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Validation of whole effluent bioassays for assessment of hydrocarbon ecotoxicity

Review of findings from Concawe / Total artificial streams research project (2007 to 2014)

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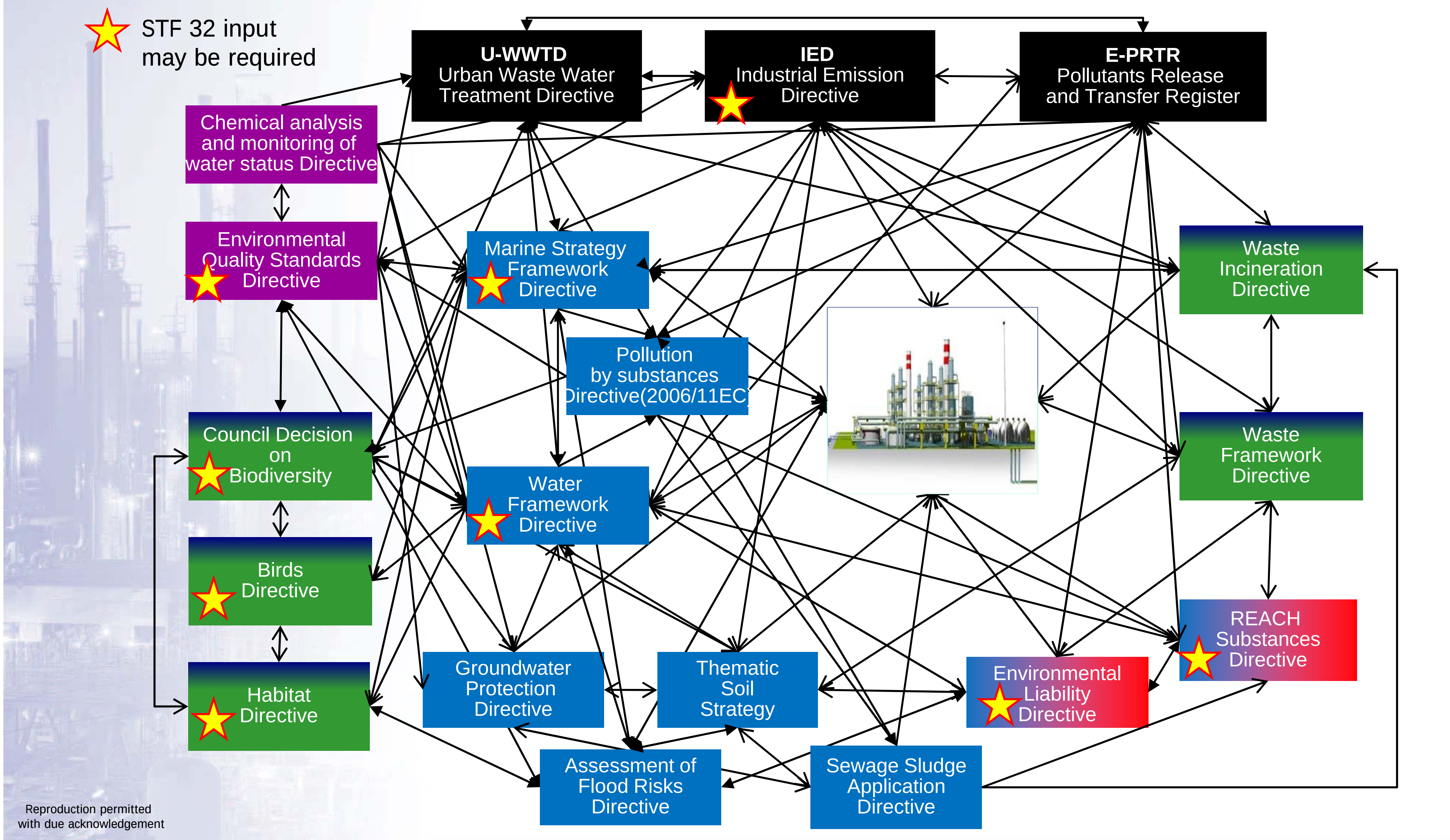
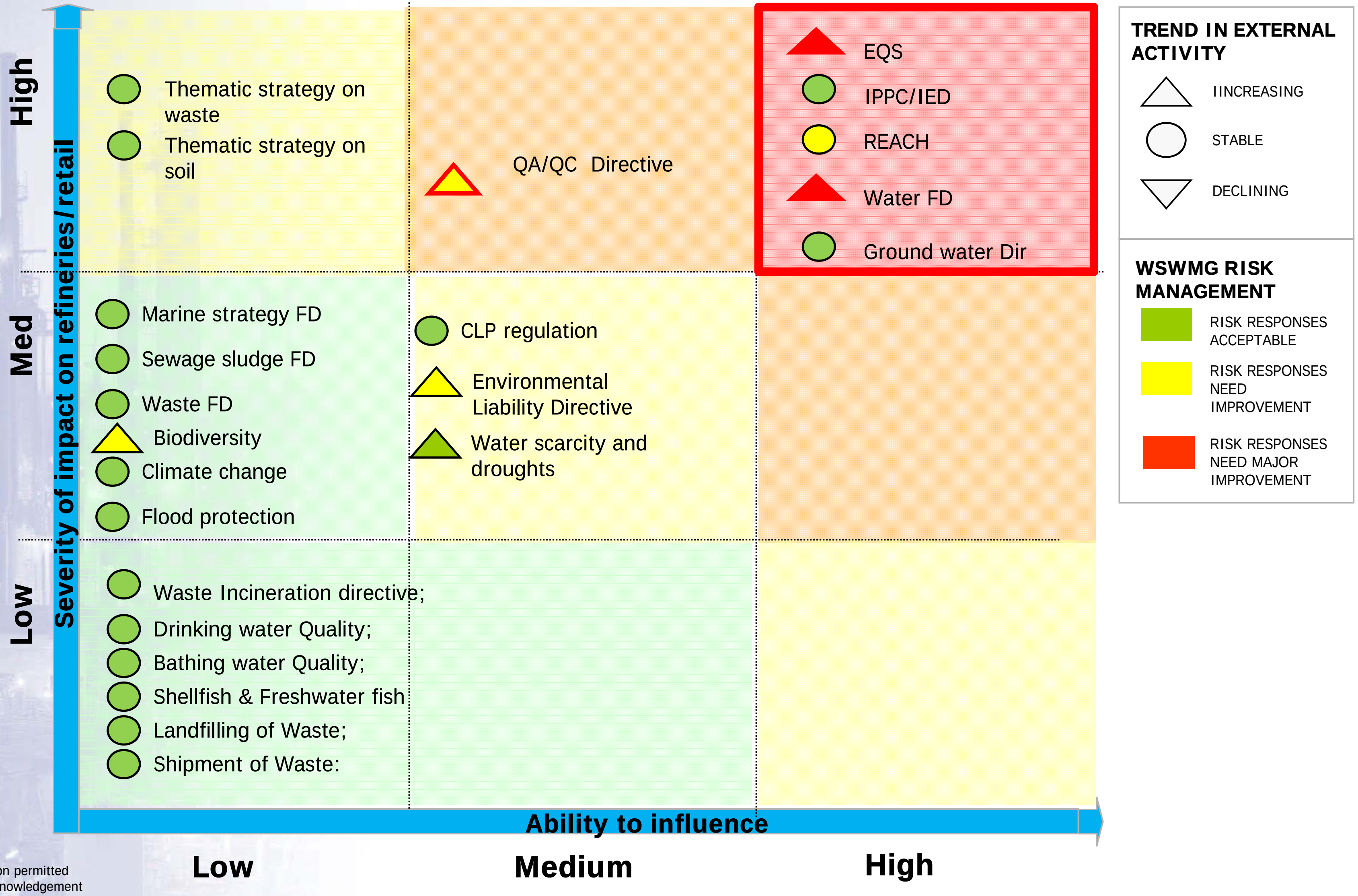
Soil, Water and Waste Legislative Environment



