

## Supplementary materials (SM)

# Assessment of levonorgestrel in leaching of a landfill and their effects over placental cell line and sperm cells

Ramiro Ríos-Sossa <sup>1</sup>, Juan José García-Londoño <sup>1</sup>, Daniel Gil-Ramírez <sup>1</sup>, Arley Camilo Patiño <sup>2</sup>, Walter D. Cardona-Maya <sup>3</sup>, Juan Carlos Quintana-Castillo <sup>4</sup> and Jhon Fredy Narváez-Valderrama <sup>1,\*</sup>

<sup>1</sup> Grupo de Investigación Ingeniar, Facultad de Ciencias Básicas e Ingeniería, Corporación Universitaria Remington, Calle 51 No. 51-27, Medellín, 050010 Colombia; ramiro.rios@udea.edu.co (R.R.-S.); juan.garcia.3750@miremington.edu.co (J.J.G.-L.); danielh.gil@udea.edu.co (D.G.-R.)

<sup>2</sup> Grupo Toxicología, Alternativas Terapéuticas y Alimentarias BIOPOLIMER, Facultad de Ciencias Farmacéuticas y Alimentarias, Universidad de Antioquia, Calle 70 # 52-21, Medellín, 050010 Colombia; arley.patino@udea.edu.co

<sup>3</sup> Grupo Reproducción, Facultad de Medicina, Universidad de Antioquia, Calle 70 # 52-21, Medellín, 050010 Colombia; wdario.cardona@udea.edu.co

<sup>4</sup> Grupo de Investigación Infettare, Universidad Cooperativa de Colombia, Calle 50A 41-20, Medellín, 050012 Colombia; juan.quintanac@ucc.edu.co

\* Correspondence: jhon.narvaez@uniremington.edu.co

## SM1. Normal sperm values

**Table S1.** Normospermia values and Consolidated effect of leached LNG on sperm motility and viability.

Progressive motility				
	0 hr	1 hr	2 hr	3 hr
Control	66 (55–78)a	61.6 (48–75)	62 (51–74)	58.8 (46–72)
Leached		62.5 (53–74)	63 (53–74)	59.8 (48–72)
LNG		63.3 (50–75)	62.8 (53–73)	59 (48–71)
Non-progressive motility				
Control	10.5 (6–14)	11.2 (8–16)	10.2 (7–18)	14.2 (8–21)
Leached		10.3 (5–15)	10.3 (5–17)	12.5 (5–19)
LNG		11.3 (5–17)	10.3 (4–17)	12.8 (4–21)
Immotile sperm cells				
Control	23.5 (16–33)	27.2 (17–40)	27.8 (18–41)	27 (20–34)
Leached		27.5 (21–36)	27 (21–36)	27.8 (21–35)
LNG		25.5 (18–37)	27 (20–36)	28.3 (21–36)
Viability				
Control	85 (78–92)	84.6 (76–90)	84 (75–90)	81.4 (74–88)
Leached		84 (75–89)	83.3 (73–91)	81.8 (73–88)
LNG		83.3 (78–90)	82 (75–89)	81 (73–87)

a: mean (Normal parameters in min and max ranges)

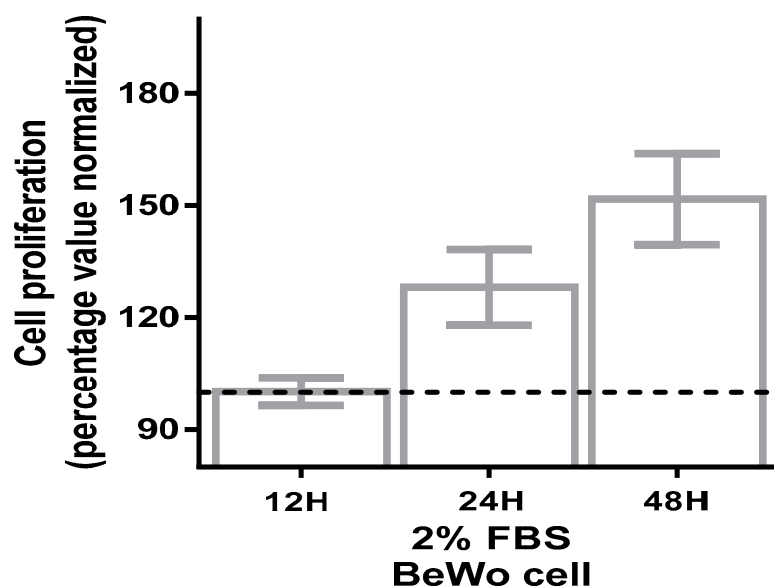
## SM2. MTT assay

**Description:** MTT Assay employ a MTT reagent used to determine mammalian cell viability. The redox potential in active mammalian cells reduces MTT to a strongly pigmented formazan

