International experts
in spill preparedness and response

imaros

Final conference

Task 3.3 – Ecotoxicity of 3 VLSFO

Fanny Chever
CEDRE

Malta, 31/05/2022

Co-funded by
the European Union

Task 3.3: Ecotoxicity

OSPAR Guidelines for Toxicity Testing of Substances and Preparations Used and Discharged Offshore.

- Adoption of an harmonised mandatory control system for use and reduction of discharges of hazardous substances in the offshore oil and gas industry
- This system states that chemical suppliers must provide the national authorities with data and information about chemicals that they plan to use.
- Tests are part of the **Offshore Chemical Notification Scheme (OCNS)**

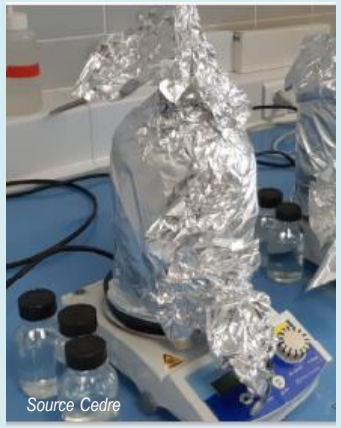
Objective:
→ Assess the potential toxicity of 3 VLSFO (IM-5, IM-14 and IM-15)

Task 3.3: Ecotoxicity – General approach

INDIRECT CONTACT (WAF)

Marine diatom *Phaeodactylum tricornutum* (ISO 10253:2016)

Marine copepod *cartia tonsa* (ISO 14669: 2003)



Water Accomodated Fraction (WAF)

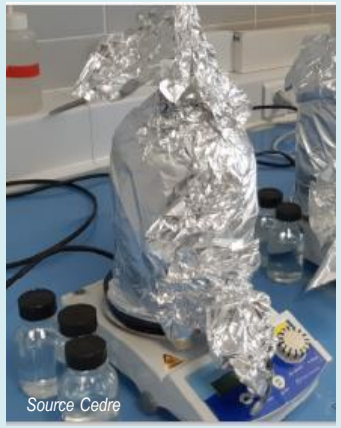
- Oil added at saturation to a known volume of seawater
- Mixing 24 h in the dark: **soluble fraction collected** and serves as a culture medium
- WAF diluted series with natural or synthetic seawater

Task 3.3: Ecotoxicity – General approach

INDIRECT CONTACT (WAF)

Marine diatom *Phaeodactylum tricornutum* (ISO 10253:2016)

Marine copepod *cartia tonsa* (ISO 14669: 2003)



Water Accomodated Fraction (WAF)

- Oil added at saturation to a known volume of seawater
- Mixing 24 h in the dark: **soluble fraction collected** and serves as a culture medium
- WAF diluted series with natural or synthetic seawater

DIRECT CONTACT

Amphipod *Corophium volutator (PARCOM protcol)*


Source Cedre


Source Cedre

Co-funded by
the European Union

Task 3.3: Ecotoxicity – Algae

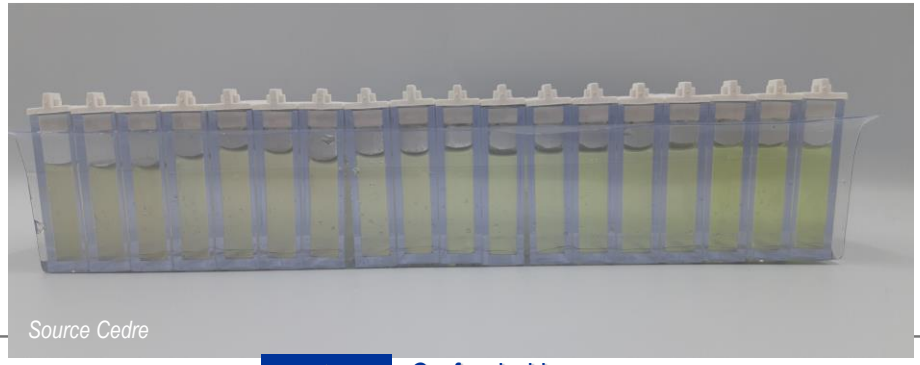
Marine diatom *Phaeodactylum tricornutum*

MicrobioTests Inc.