2013 WSWMG workshop: STF-32 issue matrix



Current and Future Legislative Drivers for Assessment of Ecological Effects

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REACH

Objective: Human health and environmental risk assessment of chemicals

Data is required to support REACH dossier risk assessments performed using PETROTOX/ PETRORISK. E.g. from bioassays and target Lipid model (direct link with potentially bioaccumulative substance PBS): data required to avoid application of overly conservative Safety factors

IED

Objective: Control and reduce the impact of industrial emission on the environment

IED implementation: Impact assessment/ prediction tools for effluent discharges (WEA/WET, PBS)

WFD

Objectives: Good chemical and ecological status of surface water bodies.

Robust, validated whole effluent assessment (WEA) methods are required for when *in- situ* monitoring of effluent effects is not possible

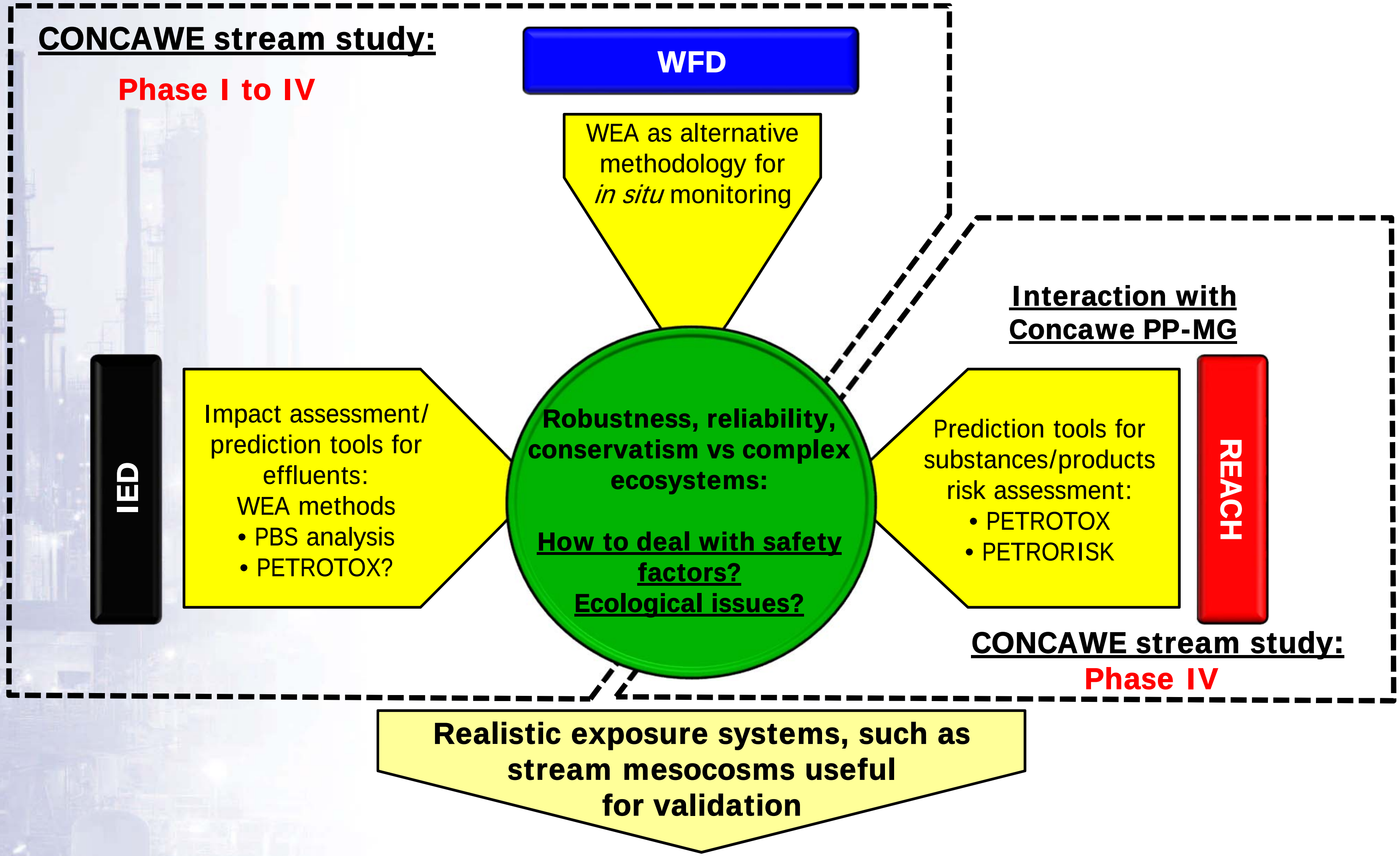
Future

- Marine Strategy Framework Directive – ecological focus
- Habitats Directive – water based ecological issues
- Definition and costing of Ecosystem Services

