Assessment of Levonorgestrel Leaching in a Landfill and Its Effects on Placental Cell Lines and Sperm Cells

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Abstract: The Buenavista landfill is located east of the city of Medellin, but it has a slope steeper than 30% and is less than 600 m away from the Piedras River, possibly influencing the quality of the drinking water in the city. Many complex residues are disposed of in this landfill, including pharmaceuticals and personal care products (PPCPs) such as levonorgestrel (LNG), which may reach water bodies via runoff and leaching. We assessed the levels of LNG in the effluent of an upflow anaerobic sludge blanket (UASB) reactor from the Buenavista landfill by uHPLC–DAD, as well as the endocrine disruptor effect of LNG on placental cell lines (BeWo) and human sperm cells. Additionally, the potential leaching of LNG was assayed under laboratory conditions using soil layers that were sampled from the Buenavista landfill. LNG was detected at levels of 315 ng·L⁻¹ in the effluents of the UASB reactor. Thus, the UASB reactor is not an efficient treatment method for the removal of recalcitrant pollutants. Additionally, we found that a layer of soil used as a cover material may adsorb more than 90% of LNG pollutants, but small amounts may still be leached, which means that a cover material is not a strong enough barrier to fully prevent the leaching of LNG. Finally, our results show that the leachate fraction decreased the levels of β-human chorionic gonadotropin, but it has a slope steeper than 30% and is less than 600 m away from the Piedras River, possibly influencing the quality of the drinking water in the city. Many complex residues are disposed of in this landfill, including pharmaceuticals and personal care products (PPCPs) such as levonorgestrel (LNG), which may reach water bodies via runoff and leaching. We assessed the levels of LNG in the effluent of an upflow anaerobic sludge blanket (UASB) reactor from the Buenavista landfill by uHPLC–DAD, as well as the endocrine disruptor effect of LNG on placental cell lines (BeWo) and human sperm cells. Additionally, the potential leaching of LNG was assayed under laboratory conditions using soil layers that were sampled from the Buenavista landfill. LNG was detected at levels of 315 ng·L⁻¹ in the effluents of the UASB reactor. Thus, the UASB reactor is not an efficient treatment method for the removal of recalcitrant pollutants. Additionally, we found that a layer of soil used as a cover material may adsorb more than 90% of LNG pollutants, but small amounts may still be leached, which means that a cover material is not a strong enough barrier to fully prevent the leaching of LNG. Finally, our results show that the leachate fraction decreased the levels of β-human chorionic gonadotropin, but not sperm motility or viability. Thus, leached LNG could trigger reproduction disorders, but further studies should be carried out to investigate its potential effects in more detail.

Keywords: cover material; soil sorption; leaching; endocrine disruptor; pharmaceutical; personal care products

1. Introduction

Pharmaceutical and personal care products (PPCPs) have been classified as emerging pollutants because of their environmental impact. Long-term exposure to these substances may affect a wide range of species, even at low concentrations [1]. Furthermore, the high persistence of PPCPs and their low removal from wastewater may increase their impact on aquatic ecosystems [2]. Traces of PPCPs have been found in drinking water supplies, which is a public health concern [3]. The implications of the toxicity of PPCPs in water include endocrine disruption (ED), which affects the equilibrium of hormone levels and leads to metabolic, neurological, and reproductive consequences in exposed species [4].

Levonorgestrel (LNG) is a pharmaceutical product widely used in Colombia as a contraceptive method; thus, complex effects similar to those of other PPCPs were found [5].
This substance is known to interact not only with progesterone receptors (PR), but also with another estrogen receptor, which may trigger complex pathways in non-targeted organisms [6]. LNG was detected in very low levels in water, leading to disruptions in the gonadotropin expression of aquatic species, such as teleost fish [7]. However, the information available on the occurrence and fate of this substance in aquatic ecosystems is limited. LNG is a steroidal molecule with strong molecular bonds, and thus, LNG is recalcitrant to degradation processes [8]. Therefore, LNG shows environmental persistence because natural conditions do not completely remove this substance. For instance, LNG is not removed by solar radiation at wavelengths close to 320 nm, and even energy radiation (UVC-265) reduces only 80% of this substance [9]. Therefore, more complex photodegradation should be applied for its removal in treatment plants.