Source Cedre

- Controls: culturing medium and algae (triplicate) for each VLSFO tested
- Reference/positive test: potassium dichromate ($K_2Cr_2O_7$) (triplicate), from 0 to 1.8 mg/L
- Algae exposed to different PAH concentrations (triplicate)
- Incubation for 3 days in an incubator, at 20°C (+/- 2 °C)

→ Optical density at 670 nm used as the parameter for algal growth inhibition
→ Inhibitory effect on the growth expressed as the concentration of a product resulting in 50 % inhibition of growth rate


Source Cedre

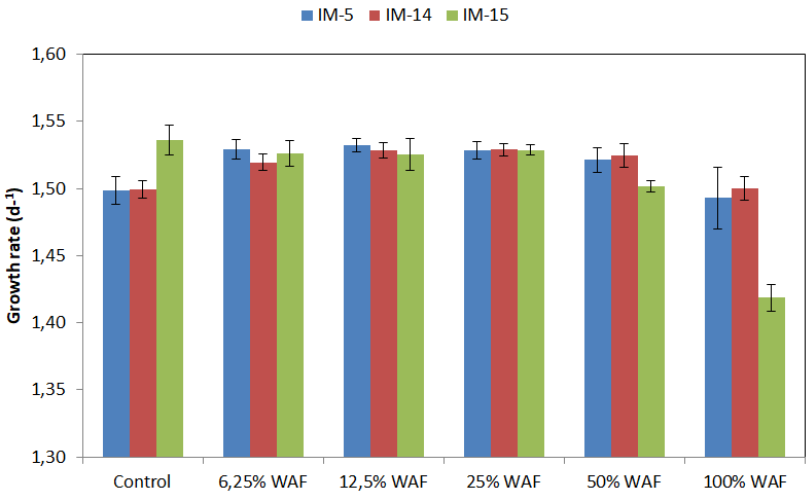
Co-funded by
the European Union

Task 3.3: Ecotoxicity - Algae

Growth rates (d^{-1}), at 72h, of the diatom Phaeodactylum tricornutum exposed to the toxic of reference (conc. in mg/L)

- $K_2Cr_2O_7$: Inhibition observed, specific growth rates decreasing from 1.50 ± 0.01 d^{-1} (control, without $K_2Cr_2O_7$) to 0.50 ± 0.01 d^{-1} (max, concentration: 31.7 mg/L $K_2Cr_2O_7$), leading to an inhibition of ~ 66 %.

Task 3.3: Ecotoxicity - Algae



Growth rates (d^{-1}), at 72h, of the diatom Phaeodactylum tricornutum exposed to the toxic of reference (conc. in mg/L) and to VLSFO

- $K_2Cr_2O_7$: Inhibition observed, specific growth rates decreasing from 1.50 ± 0.01 d^{-1} (control, without $K_2Cr_2O_7$) to 0.50 ± 0.01 d^{-1} (max, concentration: 31.7 mg/L $K_2Cr_2O_7$), leading to an inhibition of ~ 66 %.

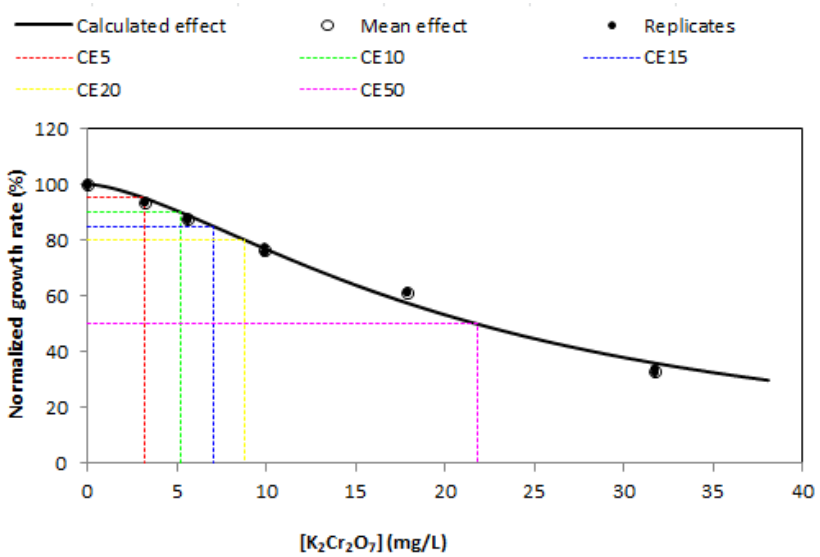
- Control ranged from 1.50 ± 0.01 d^{-1} (IM-5, IM-14 and toxic of reference) to 1.54 ± 0.01 d^{-1} (IM-15)
- No growth inhibition observed
- Only IM-15 exhibits a slight decrease for the 100 % WAF, with growth rate reaching 1.42 ± 0.01 d^{-1} for the 100% WAF condition, leading to an inhibition of 8 %.

