell survival compared to the non-irradiated control (cell viability = 100%). The values correspond to an average of eight replicates.

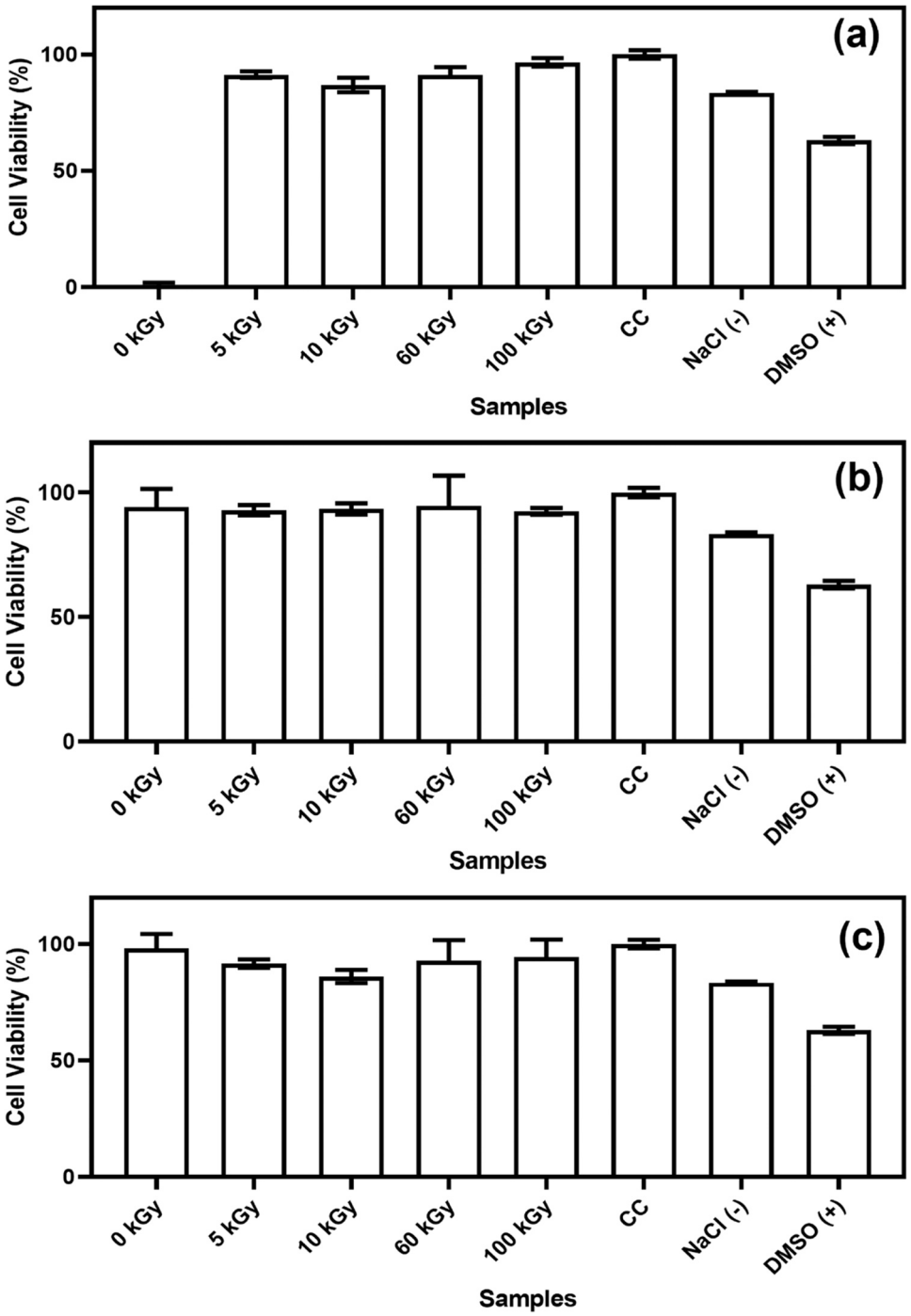

Fig. 7. Effect of γ-radiolysis of PURE I-LNG (a), PURE I-GES (b), and RPW (c) solutions submitted to doses of 0, 5, 10, 60, and 100 kGy on NIH-3T3 cells viability, expressed as the percentage of cell survival compared to the non-irradiated control (CC-cell control, corresponding to cell viability = 100%). The values correspond to an average of eight repetitions.

was 3.29 μg mL^{-1} for MTX, demonstrating its toxicity to NIH/3T3 cells. NIH/3T3 cells exposed to treated samples showed 100% of cell viability, with no formation of cytotoxic by-products.

3.3.3. *Effect of γ-radiolysis on estrogenic activity*

Fig. 8 shows the evolution of estrogenic activity, evaluated by the YES assay, as a function of absorbed dose. E2 was used as a standard to obtain a dose-response curve (Fig. S1). In the case of LNG ($[LNG]_0$ = 1.70 ± 0.08 mg L^{-1}), treatment with γ-radiolysis using 0.5 kGy and 10 kGy h^{-1} resulted in a slight increase in the estrogenic response, which is associated with the transformation products formed following 16% LNG degradation. The use of higher doses, for which LNG removals of 77.5% (5 kGy) and 86.4% (5 kGy) were achieved (Fig. 3), allowed the estrogenic activity to be removed to values below the detection limit of the YES assay (2.3 ng L^{-1} E2-EQ).