Many PPCPs, including LNG, are disposed of in landfills, which could be a source of water pollution due to leaching [10]. The leaching that occurs in this landfill is a risk for long-term exposure in many people, but the effects are still unknown. For instance, some landfill leachates containing heavy metals induced genotoxic and cytotoxic effects in the peripheral blood erythrocytes extracted from Wistar strain rats, which showed the impact of landfills on biota [11]. Furthermore, the leachate may contain more complex substances, including PPCPs. However, leaching impacts may be reduced when the interaction of pollutants with a soil layer that is applied for covering waste residues in landfills (cover material) increases. Thus, the selection of an appropriate cover material decreases soilborne pollutants due to the soil layer working as a natural barrier. In previous research, Yang et al. found that hydrophobic partitioning is the main pathway for the sorption of progestins in soil [12]. For instance, the organic fractions in the soil layer may affect LNG leaching from landfills. The leached fraction of LNG may trigger ED in biota, among other complex implications in exposed organisms. For example, it has been found that levels between 3.3 and 40 ng·L⁻¹ of LNG can reduce fish reproduction by the masculinization of females, changes in gonad histology, and disruption to hormone levels [13]. However, LNG toxicity in human reproduction has not been deeply studied. In a previous report, we showed that LNG and its photodegraded fractions decrease human chorionic gonadotropin (β-hCG) hormone levels in placental cell lines. This may cause problems to occur during gestational development [9].

The Buenavista landfill, which is located in Antioquia, Colombia, has been found to contain expired PPCPs, including painkillers, contraceptives, and antibiotics, among others. Thus, this landfill could be a source of PPCPs leaching into the Piedras River, which plays an essential role in the supply of drinking water to the city of Medellín. However, an appropriate cover material to reduce the transport of leachate pollutants, as well as treatment, may reduce their impact in natural water. To this end, an upflow anaerobic sludge blanket (UASB) reactor is used for the treatment of leachates produced by the Buenavista landfill. However, the UASB reactor, among other conventional treatments, is not sufficient for persistent organic pollutant removal because they are extremely resistant to biological degradation processes, and more specific advanced oxidation processes should be included for the treatment of pollutants [14].

The aim of this study was to assay the levels of LNG from the effluent of a UASB reactor used in the Buenavista landfill to evaluate possible transport by leaching from cover materials. Additionally, we studied the effects of the cover material used in this landfill on LNG adsorption to approximate a better material selection for reducing leaching. Finally, we studied the endocrine disruptor effect of LNG on the BeWo cell line and human sperm cells by in vitro assay. The results may be relevant for future studies on the assessment of risk due to the long-term exposure of PPCPs disposed of in landfills and their impact on bodies of water.
2. Materials and Methods

2.1. Chemicals and Materials

A stock solution of LNG was prepared at a concentration of 10.5 mg·L⁻¹ from the commercial pharmaceutical Cerciorat® containing 1.5 mg per pill (98–102% USP29). Then, seven pills were macerated and diluted in Milli-Q water. The stock solution was stored at −20 °C. Solvents, including methanol and acetonitrile (HPLC grade), fetal bovine serum (FBS), and Ham-F10 medium, were purchased from Sigma-Aldrich (San Luis, MO, USA). The water was purified in a Thermo Scientific® Barnstead 50131217 GenPure TM UV/UF for Type I Milli-Q water (deionized). The buffer solution for the mobile phase was prepared with 0.05% formic acid in deionized water. All solvents were filtered through cellulose nitrate (with pore sizes of 0.2 µm) and degassed in an ultrasonic bath for 10 min at 25 °C. Finally, the antibodies for β-hCG and progesterone analysis were provided by Medife S.A.S (the provider for Roche Colombia).