Task 3.3: Ecotoxicity - Algae

LC_{50} determintation

Given the low impact of the 3 VLSFO on the growth rate, linear model could only be fitted for the toxic of reference

LC_{50} ($K_2Cr_2O_7$) = 21.7 mg/L



Normalised growth rate (in d^{-1}) calculated with the positive substance $K_2Cr_2O_7$ and determination of the LC_{50} with the Regtox programme

→ For the highest quantity of oil tested, LC_{50} at 72 hours could not be determined for the 3 VLSFO tested

LC_{50} > 25.6 µg .L^{-1} PAH for IM-5
LC_{50} > 12.9 µg .L^{-1} PAH for IM-14
LC_{50} > 156.1 µg .L^{-1} PAH for IM-15

Task 3.3: Ecotoxicity - Copepods

- Controls: copepods exposed to seawater (12 replicates)
- Positive control: copepods exposed to 3,5-dichlorophenol (1 $mg.L^{-1}$) (12 replicates)
- 5 WAF prepared by dilution
- 5 copepods exposed to each test vessel (4 replicates)
- After 48h exposure at 20°C, survival of the test organisms assessed and LC_{50} calculated

Preparation of the control and the reference substance flasks

Observation and counting of copepods

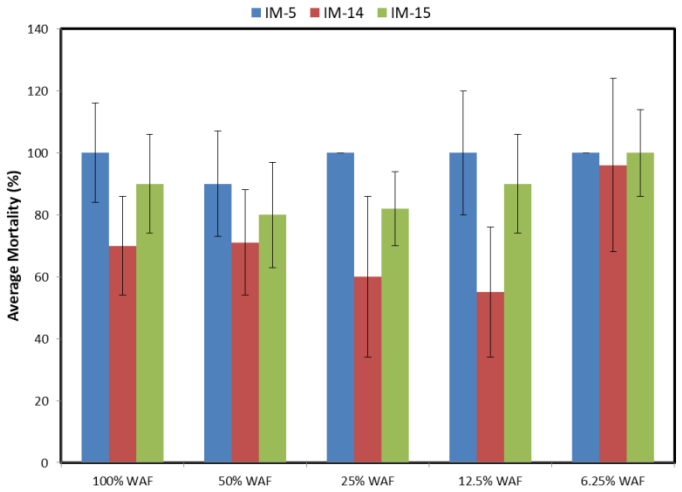
Co-funded by
the European Union

Task 3.3: Ecotoxicity - Copepods

- Controls: after 24 hours and 48 hours, mean mortality was of 7% and 21 % respectively (limit at 10%)
- Positive control: mean mortality of 79 % (range 20% - 80 %)
- High sensitivity of the organisms (travel conditions)

Task 3.3: Ecotoxicity - Copepods

- Controls: after 24 hours and 48 hours, mean mortality was of 7% and 21 % respectively (limit at 10%)
- Positive control: mean mortality of 79 % (range 20% - 80 %)
- High sensitivity of the organisms (travel conditions)



- Copepods exposed to dissolved compounds of the 3 VLSFO exhibited a mortality rate close to 100% even for the lowest concentration tested

LC_{50} 48 hours : < 4.3 µg $.L^{-1}$ PAH for IM-5
LC_{50} 48 hours: < 0.75 µg $.L^{-1}$ PAH for IM-14
LC_{50} 48 hours: < 10.2 µg $.L^{-1}$ PAH for IM-15