Background to Streams Study

- WEA can provide a clear indication of the combined effects of all the constituents present in what are often poorly characterized and complex effluents.
- Such assessments can be difficult or impossible to obtain from analyses of data for individual effluent constituents.
- However, this should not be taken to imply that WEA techniques are simple to apply in all cases: Case studies presented in **Concawe report 1-12** (Assessment of refinery effluents and receiving waters using biologically-based effect methods) show that the use of biological methods for assessment of refinery effluent and receiving waters ecotoxicity may be complicated by the following factors:
  - Timescale over which effects develop vs temporal variation in effluent/ receiving water quality
  - Difficulty in associating observed ecological effects to substances, or groups of substances
- If the WEA methods used are inappropriate or incorrectly applied there is a high probability of drawing incorrect conclusions and this can lead to, for example, reputational issues with regulators or demands for unjustified risk reduction
- The streams study research is designed to address the above issues, so that WEA techniques may be applied with greater confidence to refinery effluents

Core Deliverables of Streams Research Project



Project Timeline: Phase I to IV

Period	Phase	Setting	Objective
2009	Phase 1: Preliminary experiments	In Laboratory	Is it possible to store effluent, and is it possible to upscale?
2010-2011	Phase 2: Feasability study	Stream mesocosms	Develop facility to ship effluent from refinery to facility and deploy in streams. Perform dose-response test
2011-2012	Phase 3: WEA	Stream mesocosms	Understand whether effects in WEA bioasssays under or overestimate those in stream mesocosms
2014-2015	Phase 4: WEA	Stream mesocosms	Assess degree of conservatism required in safety factors applied to WEA data Use output of phase 4 to support models used in REACH chemical safety assessments

CONCAWE stream study Phase 1: Effluent storage and preservation trials

	Storage at room temperature	Storage at room temperature + Stirring	Storage at 4°C	
With headspace				→ Bioassays
With no headspace + light				→ Bioassays
With no headspace and no light	YES	YES	YES	→ Bioassays

- Following extensive laboratory testing, it was determined that the best storage method for phase 2 was the use of flexible tanks made of plastomer-coated materials with no light or headspace. This ensured that the effluent was of consistent compositional quality

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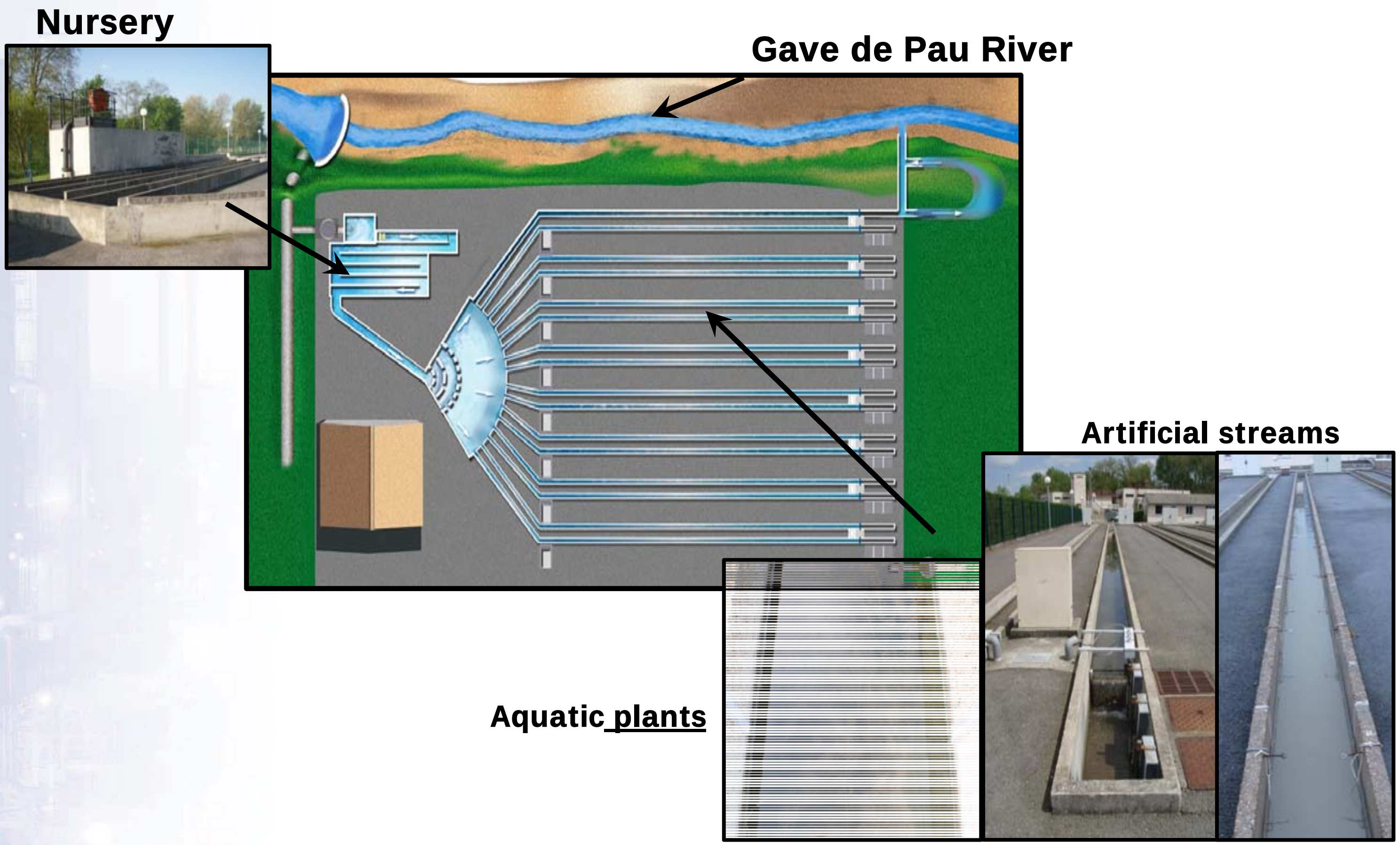
Phase 2: Feasibility assessment of testing effluents in outdoor stream mesocosms