2.2. Soil Layer Sampling and Monitoring of LNG in the Effluent of the UASB Reactor from the Buenavista Landfill

Different soil layers used as cover materials were sampled from the Buenavista landfill, located in La Union city, Antioquia, at the following coordinates: 5°57′31.37″ N 75°19′41.76″ W (latitude and longitude, respectively). These soil layers were identified at different depths from a complete soil profile that was mixed to obtain the cover material to bury the solid waste. The soil properties of the layers differed in terms of color, texture, structure, and thickness. Three layers were identified: Layer 1 (L1), Layer 2 (L2), and Layer 3 (L3). Samples of the soil layers were individually taken and stored at 4 °C until physicochemical analysis. They were sterilized by autoclaving at 120 °C for 20 min in order to reduce the microbial activity and thus avoid biodegradation. A sample of leachate was taken from the effluent of the UASB reactor in the Buenavista landfill to assay the LNG levels. This sample was filtered through a 0.2 µm membrane and analyzed using an ultra-high-performance liquid chromatography–diode array detector (uHPLC–DAD). The LNG was confirmed by the UV spectrum at its retention time, which was in agreement with the standard UV spectrum presented in the literature. The sample was spiked via the standard addition method.

2.3. Leaching Analysis of LNG from the Soil Layers under Laboratory Conditions

The experiment was carried out inside packed columns of polyvinyl chloride (PVC) using each of the soil layers in duplicate (with a diameter of 2.54 cm and a length of 30 cm). Then, 50 mL of the LNG stock solution was passed through the columns of PVC. The volume of the standard solution was calculated according to the average precipitation around the sampling site per area. Next, 50 mL of deionized water was used in the soil layers as a negative control. All leachate fractions were collected after 24 h in conical flasks to measure the volume, and they were centrifuged at 3000 rpm for 10 min at 25 °C for cleaning. Finally, 1 mL of the sample was injected into the uHPLC–DAD system using the recommended method.

2.4. Validation Parameters for LNG Analysis by uHPLC–DAD

We analyzed 10.5 mg·L⁻¹ of the stock solution of LNG by uHPLC–DAD (Thermo Scientific Dionex UltiMate 3000) at a wavelength of 245 nm. The LNG was identified using the UV spectrum at its retention time. The LNG was separated on a C18 Hypersil Gold column (5 µm × 150 × 4.6 mm). A volume of 20 µL of the LNG solution was injected and separated using a mobile phase with a ratio of 85:15 (v/v) of buffer–acetonitrile, by applying a flow of 0.450 mL/min⁻¹. After 10 min, the mobile phase was modified at a ratio of methanol–acetonitrile of 50:50 (v/v), under a flow of 0.500 mL/min⁻¹. The mobile phase was set back to initial conditions (85:15 (v/v) of buffer–acetonitrile). Finally, a calibration curve was plotted between 1 and 10 ppm, and the validation parameters, such as the limit
of detection (LOD), the limit of quantification (LOQ), and recovery were estimated. See the Supplementary Materials (SM) for more details.

2.5. Assessment of Leached Fractions in Human Sperm Cells

The experimental in vitro assays included five fresh semen samples from healthy individuals, collected by masturbation in a sterile container after sexual abstinence for 3 to 5 days. The normozoospermia for semen parameters is presented in Supplementary Materials SM1. The sperm motility and viability were quantified for each seminal sample following the guidelines established in the manual for seminal analysis published by the World Health Organization [15] before and after incubating an aliquot of $4-5 \times 10^6$ sperm cells with 100 $\mu$L of the leachate samples from the effluents of the UASB reactor for three hours. Both sperm parameters were quantified at the initial time (0 h) and 3 h after incubation (once every hour). The negative control was incubated with only 100 $\mu$L of PBS, while the leachate sample was incubated with 100 $\mu$L of samples from the UASB reactor. Additionally, the direct effect of the LNG was tested with 100 $\mu$L of the stock solution.