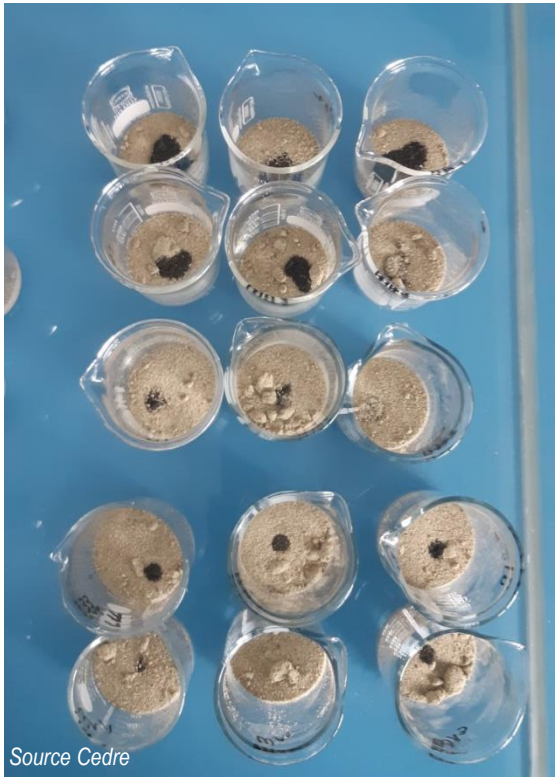
Task 3.3: Ecotoxicity - Amphipods

Sediment bioassay : Amphipod *Corophium volutator*

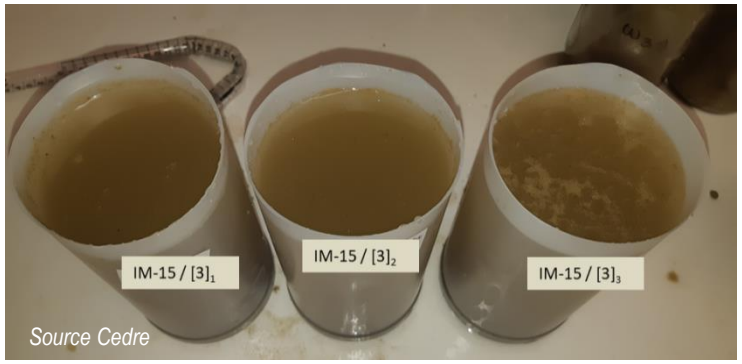
- Test organisms (20) added to 1 L beaker with a range of oil concentrations spiked sediment (oil directly added to dry sediment),
- Nine tanks contained blank
- Positive control: fluoranthene (dissolved in acetone)
- Four flasks contained acetone blank
- Test run for 10 days under continue light, at 15°C


Source Cedre

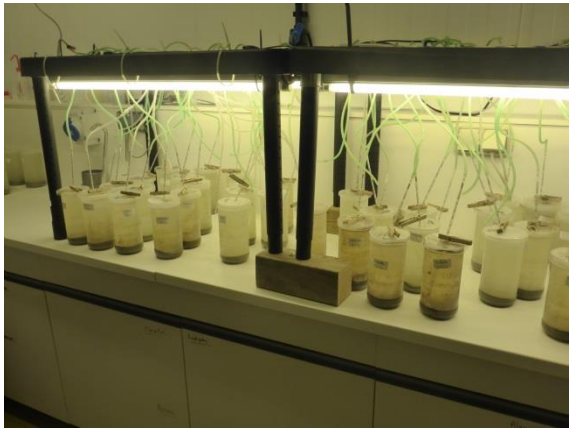
Task 3.3: Ecotoxicity - Amphipods



Sediment preparation (before and after oil mixing)



Addition of 700 mL of seawater



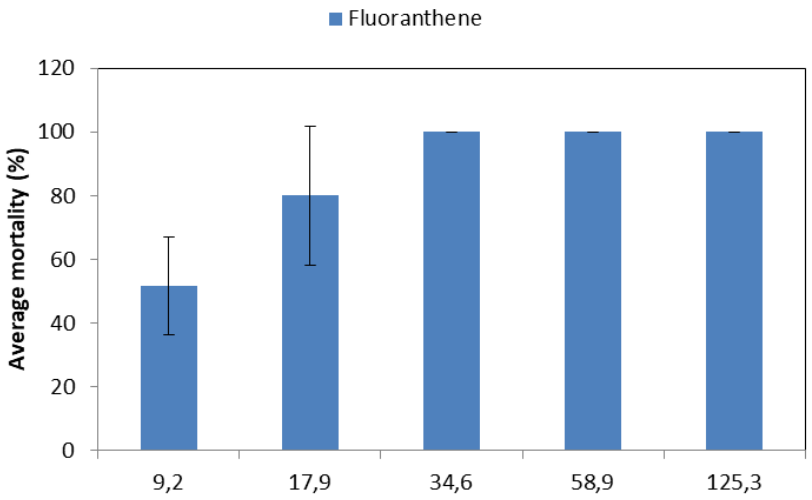
Incubation

Task 3.3: Ecotoxicity - Amphipods

Mortality recording after 10 days experiment (after sediment sifted thought sieve)

- Result expressed as the concentration of a product giving 50 % mortality, LC_{50}

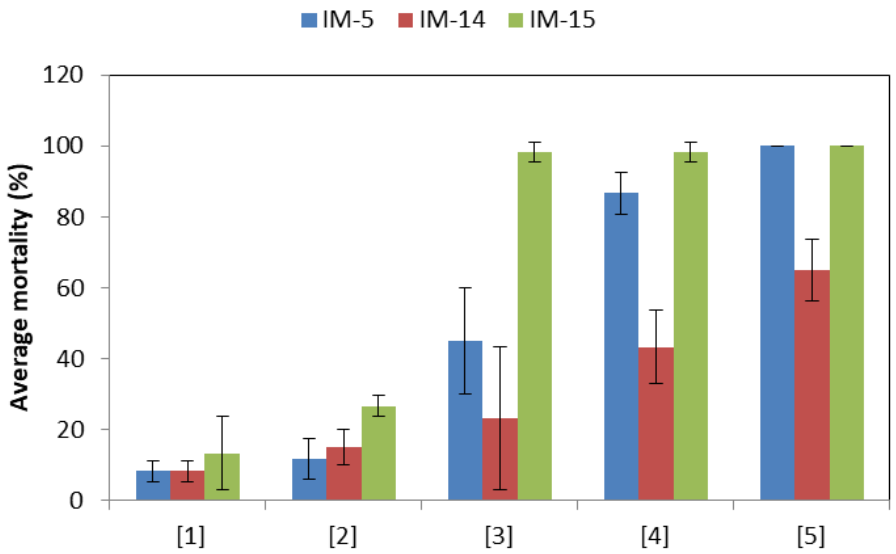
Task 3.3: Ecotoxicity - Amphipods



(concentration in mg/kg)