TOTAL stream mesocosms (Lacq)



- Dynamic system: continuous water flow
- Open system: the water flows from the Gave de Pau continuously

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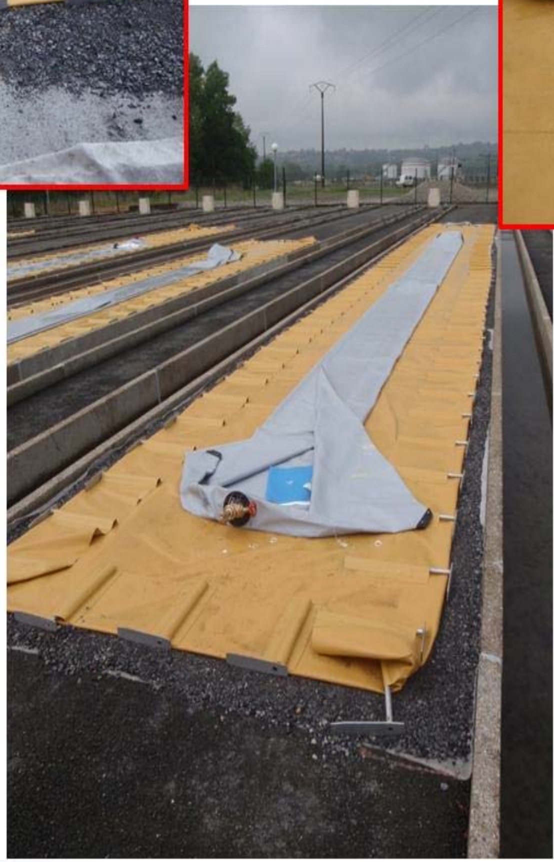
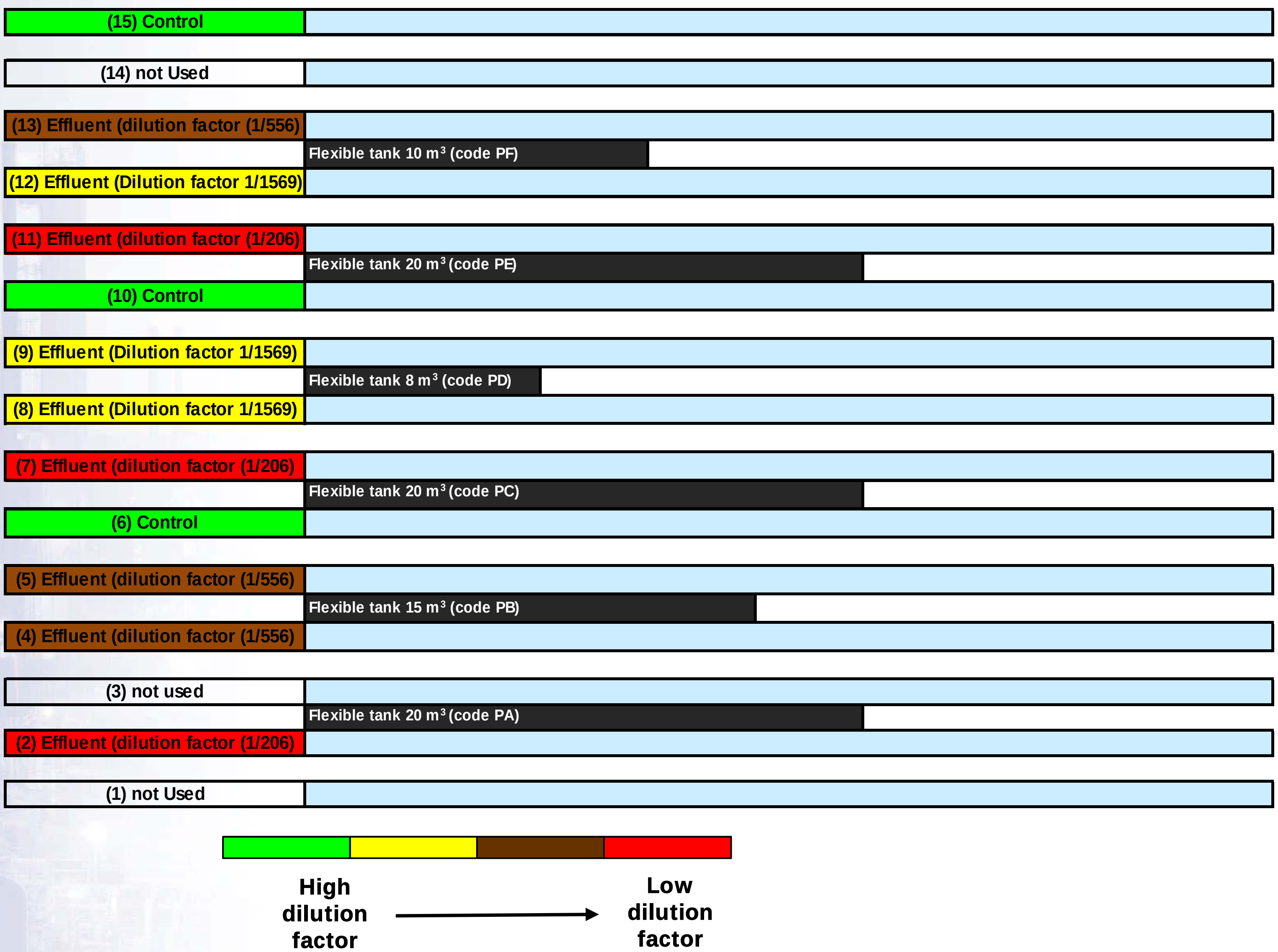
Phase 2: Development of flexible tanks made of plastomer-coated materials