2.6. Effects of Leachate Sample from the UASB Reactor on BeWo Cells and Their Production of $\beta$-hCG

The BeWo cell line was supplied by ATCC® CCL-98™, and it was cultured using Roswell Park Memorial Institute (RPMI)-1640 medium with 2% fetal bovine serum under a 5% CO$_2$ atmosphere at 37 °C and 95% humidity (confluence > 70% and viability > 90%). A negative control was prepared with 100 $\mu$L of PBS in 5 mL of medium containing $5 \times 10^5$ cells and similar conditions. It was tested with 100 $\mu$L of the leachate sample from the UASB reactor. Additionally, 100 $\mu$L of the LNG stock solution was assayed. All experiments were carried out in duplicate for 24 h. A colorimetric assay (MTT assay) was carried out for cell viability according to Lomonte et al. [16]. For more details, see the Supplementary Materials (Supplementary Materials SM2). The analysis of the levels of $\beta$-hCG hormone in the medium was assayed after 24 h by ElectroChemiLuminescence using the COBAS® 411 Integrated System (HITACHI) analyzer for the immunoassay test. A total of 1 mL of the medium was taken out from each experimental well and added to a conical Eppendorf tube for the analysis of progesterone and $\beta$-hCG using the COBAS® 411 device. The UV–vis absorption and photoluminescence were recorded on the COBAS® 411 device. This device was supplied by the Hospital Marco Fidel Suarez (Bello, Colombia).

2.7. Statistical Analysis and Graphs

All graphs were plotted using GraphPad Prism 7.0 software (GraphPad Software Inc., San Diego, CA, USA). For linearity, homoscedasticity analysis was carried out, and the R square was found. The limit of detection (LOD) and the limit of quantification (LOQ) were determined by the signal–noise method, and five blanks were analyzed by uHPLC–DAD via the method presented above. Finally, the ED effects, due to changes in $\beta$-hCG and sperm, were defined using case–control methods via the application of an unpaired t-test analysis.

2.8. Ethical Statements

The BeWo cell line experiments do not require ethical review and approval because this line is obtained as a commercial immortalized from placental choriocarcinoma (ATCC® CCL-98) which did not include human experimentation. However, ethical approval for the semen analysis was obtained from the Bioethics Committee of the School of Medicine at Universidad de Antioquia (F-CBI-012, 14 February 2019), and the patients signed an informed consent form.
3. Results

3.1. Validation Parameters for LNG Analysis by uHPLC–DAD

The chromatography method allowed for the separation of the LNG at a retention time of 17.63 min. The quality parameters, for quantification purposes, are presented in Table 1.

| LNG | $R_t$ (min) | $r^2$ | LOD (ppm) | LOQ (ppm) | Linearity range (ppm) | Linear equation | $\log K_{ow}$ $[17]$ |
|-----|-------------|-------|----------|----------|----------------------|-----------------|------------------|
|     | 17.63       | 0.998 | 0.09     | 0.30     | 0.3–10.5             | $y = 0.7271x + 0.6011$ | 3.48             |

The UV wavelength for LNG detection and quantification was 254 nm. The $n$-octanol-water partition coefficient ($\log K_{ow}$) is included.

The linearity was analyzed by homoscedasticity and $r^2$, which produced a good fit in a linear model, and the slope was significantly non-zero ($p$-value < 0.05). For more details, see Supplementary Materials SM3.

According to the uHPLC–DAD method, the LOQ appeared to be sensitive. However, some integrated techniques for sample extraction may increase its sensitivity. For example, Huang et al. applied dispersive liquid–liquid microextraction for LNG analysis in natural water, obtaining a sensitivity 100 times higher [18].

3.2. Analysis of LNG in the Effluent of the UASB Reactor from the Buenavista Landfill

The UV spectrum obtained at a retention time of 17.63 was in agreement with the spectrum found in the literature; therefore, the sample showed the presence of LNG (see Supplementary Materials SM3). Additionally, the standard addition appeared to increase at the same time in the spiked samples. Therefore, the leachate sample from the Buenavista landfill showed small amount of LNG at 315 µg·L$^{-1}$, which was calculated by linear regression (see Figure 1). A similar method was developed to determine the levels of LNG in tap water, lake water, and river water samples using uHPLC–DAD [18]. However, the authors applied dispersive liquid–liquid microextraction for analysis to increase the sensitivity of the methods. In our work, we analyzed samples by direct injection because we consider that more polluted water (leached water), and thus higher levels of pollutants, do not require a more difficult extraction process.