- Control mortality: 5 %
- Mortality observed for amphipods exposed to the fluoranthene. The lowest concentration tested led to a mortality of 52 %

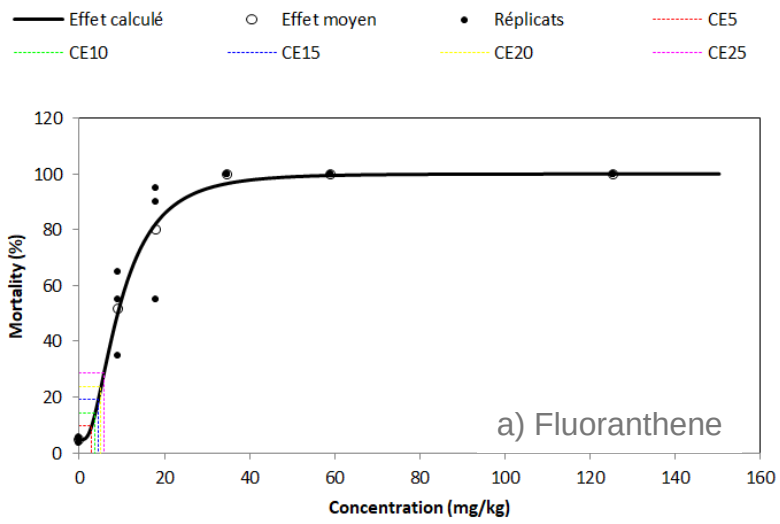
Task 3.3: Ecotoxicity - Amphipods

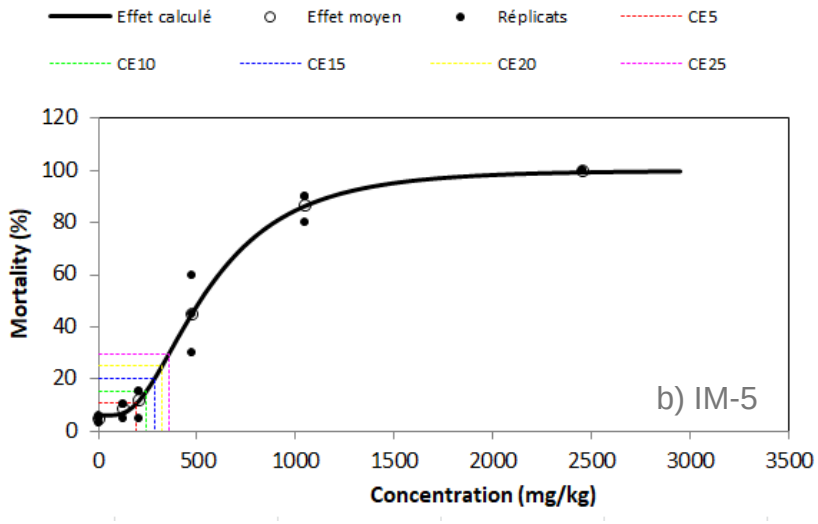


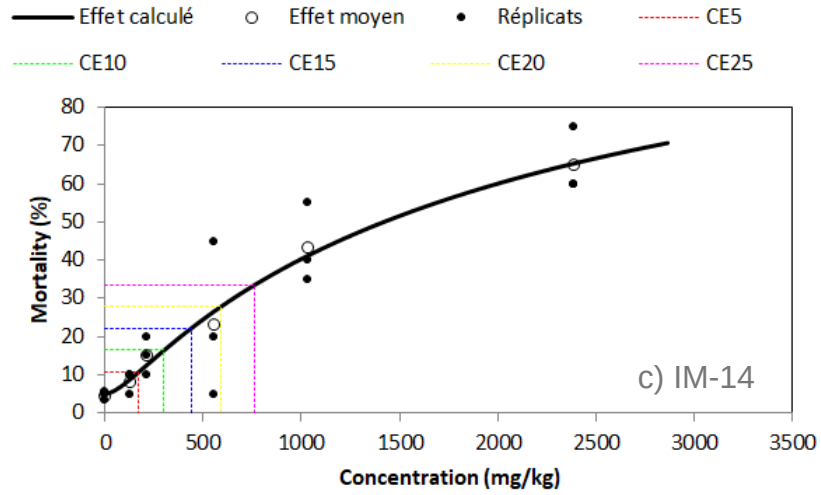
[1] : ~ 100 mg/kg
[2]: ~ 200 mg/kg
[3]: ~ 500 mg/kg
[4]: ~ 1 000 mg/kg
[5]: ~ 2 400 mg/kg