In contrast, 5- and 28-fold increases in residual estrogenicity of GES solutions were obtained at doses as low as 0.5 and 5.0 kGy, respectively, for which hormone removals of about 6% and 99.5% were achieved, highlighting the remarkable impact on E2-EQ values of by-products formed through combined oxidative and reductive degradation pathways. These products could be further degraded at 10 kGy, resulting in about 94% reduction in the estrogenic activity of the solution; however, higher doses would be required to achieve E2-EQ values below the detection limit of the assay.

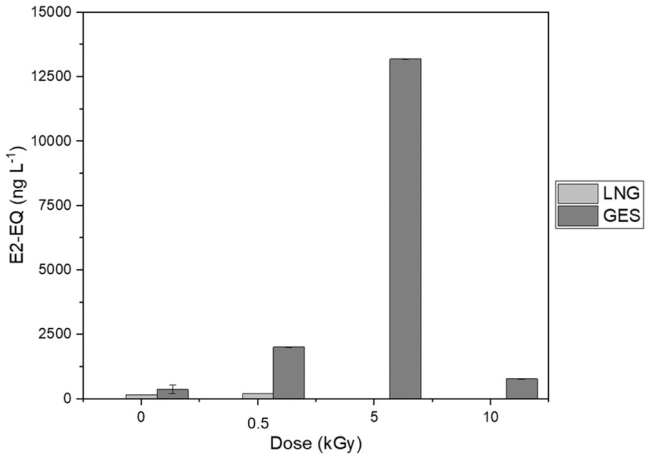

Fig. 8. Results of estrogenic activity (E2-EQ) before and after γ-radiolysis of progestins solutions irradiated at 0.5, 5, and 10 kGy, at a dose rate of 10 kGy h^{-1}. $[LNG]_0$ = 1.70 ± 0.08 mg L^{-1}; $[GES]_0$ = 8.06 ± 0.01 mg L^{-1}. Measurements performed with PURE I solutions. Error bars correspond to n = 3 replicates.

4. Conclusions

Gamma-rays and electron beam radiation can promote the effective radiolytic degradation of the progestin hormones levonorgestrel (LNG) and gestodene (GES) in different aqueous matrices. Gamma-rays proved to be the best radiation source, achieving complete removals of LNG and GES depending on initial concentration, absorbed dose and dose rate. The influence of the absorbed dose was observed by monitoring progestins, indicating that doses up to 10 kGy promoted a reduction in progestins concentrations below their respective limits of detection by the UFLC (ultra-fast liquid chromatography) method used (0.02 mg L^{-1} for LNG and 0.06 mg L^{-1} for GES). The initial concentration of hormones impacted the removal efficiency, due to competition for reactive species. The kinetic behavior of ionizing-induced degradation, for both hormones, followed a *pseudo-first*-order reaction, which allowed the determination of dose constants.

The comparison between the radiolytic removal of LNG and GES allowed the identification of a greater recalcitrant character of LNG and its affinity for reductive reactive species, unlike GES, for which the tests with scavengers suggest combined oxidative and reductive degradation. Furthermore, the use of low doses, such as 2.0 kGy, promoted the removal of 65% and 93% for LNG and GES, respectively. The radiolytic removal of progestins in complex matrices was highlighted for high doses, such as 60 and 100 kGy.

With regard to acute toxicity to *Daphnia similis*, non-irradiated LNG and GES solutions can be classified as acutely toxic. Interestingly, the toxicity of LNG and GES solutions following γ-radiolysis was dose-dependent, with GES solutions showing a 41.8% decrease in toxicity at 5 kGy and a three-fold increase at 60 kGy and 100 kGy, for example, which can be related to the transformation products formed in each case. The initial cytotoxic character of LNG to NIH-3T3-L1 cells was removed by water radiolysis, while non-irradiated or irradiated GES solutions did not show any cytotoxic effects. Finally, the evolution of estrogenic activity, evaluated by the Yeast Estrogen Screen (YES) assay, proved to be strongly dependent on the hormone and the applied dose, which may be related to the transformation products generated by the degradation pathway prevalent in each case.

Based on these results, it can be concluded that the use of water radiolysis can be a viable alternative for the removal of progestins from water and pharmaceutical effluents, successfully targeting different biological indicators of environmental importance.

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CRediT authorship contribution statement

Juliana Mendonça Silva de Jesus: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Flávio Kiyoshi Tominaga:** Methodology, Validation, Formal analysis. **Allan dos Santos Argolo:** Methodology, Validation, Formal analysis. **Sueli Ivone Borrely:** Methodology and Validation. **Ana Cristina Gomes Nascimento:** Methodology, Validation, Formal analysis. **Daniel Perez Vieira:** Methodology, Validation, Formal analysis. **Daniele Maia Bila:** Methodology and Validation. **Antonio Carlos Silva Costa Teixeira:** Conceptualization, Validation, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition All the authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data Availability

All data generated or analyzed during this study are included in this published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psep.2022.04.021.

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