![Figure 1. LNG analysis by uHPLC–DAD. The dashed line shows the analysis of the LNG standard. The black line shows the analysis of the leachate sample, and the horizontal dashed line shows the spiked sample.](image)

According to the detected levels, this pharmaceutical product may be transported through the cover material used in landfills. This substance has been detected in wastewater and drinking water treatment plants worldwide at levels between 3.6 and 78.95 ng·L$^{-1}$ [13,19]. Although the $\log K_{ow}$ presented for LNG in Table 1 indicates a possible lipophilic interaction, the
soil is the final barrier that can trap molecules inside. The soil sorption and interaction are presented in Section 3.3 in order to explain the leaching process.

Consequently, the risk assessment for LNG exposure may be considered for public health studies because people can ingest low levels of these substances that are not entirely removed, as was previously found [20]. Progestins may accumulate in exposed organisms because of their lipophilic properties. For instance, LNG was reported to accumulate in the blood plasma of rainbow trout, reaching levels of 8.5–12 ng·mL$^{-1}$ when the species was exposed to treated wastewater effluent (at a concentration of 1 ng·L$^{-1}$ of LNG) for 14 days [21]. This concentration exceeds the plasma concentrations of the therapeutic doses used in humans (2.4 ng·mL$^{-1}$).

On the other hand, LNG may bioaccumulate and biomagnify in all tropic chains. For instance, this substance can cause severe consequences in the gestation process and embryonic development of zebrafish by bioaccumulation [22].

3.3. Assessment of Leaching Processes for LNG in Soil Layers Used as Cover Materials in the Buenavista Landfill

To approximate a possible explanation as to why LNG may be found in the effluent of the UASB reactor located at the Buenavista landfill, a leaching experiment was carried out. First, physicochemical analysis of the soil layers was performed. See Table 2 for details.

### Table 2. Soil properties. CEC: cation exchange capacity.

| Property                  | Layer 1 (L1) | Layer 2 (L2) | Layer 3 (L3) |
|---------------------------|--------------|--------------|--------------|
| Soil texture              | Clay sandy loam | Sandy loam  | Loam         |
| % Clay                    | 50           | 36           | 44           |
| % Silt                    | 18           | 26           | 44           |
| % Sand                    | 32           | 38           | 12           |
| pH                        | 5.6          | 5.1          | 5.1          |
| Org. matter (%)           | 4.1          | 0.15         | 0.12         |
| Al (cmol kg$^{-1}$)       | –            | 1.4          | 2            |
| Ca (cmol kg$^{-1}$)       | 0.2          | 0.1          | 0.1          |
| $\rho$                    | 1.0          | 2.0          | 8            |
| CEC (cmol kg$^{-1}$)      | 0.2          | 1.5          | 2.1          |
| Soil water infiltration (%)| 75.6         | 42.6         | 31           |

Overall, the soil layers showed high clay and silt content, which is related to the high soil sorption of PPCPs [23]. Although low amounts of organic material were found in the soil layers, lipophilic interactions may occur (Table 2). For instance, the humic acid in organic matter may increase the soil–pollutant interaction [24]. Additionally, the cation exchange capacity (CEC) in the soil allows for electrostatic interactions with pollutants due to the charges and ionizable states of molecules.

The water infiltration in the soil was found to be between 31% and 75.6%. The highest level of infiltration was found in L1, which may play an essential role in the transport of pollutants by soil–water partitioning. For instance, intensive rainfall events in the landfill may increase the leaching of pollutants [10].

Second, the leaching of LNG was tested in all soil layers. According to Figure 2a, more than 90% of LNG pollutants were adsorbed in all layers used as a cover material, but a higher level of soil sorption of LNG was found in L2 and L3. Some physicochemical characteristics in soil layers may increase the LNG–soil interaction. For instance, the lipophilic properties of LNG may induce more sorption in organic fractions and decrease the water interaction. Thus, higher amounts of LNG may remain for a long period of time in cover material. For instance, according to Ahmed et al., nonpolar substances are adsorbed into soil more than polar substances due to lipophilic affinity [25]. To understand the influences of the soil physicochemical properties of pollutant sorption, a correlation
plot is provided in Figure 2. Some properties, such as the CEC, water infiltration, and silty fraction showed a good correlation (Pearson $r > 0.99$, $p$-value $= 0.1910$).