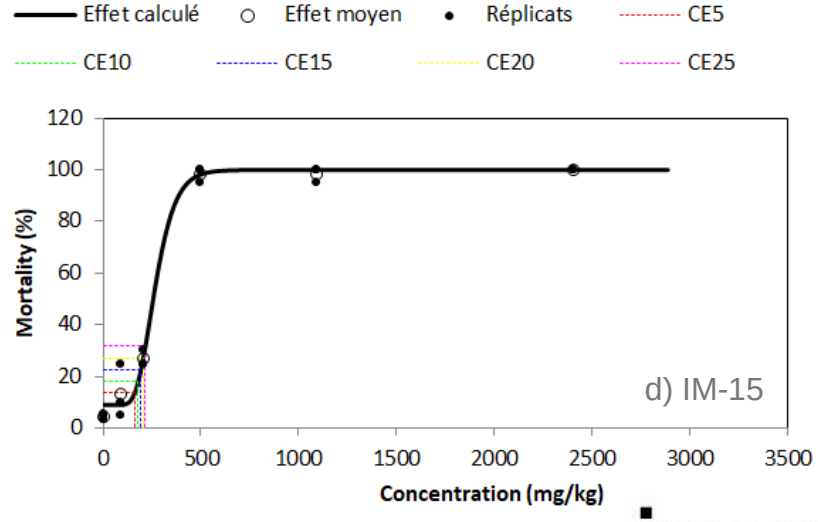
- Amphipods mortality increased with oil concentration (above 50% for the highest concentrations for all VLSFO).