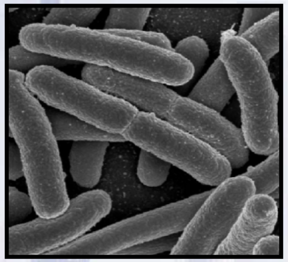
- One effluent stored in all flexible tanks (volumes 8, 10, 20 m^3)
- 3 control streams
- 3 dilutions tested (dosing for 21 days)

Phase 2: Experimental design

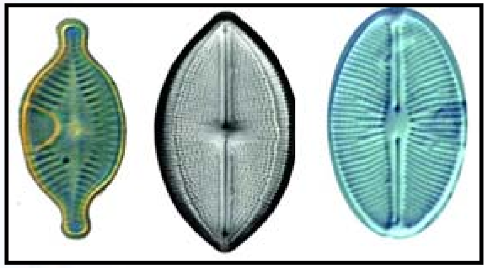


Phase 2: Bioassays and Chemical Analysis

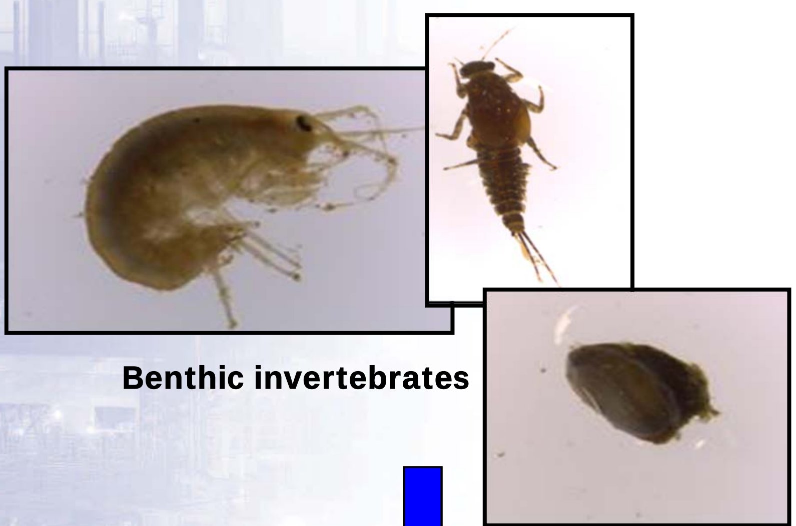
Biological analysis in the streams



Bacteria



Diatoms



Benthic invertebrates

Ecological impact assessment

Physical and chemical analysis in the flexible tanks to confirm input flux:

- BOD_5 and COD
- SPME (Potentially Bioaccumulative substances: PBS)
- Metals

Physical and chemical analysis in the streams to confirm water quality and effluent dose:

- pH, O2, conductivity
- BOD_5 and COD
- Metals

Exposure assessment

Phase 2 : Results and conclusions

- This first series of experiments performed in the stream mesocosms did not provide evidence of a clear dose response because pure effluent was not toxic enough
- Only slight effects determined at the lowest dilution.
- Not possible to clearly conclude whether the results of the WET assays overestimated or underestimated the impact to aquatic ecosystem.

⬇

- Minimum dilution factor in the stream mesocosms = 140
- Relatively low hydrocarbon concentrations measured in the effluent regarding dilution factor to be tested in stream mesocosms

⬇

- Fortification of some of the effluent samples with an appropriate petroleum distillate adopted for phase 3 so as to increase the contaminant concentrations (Potentially bioaccumulative substances: PBS)

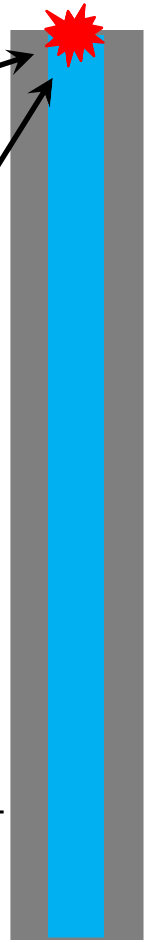
Phase 3: Understanding and comparing the biological responses in effluents and mesocosms