Figure 2. Analysis of soil sorption of LNG by leaching analysis: (a) leaching of LNG from soil layers used as cover materials; (b) effects of water infiltration on soil layer sorption; (c) effects of the silty fraction on soil layer sorption; (d) effects of CEC on soil layer sorption.

The water infiltration measured in the soil layers was indirectly correlated with the soil sorption of LNG (Figure 2b). Therefore, the leaching of pollutants depends on water infiltration, which occurs due to hydrogen bonds, polar interactions, and even liquid–solid partitioning. LNG shows a hydroxyl group that may interact with water by hydrogen bonds, but due to the high $\log K_{ow}$, only a lower amount can remain in water at equilibrium, which explains why less than 8% of the LNG was transported through the column. For instance, some LNG ketone and hydroxy groups may interact in soil, increasing LNG uptake in soils, and inducing hydrogen bonding and pore-filling [12]. Unfortunately, desorption of synthetic progestin may be induced by rainfall, and thus, a low amount may be transported by leaching. Therefore, selecting an appropriate cover material and ensuring adequate operating conditions is critical to reducing LNG leaching from landfills.

The silty fraction showed a direct relationship with the soil sorption of LNG (see Figure 2c). Therefore, a higher silty fraction may increase the soil sorption of steroid molecules because the size particles may uptake substances with a higher molecular weight, operating as size exclusion chromatography. According to Yang et al., the relatively high surface area of soils and sediment favored the uptake of some estrogens with similar molecular structures of LNG, such as altrenogest, drospirenone, and medroxyprogesterone acetate [26]. However, the soil layers studied in this paper contain small amounts of clay,
which may play an important role in potentializing the soil sorption of pollutants due to the small grain size [27].

Similarly, the CEC showed a direct relationship with the soil sorption of LNG (see Figure 2d). Thus, higher values of CEC increased its soil sorption. According to Tang et al., the equilibrium time for the soil sorption of LNG took a shorter amount of time at lower CEC values because LNG may fill the vacant sites in soils more quickly in order to reach saturation [2].

According to our results, the grain size, the CEC, and water infiltration may play essential roles in the leaching fractions of LNG. Some soil-borne LNG levels may induce chronic toxicity with long-term exposure in aquatic organisms and possibly overexposed humans. However, more data for the correlation between the soil sorption of LNG in cover materials and the soil properties should be included in future studies.

3.4. Assessment of Leachate Sample in Placental Cell Lines (BeWo) and Sperm Cells

Both the LNG and leached LNG from soil layers under laboratory conditions did not show a change in the conventional sperm parameters, including progressive motility (Figure 3a), non-progressive motility (Figure 3b), immotile sperm cells (Figure 3c), and viability (Figure 3d) compared to the negative controls.

The findings of this study show that the sperm motility and viability of seminal samples were not affected when they were in contact with LNG. However, chronic exposure to this substance, even at low concentrations, may alter some species’ reproductive capacities. For instance, low amounts of LNG may result in reproductive disorders in male zebrafish in progestin-contaminated aquatic environments due to LNG causing a significant decrease in the plasma concentrations of 11-ketotestosterone (11-KT) or estradiol (E2) in males exposed to between 10 and 100 ng·L$^{-1}$ of LNG [28]. Additionally, previous studies have shown...
a reduction in sperm kinematics and capacitation in ovine spermatozoa when exposed to estrogens such as 17β-estradiol and progesterone [29]. On the other hand, more long-term exposure to LNG may affect sperm kinematics in male fathead minnow (Pimephales promelas) [30].

The BeWo cell line was applied to assess possible changes in β-hCG production because this hormone is related to corpus luteum rescue in trophoblastic cell implantation. Significant differences (SD) between the negative control and the stock solution of LNG or the leachate sample were found by applying an unpaired t-test. Our results show that LNG and leached fractions decreased β-hCG in the cell medium after BeWo cell line exposure (see Figure 4). This could be a result of the ED effect because the MTT assay did not show differences in viability during the test. This effect is related to changes in β-hCG production and not to acute toxicity due to the reduction in cell numbers.

