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Article

Use of Sediqualsoft® to Determine the Toxicity of Cigarette Butts to Marine Species: A Weather Simulation Test

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S.1. Materials and Methods

Cigarette Composition

Below is the composition of the cigarettes used for the experiment:

ECB Tobacco = 203.3 mg/stick (nicotine = 0.5 mg/stick)
Glycerol = 47.1 mg/stick
Water = 36.1 mg/stick
Cellulose = 10.5 mg/stick
Guar gum = 6.1 mg/stick
Propylene glycol = 2.4 mg/stick
Natural and artificial flavourings = 0.638 mg/stick
Filtration material = 357.5 mg/stick
Paper and wrappers = 100.9 mg/stick
Tipping paper and tipping paper inks = 20.2 mg/stick
Adhesives = 10.8 mg/stick

CCB* Tobacco = NA (nicotine = 0.7 mg/stick)
Water = NA
Propylene glycol = NA
Sugars (sucrose and/or invert sugar) = NA

Glycerol = NA
Natural and artificial flavouring = NA
Filtration material = NA
Paper and wrappers = NA
Tipping paper and tipping paper inks = NA
Adhesives = NA

*NA = Non-Available. About the CCB the producer doesn't explain the single concentration of the stick components.

Artificial Sea Water (ASW) Composition. Quantity expressed in g/L.

1- $NaCl$ = 22.0
2- $MgCl_2 * 6H_2O$ = 9.7
3- Na_2SO_4 = 3.7
4- $CaCl_2 * 2H_2O$ = 1.32
5- KCl = 0.65
6- $NaHCO_3$ = 0.2
7- H_3BO_3 = 0.023

Composition of the Nutrients used for *P. tricornutum* Test:

S1 = $FeCl_3 * 6H_2O$ = 48 mg/L
$MnCl_2 * 4H_2O$ = 144 mg/L
$ZnSO_4 * 7H_2O$ = 45 mg/L
$CuSO_4 * 5H_2O$ = 0.157 mg/L
$CoCl_2 * 6H_2O$ = 0.404 mg/L
H_3BO_3 = 1140 mg/L
Na_2EDTA = 1000 mg/L

S2 = Thiamin hydrochloride = 50 mg/L
Biotin = 0.01 mg/L
Vitamin B_{12} (cyanocobalamin) = 0.10 mg/L

S3 = K_3PO_4 = 3.0 g/L
$NaNO_3$ = 50.0 g/L
$Na_2SiO_3 * 5H_2O$ = 14.9 g/L

Preparation of Leachates and Elutriates

Before exposure to target species, in some case parameters were corrected to comply with the standardized requirements of the eco-toxicological tests. In detail, pH was corrected by the addition of few microliters of NaOH and HCl 1M. Corrections are specified in Table 1 by means of *.

Table S1. Experimental conditions required by the applied methods.

Species	Endpoint	Type	Method	Salinity PSU	Temperature °C	Test duration	Illumination
A. fischeri	Inhibition of bioluminescence	Acute	UNI EN ISO 11348-3:2019	> 20	15 ± 1	15 minutes 30 minutes	-
P. lividus	Larval development	Chronic	EPA/600/R-95-136/Sezione 15 + ISPRA Quaderni Ricerca Marina 11/2017	35 ± 1	18 ± 1	72 hours	Darkness
P. lividus	Fertilization	Acute	EPA/600/R-95-136/Sezione 16 + ISPRA Quaderni Ricerca Marina 11/2017	35 ± 1	18 ± 1	40 minutes (20 min exposition + 20 min fertilization)	-
P. tricornutum	Growth inhibition	Chronic	UNI EN ISO 10253:2017	30 ± 1	20 ± 2	72 hours	24/24 h light 6000-10,000 lux from both side

Table S2. Endpoint values obtained from the analyses of negative and positive controls during experiments.

Species	Negative Control			Positive Control		
	Medium	Mean	Standard Deviation	Substance	Value	Standard Deviation Confidential limit
A. fischeri	ASW	0.0 %	0.0	3,5-dichlorophenol 3.4 mg/L	15′: 37.46% 30′: 36.11%	15′: 1.88 30′: 2.27
P. lividus (Larval development)	ASW	5.67 %	1.15	Copper (II) nitrate	EC_{50}: 28.26 µg/L	26.90–29.69 µg/L
P. lividus (Fertilization)	ASW	0.0 %	0.0	Copper (II) nitrate	EC_{50}: 57.43 µg/L	33.58–98.24 µg/L
P. tricornutum	algal culture medium	0.0 %	1.61	Potassium dichromate	EC_{50}: 21.61 mg/L	16.78–27.83 mg/L