Phase 3: Experimental design-I

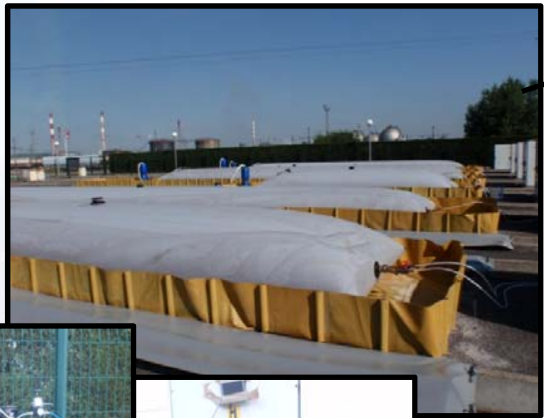
Transport:
stainless trucks

Dilution factor: 140

Artificial streams



Effluents

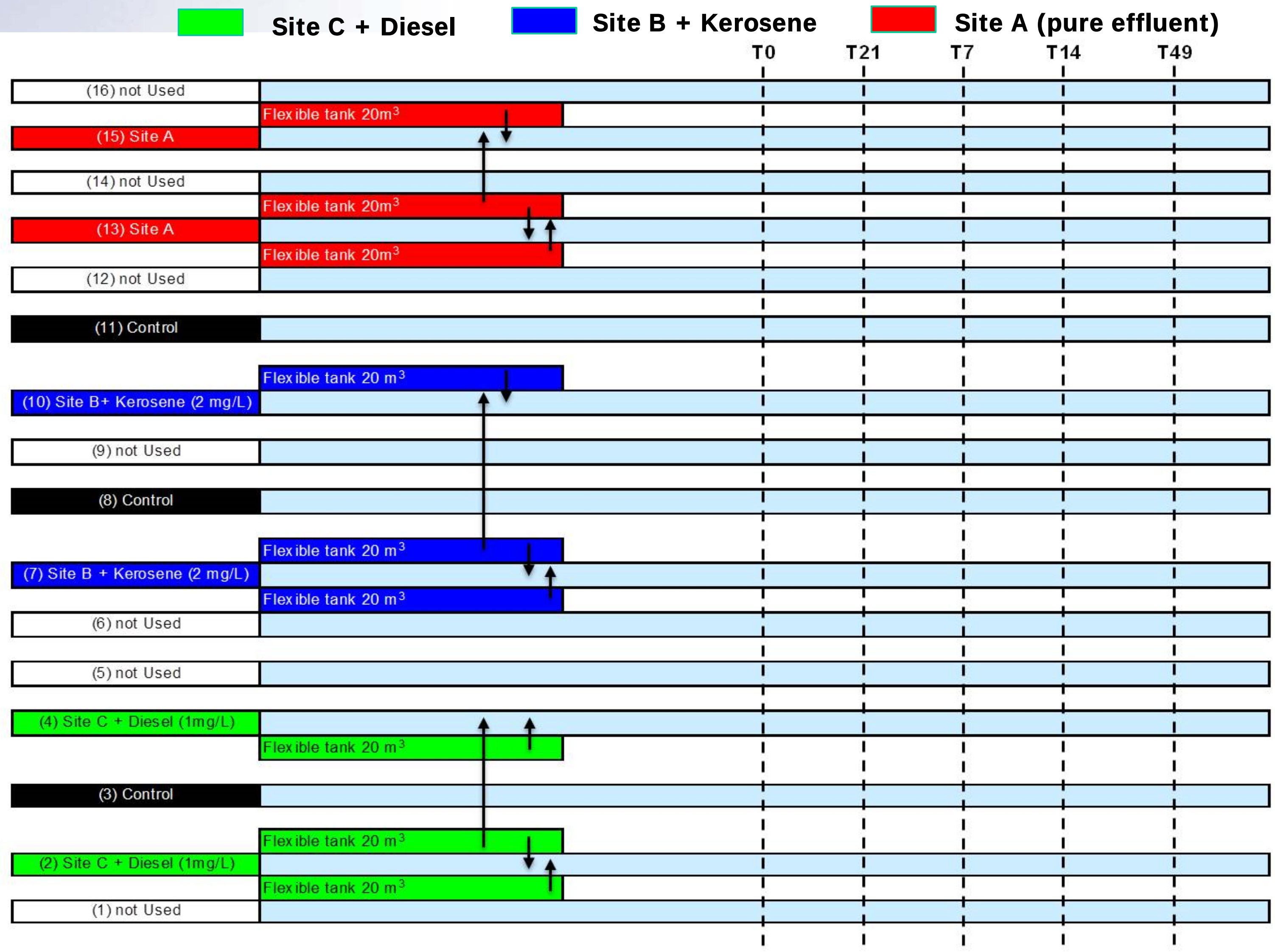


Injection system

Diesel fortification: 1 mg/L
Kerosene fortification: 2 mg/L

Cut fortification

Phase 3: Experimental design-II



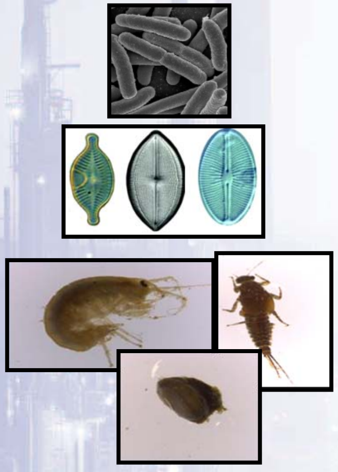
Dosing for 21 days

Phase 3: Bioassays and Chemical Analysis

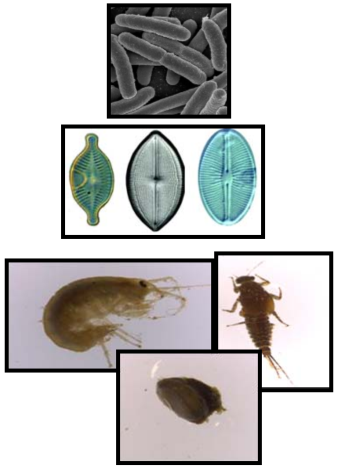
Day -60 | Day 0 | Day 21 | Day 49

Reference → **Effluent Dosing (flow through)** → **Recovery**

In the streams



In the streams



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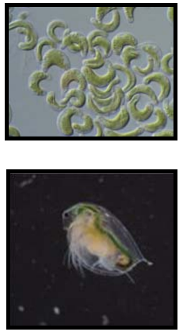
pH, O_2, conductivity