In a previous study, we found that low levels of LNG and its degradation products may induce a similar reduction in β-hCG levels after the exposure of placental lines to those fractions [9].

On the other hand, previous reports have shown that LNG may work as an ED in the reproductive functions of fish and amphibians, which causes the gene expression of pituitary gonadotropins and gonadal steroidogenic enzymes [7,31]. Furthermore, these alterations induce estrogenic effects in fathead minnows (males and females) exposed to different concentrations of LNG (up to 3124 ng L⁻¹) for 28 days [32].

According to the biological activity of progestins, it is known that they act intracellularly through the binding and activation of the progesterone receptor (PR), which serves as a transcription factor to induce genomic effects [33]. These nuclear receptors are evolutionarily conserved in vertebrate animals and, thus, indirect exposure in water at low levels could induce similar pathways [4].
3.5. A Possible Implication of Leached LNG in Medellín, Colombia

The Buenavista landfill is located 600 m away from the Piedras River. This landfill has a slope steeper than 30% and, thus, runoff and leaching may occur. Therefore, the quality of the river may be impacted by pollutants transported from the landfill (for more details, see Supplementary Materials SM4). For instance, we detected LNG in effluents of the UASB reactor in the Buenavista landfill, and this substance may reach the water body. Therefore, the levels of LNG should be monitored in this river. A percentage of water from the Piedras River is pumped to the La Fe reservoir, one of the most important drinking water reservoirs for the Valle del Aburrá in Antioquia (Colombia), supplying potable water to more than two million people. The natural water is purified in La Ayurá, a drinking water treatment plant (DWTP) located in Medellín before its distribution. Thus, the exposure risk for people may depend on five factors, including: (1) the final disposal of PPCPs in the landfill; (2) the cover material sorption; (3) the leaching treatment at the UASB reactor; (4) natural degradation; and (5) purification in the DWTP (La Ayurá). However, regarding the first factor, we found many empty pharmaceutical blisters and plastic containers in the Buenavista landfill, which shows that this place is the final destination for many PPCPs. Secondly, we found that cover materials may allow for the leaching of LNG at values lower than 8% (under laboratory conditions). Furthermore, the levels of LNG in the effluents of the UASB reactor indicate that the cover material interaction and even the leaching treatment do not sufficiently reduce the levels of LNG. Recalcitrant pollutants, such as PPCPs, are not efficiently removed from wastewater treatment plants (WWTPs) by conventional processes. For instance, Botero-Coy et al. detected levels of antibiotics above 1 µg L\(^{-1}\) in the effluents of WWTPs in cities such as Bogotá, Medellín, and Florencia, which means that these pollutants are not completely removed by conventional methods [34]. Thus, the design of treatment plants for leaching water and wastewater should include advanced oxidation, among other tertiary treatment processes for the removal of pollutants.

Natural degradation is not efficient enough to remove LNG in natural water. In a previous report, we found that solar energy for the photolysis of LNG does not have a strong enough effect to enable its degradation under natural conditions [9]. Thus, LNG fractions may remain in the water for a long time. However, biodegradation should be considered in future studies. Although information about the biodegradation and biotransformation of LNG as well other contraceptives is still unknown, its half-life in non-sterile soil is ten times higher than in sterile soil and, thus, microorganisms may play an important role in its natural degradation [35]. According to our results, LNG was detected in the effluent of the UASB reactor; through runoff, this fraction could reach the natural water in the Piedras River. Thus, the exposure risk for people in Medellín may depend on the DWTP, which applied coagulation and chlorination during treatment. However, these conventional methods are not efficient for the removal of pollutants, as discussed above. Thus, LNG exposure could have some endocrine effects on the biota and exposed population. Although the effects of short-term exposure were not found in sperm cells by in vitro assay in this study, long-term exposure to LNG should be considered because of the risks of continuous exposure at low levels. According to Elisabeth Carlsen et al., the quantity of semen has decreased by fifty percent in the last 50 years from 113 × 10\(^6\) mL\(^{-1}\) [36]. Although the exact causes of this decrease have not been confirmed, complex xenobiotic exposure may be related to the reduction in sperm count. For instance, recently, more PPCPs have been found in bodies of water, and reproduction disorders in exposed aquatic species have increased [1]. According to the US Environmental Protection Agency (EPA), steroidal hormones, including LNG, may induce feminization, among other reproductive dysfunctions, in non-target organisms [37].