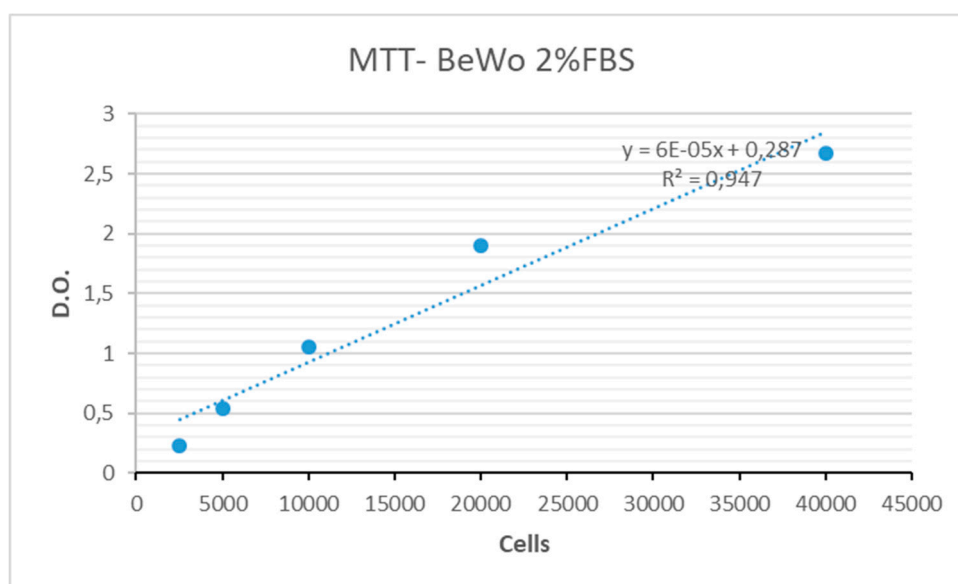
product. After solubilization, the absorbance of the formazan can be measured with a microplate absorbance reader.