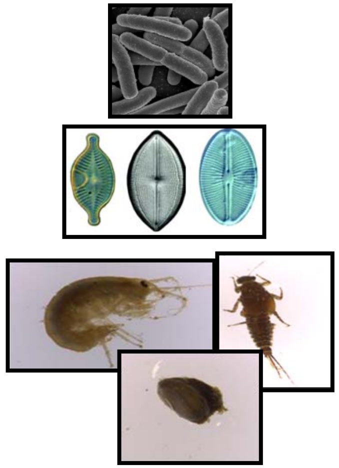
Effluent tested In the laboratory



+

BOD_5, COD

In the streams

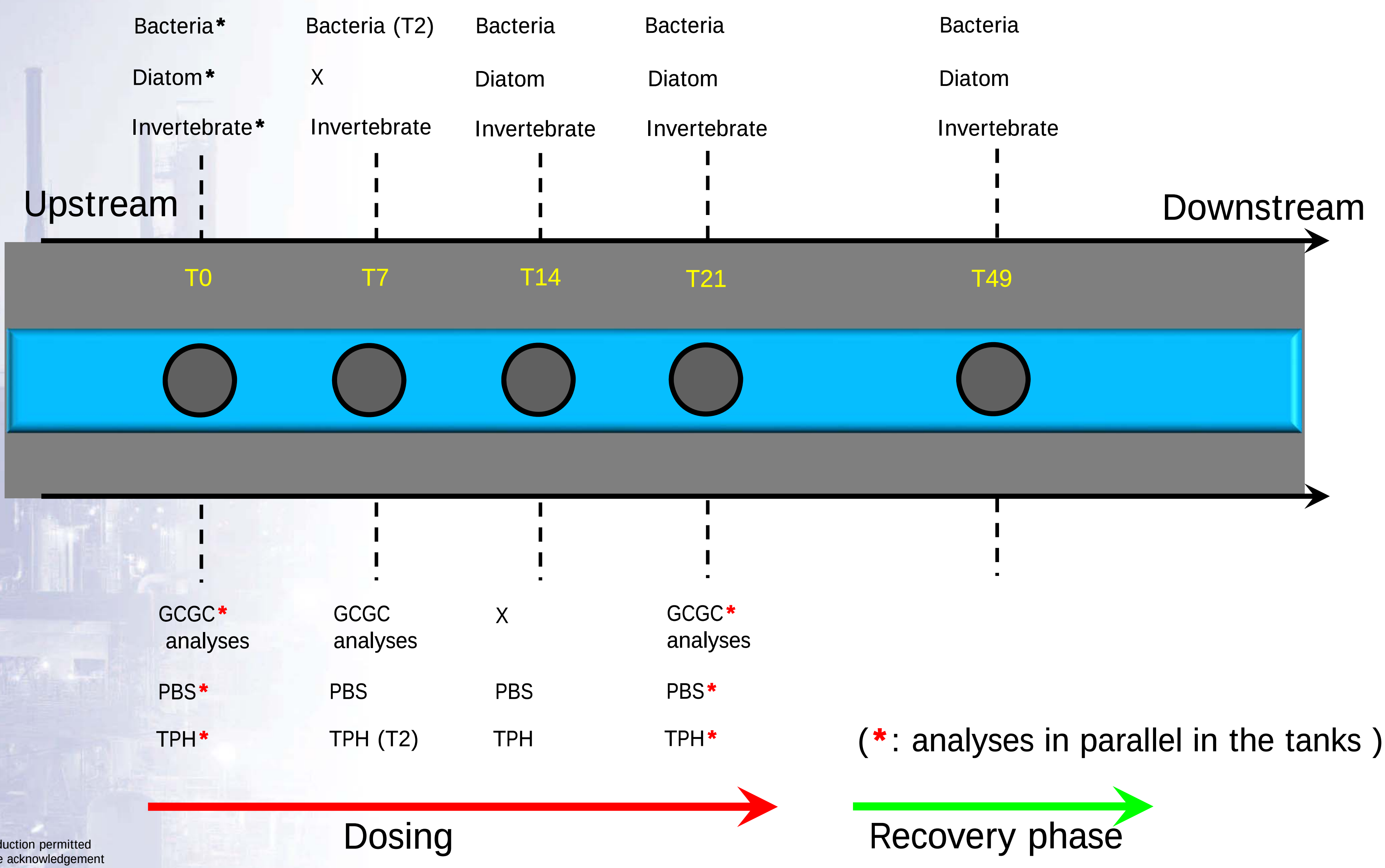


Chemical analysis

TPH, PBS, 2DGC

TPH, PBS, 2DGC

Phase 3: Sampling design



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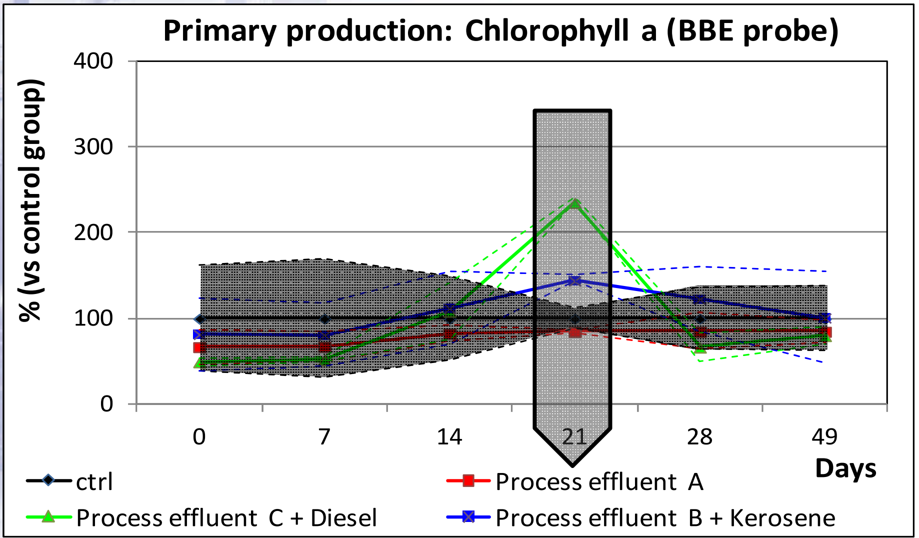
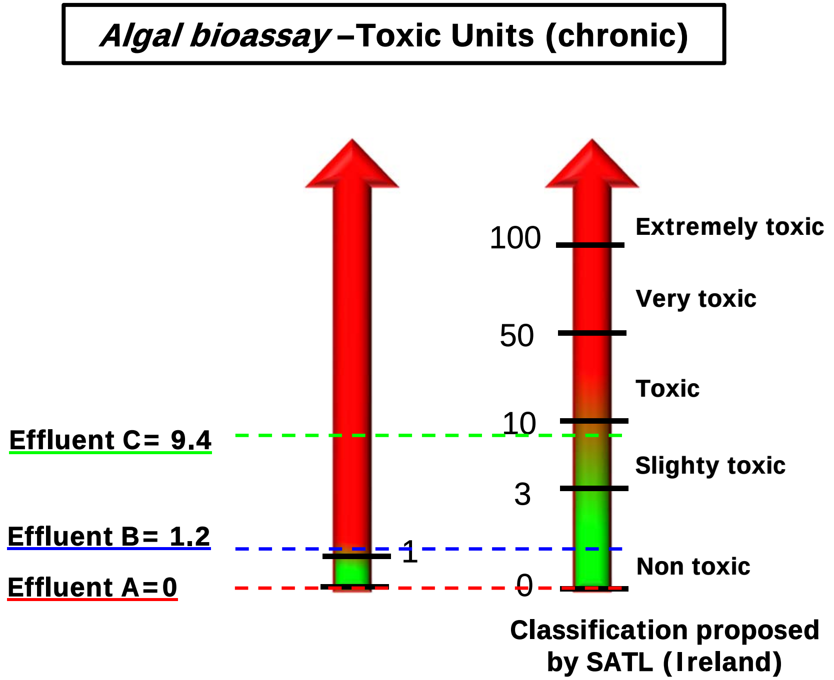
Phase 3: Results- I