Finally, in this study, we found that leached fractions of LNG decreased the levels of \(\beta\)-hCG in a placental cell line, which indicates possible effects on implantation pathways. Therefore, more studies should be carried out on human ED due to low levels of unintentional exposure to contraceptives that may leach from landfills. Expired products may be disposed of in the Buenavista landfill, and soil sorption may be the final barrier.
preventing pollutants from ending up in surface water. However, we found that the cover material allowed the passage, through the soil layer, of small amounts of freely dissolved concentration of LNG, which may affect the endocrine systems of exposed species, even in humans. Future studies should focus on reducing the disposal of PPCPs in landfills and developing an efficient cover material for the sorption of pollutants to avoid exposure to soil-borne LNG.

4. Conclusions

Many expired or partially consumed PPCPs are disposed of in landfills. In these places, the soil layers used as cover materials influence the sorption capacity of these pollutants. This interaction may affect the transport of these substances, allowing them to reach water bodies, inducing imbalances in exposed ecosystems and, consequently, increasing the risk to public health. The soil layers used in the Buenavista landfill contain high amounts of clay and silt, which increase LNG–soil interactions. However, LNG is not completely adsorbed and, as a result, it was detected in leachate samples. The samples that contained LNG and the LNG stock solution affected the production of β-hCG in placental cell lines. Therefore, indirect exposure to LNG may cause problems during gestational development.

On the other hand, the levels of LNG detected in the leachate samples did not affect the seminal parameters of sperm cells. However, studies on the effects of chronic toxicity on the andrological pathways should be carried out to assess the outcomes of long-term exposure to LNG in drinking water. This was a preliminary study carried out to understand the influence of the Buenavista landfill as a source of PPCPs in the Piedras River due to leaching.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14060871/s1, SM1. Normal sperm values, SM2. MTT assay, SM3. UV spectrum of LNG, SM4. Analysis of PPCPs in the landfill.

Author Contributions: Conceptualization, A.C.P. and J.C.Q.-C.; methodology, R.R.-S., W.D.C.-M. and J.J.G.-L.; validation, D.G.-R.; formal analysis, writing—original draft preparation and supervision, J.F.N.-V. All authors have read and agreed to the published version of the manuscript.

Funding: The materials and methods were supported by grants from Ministerio de Ciencia y Tecnología MINCIENCIAS (Project code: 13657757707). Additionally, this paper was supported by Corporación Universitaria Remington and Universidad Cooperativa de Colombia (Project code: 400000098).

Institutional Review Board Statement: Informed consent was obtained from all subjects involved in the study.

Informed Consent Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethic Committee of the Faculty of Medicine-University of Antioquia (Acta de aprobación No. 003, date: 14-02-2019).” for studies involving humans. Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank the Ministerio de Ciencia y Tecnología MINCIENCIAS for funding the project entitled “Potencial de bioacumulación de agroquímicos y contaminantes persistentes en una cuenca del oriente antioqueño: Evaluación de un problema de salud pública”. The authors also thank the authors of the research project entitled “Ensayo de disrupción endocrina de micro-contaminantes acumulados en prótesis mamarias de silicona por medio de passive dosing y expresión de la hormona β-hCG en la línea celular BeWo” which was funding by Universidad Cooperativa de Colombia and Corporación Universitaria Remington. Additionally, the authors thank the Hospital Marco Fidel Suarez (Bello-Antioquia) for analyzing the levels of β-hCG in the samples and for their support.

Conflicts of Interest: The authors declare that there is no conflict of interest with regard to the published results.
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