**Assay:**

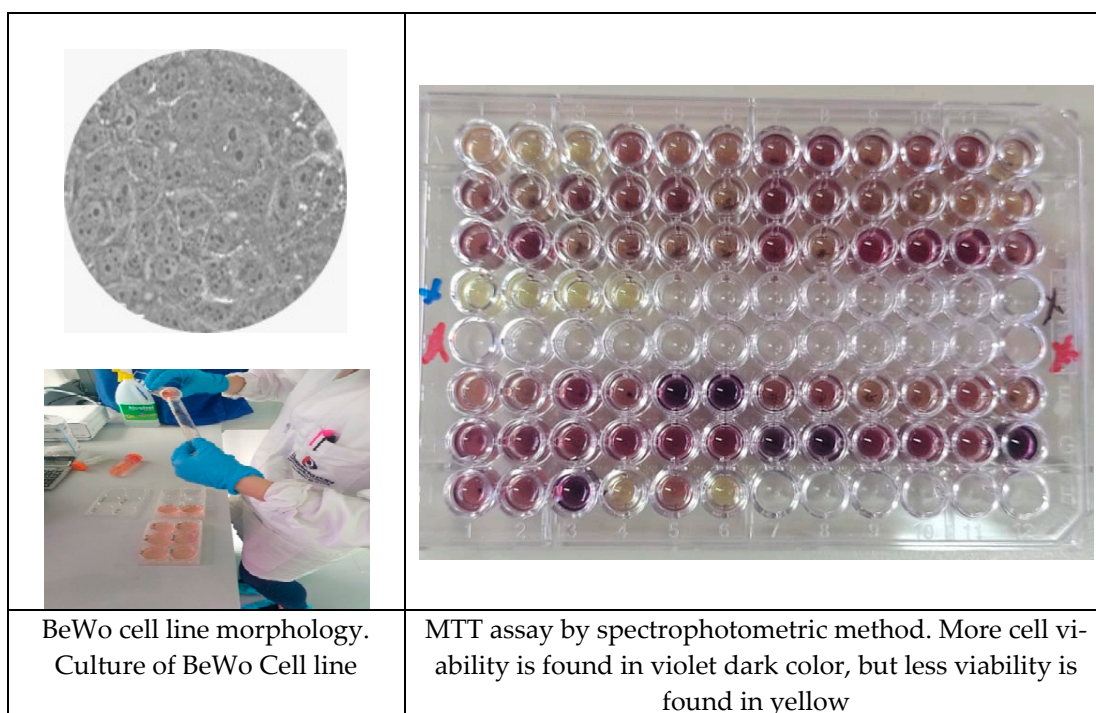
1. For adherent cells (BeWo), the medium was removed and this was replaced with 100  $\mu$ L of fresh culture medium.
2. Then 10  $\mu$ L of MTT solution (12 mM) was measured in each well (96-well dishes). Additionally, negative control using 10  $\mu$ L of the MTT solution into 100  $\mu$ L of medium was carry out.
3. The MTT assay was incubated at 37 °C, 5% CO<sub>2</sub> and 95% humidity for 4 hours.
4. During this time, the cell wells were monitored under the microscope to analyze the formation of formazan crystals, that is, when a purple precipitate is formed. Once the time, the solution was removed, trying to leave a 25  $\mu$ L remnant in each well.
5. Then, 50  $\mu$ L of DMSO was added to each well and those were mixed using a micropipette.
6. The assay was Incubated at 37 °C, 5% CO<sub>2</sub>, and 95% humidity for 10 minutes.
7. Finally, the plate was inserted into the microplate reader, shaken for 10 seconds and the absorbance (optical density-OD) was read at 540 nm.



**Figure S1.** Results of BeWo cell proliferation.



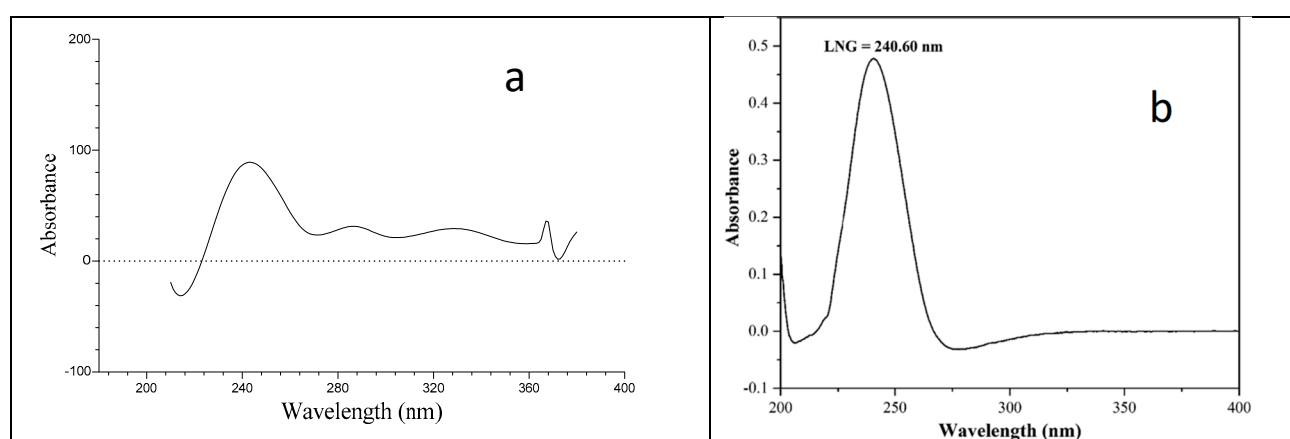
**Figure S2.** Result of curve calibration for cell viability and proliferation.