Task 3.3: Ecotoxicity - Amphipods



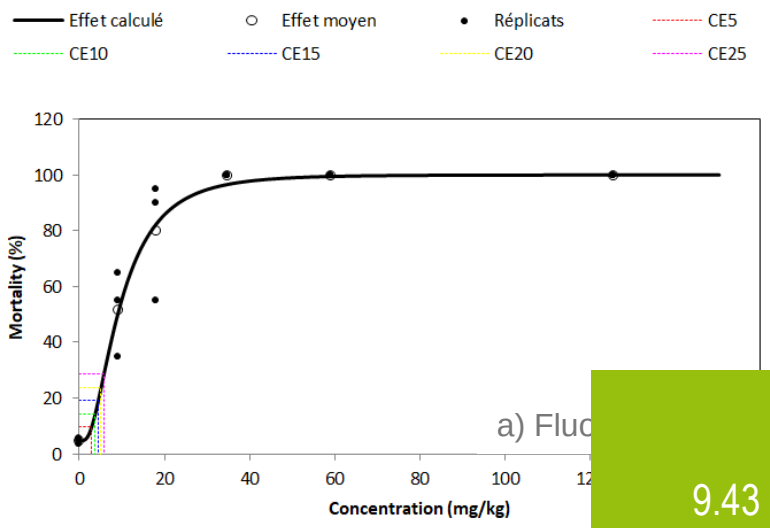


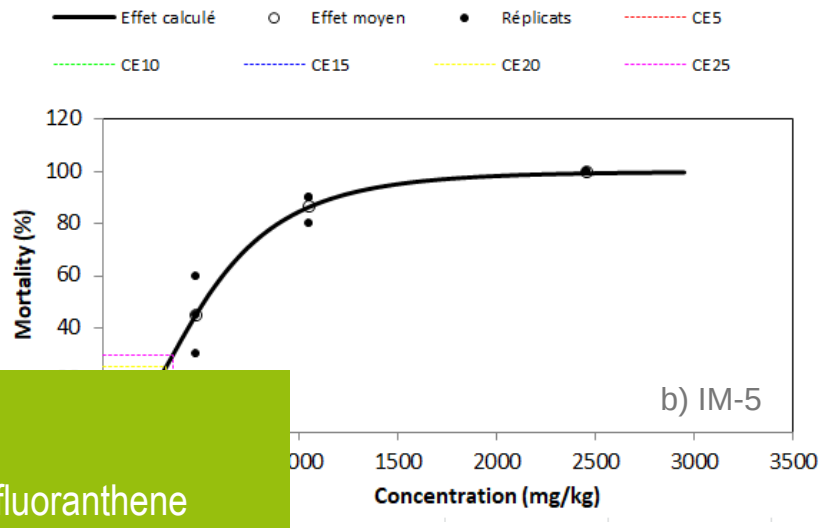




RegTox software

Task 3.3: Ecotoxicity - Amphipods





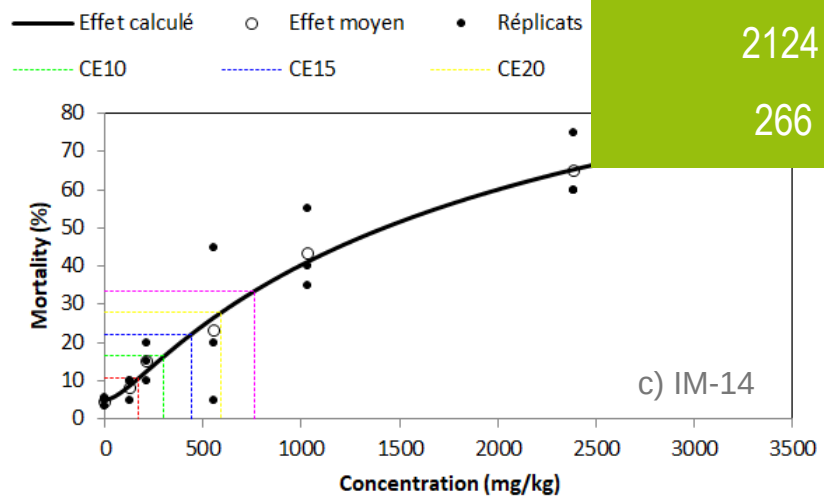
LC_{50}

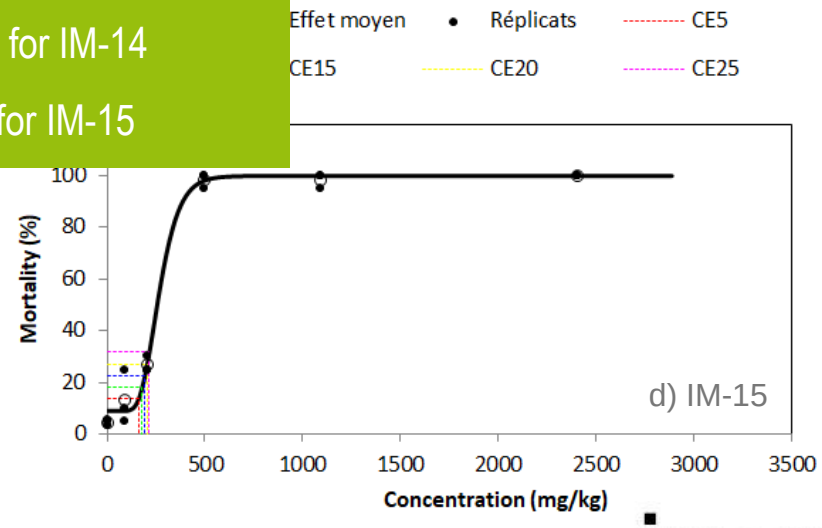
9.43 $mg.kg^{-1}$ for fluoranthene

542 $mg.kg^{-1}$ for IM-5

2124 mg.kg-1 for IM-14

266 $mg.kg^{-1}$ for IM-15

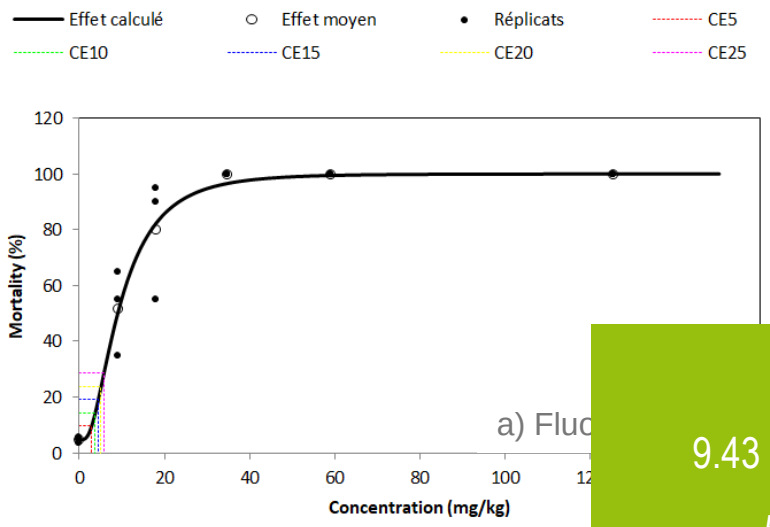




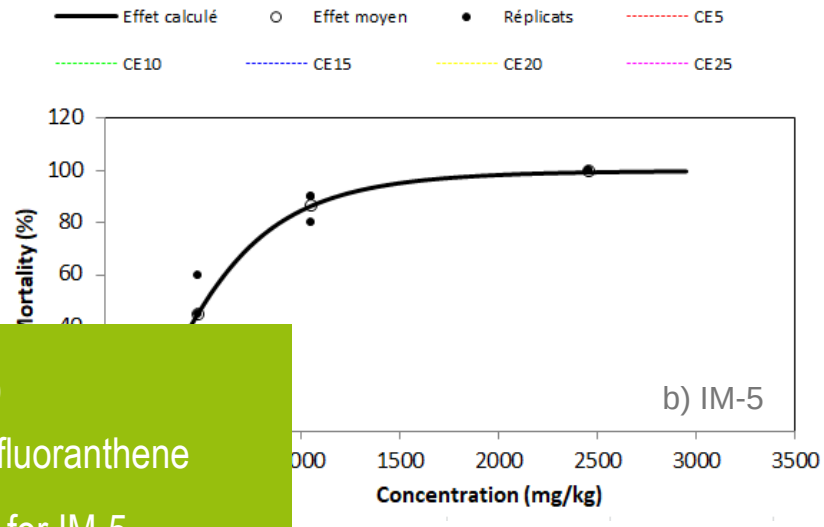
RegTox software

Co-funded by the European Union

Task 3.3: Ecotoxicity - Amphipods



a) Fluo



b) IM-5

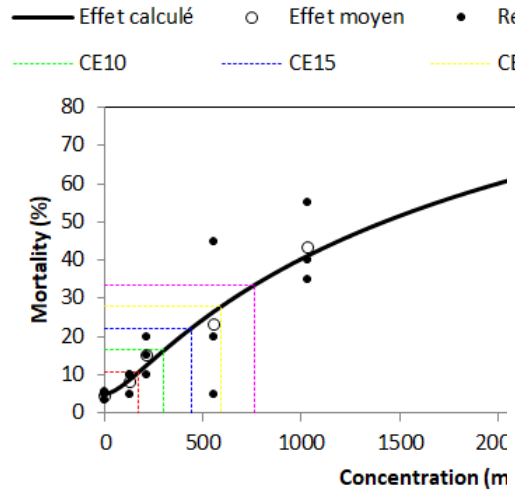
LC_{50}

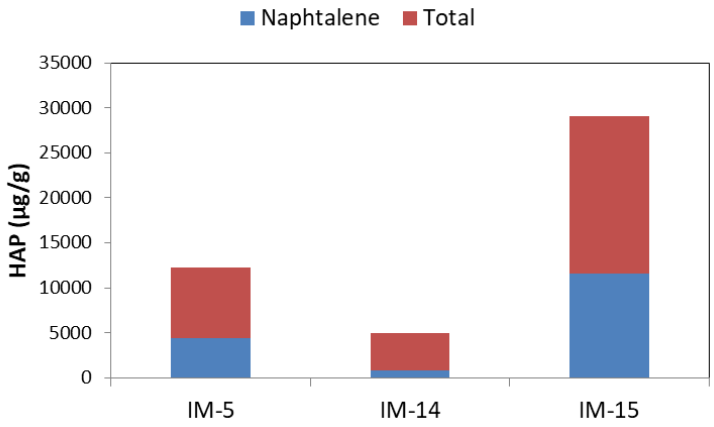
9.43 $mg.kg^{-1}$ for fluoranthene

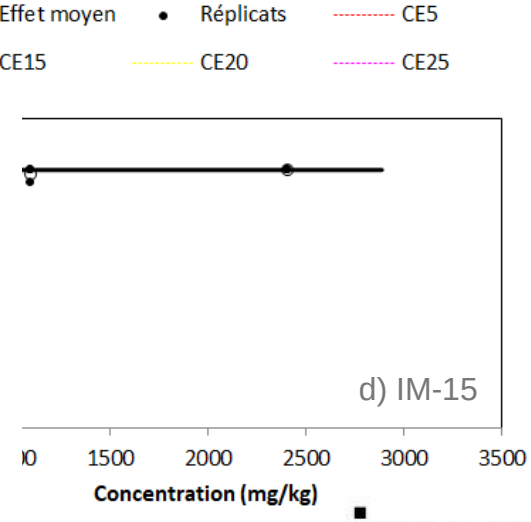
542 $mg.kg^{-1}$ for IM-5

2124 $mg.kg^{-1}$ for IM-14

266 $mg.kg^{-1}$ for IM-15







d) IM-15

RegTox software

Cedre

Co-funded by the European Union

imaros

Task 3.3: Ecotoxicity - Conclusion

- Algae: LC_{50} 72 hours could not be calculated and is greater than the maximum concentration tested.
- Copepods: High sensitivity of the organisms. Mortality rate close to 100% even for the lowest concentration tested
- Amphipods: CL_{50} IM-15 < CL_{50} IM-5 < CL_{50} IM-14 (in link with PAH quantification)

Potential impact of VLSFO on marine organisms (leaving in the water column and in the sediments)

Necessity to recover the oil from the water surface and to clean the shoreline as much as possible

imaros

Final conference

Task 3.3 – Ecotoxicity of 3 VLSFO

Fanny Chever
CEDRE

Malta, 31/05/2022

Co-funded by
the European Union