**Figure S3.** Laboratory analysis.

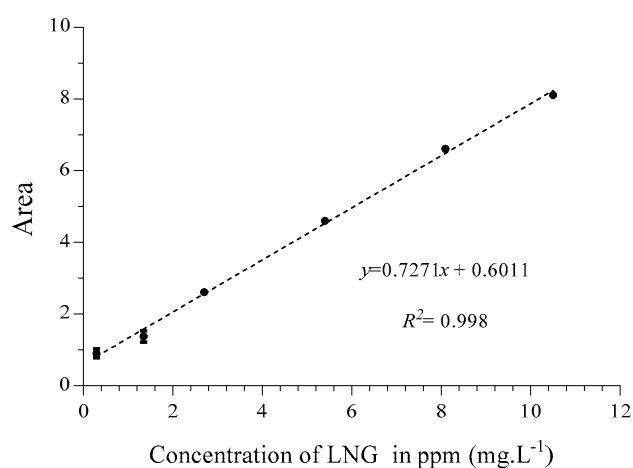
### SM3. UV spectrum of LNG

**Description:** To identify the LNG in samples the UV spectrum obtained by uHPLC-DAD during analysis was compared to this obtained in literature



**Figure S4.** (a). UV spectrum of LNG by uHPLC. (b). UV spectrum of LNG (Literature). Both spectrum showed the maximum UV absorption in 240 nm.

### Linearity for LNG

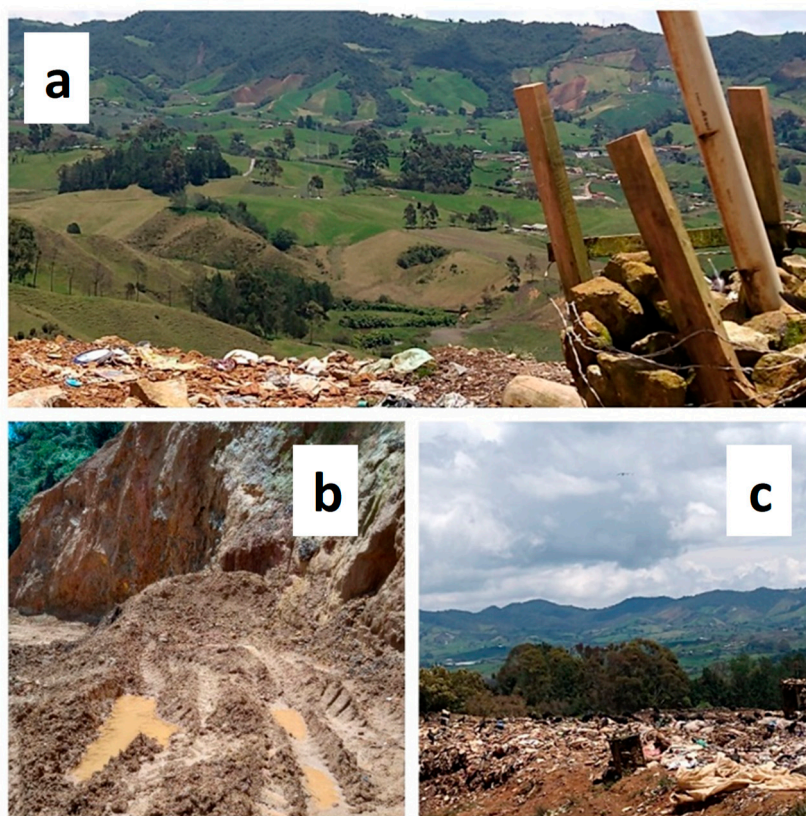


**Figure S5.** Linearity for LNG.  $n = 3$ . Linearity range between 0.3–10.5 ( $\mu\text{g L}^{-1}$ ).

### SM4. Analysis of PPCPs in the landfill



Influence of Buenavista landfill over Piedras river. This landfill shows a slope higher than 30% and thus rainfall and leaching processes may be done



**Figure S6.** (a). Landfill Buenavista: slope steeper than 30% and is less than 600 m away from the Piedras River. (b). Cover material extraction for covering wastes in Buenavista Landfill. (c). Pharmaceutical blister residues in Buenavista landfill.



**Figure S7.** Many pharmaceutical blisters are found in Buenavista Landfill.



**Figure S8.** Anaerobic sludge blanket reactor (ASBR) from Buenavista landfill.