Streams result: in-situ mesocosms



Significant chronic toxicity

Lab Bioassays- WEA test



Phase 3 results- II

	Effluent A		Effluent B fortified with Kerosene		Effluent C fortified with Diesel	
	Stream mesocosms	Bioassays	Stream mesocosms	Bioassays	Stream mesocosms	Bioassays
Invertebrate acute effect	0	0	0	0	0	+
Invertebrate chronic effect	0	0	+	+	+	+
Primary production acute effect	0	0	0	0	0	+
Primary production chronic effect	0	0	+	+	+	+
Bacteria	0	+	+	+	+	+

- Examples of good agreement between WEA bioassay and stream mesocosm outcomes
- Examples of where WEA bioassays are conservative in comparison with the outcomes measured in stream mesocosms

Phase III: Conclusions

Streams:

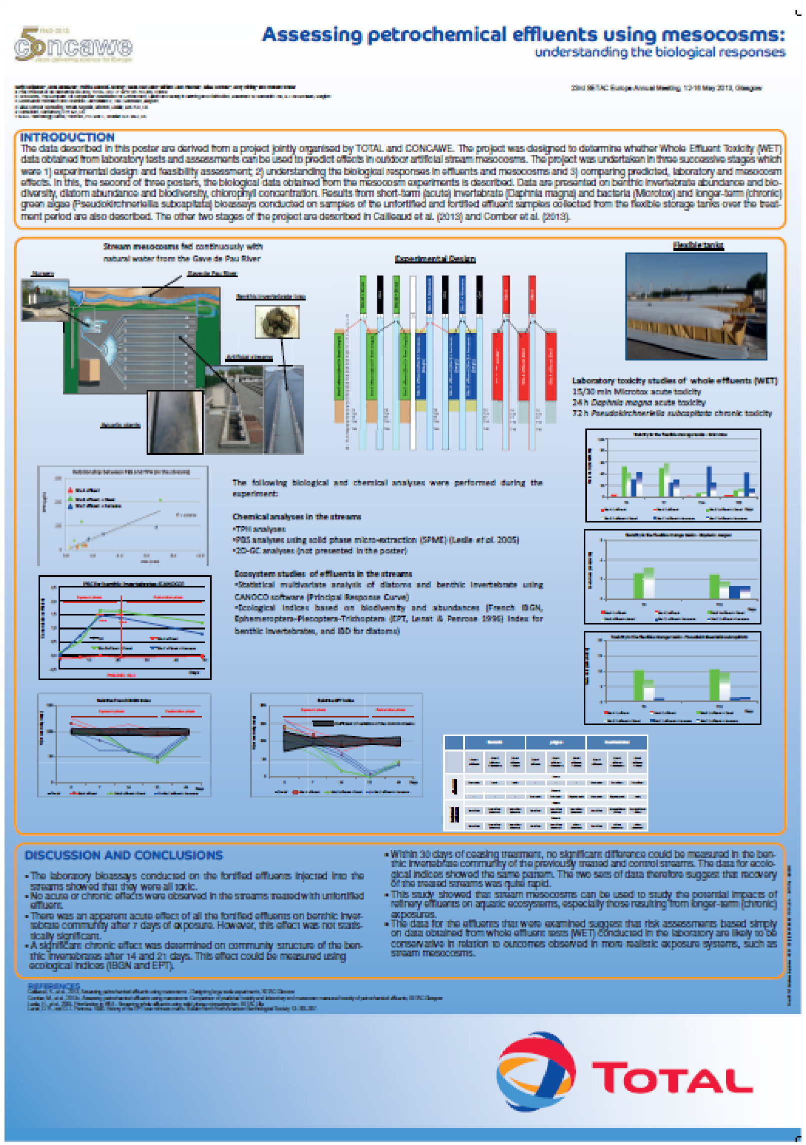
- Unamended refinery effluent (A) had no observed impact on both benthic invertebrate and primary production in stream mesocosms (probably due to dilution)
- Effluents dosed with kerosene (B) and diesel (C) had no short term effect but significant long term effect on both benthic invertebrate and primary production in stream mesocosms.
- The stream communities showed signs of significant recovery (or had completely recovered) within 30 days of ceasing effluent input (and dosing)

WEA bioassays:

- Effluent A exhibited no acute or chronic toxicity in any of the three tests
- Effluent B exhibited chronic toxicity to both crustacean and microalgae but no acute toxicity (except in Microtox)
- Effluent C exhibited both acute and chronic toxicity to crustacean and microalgae.

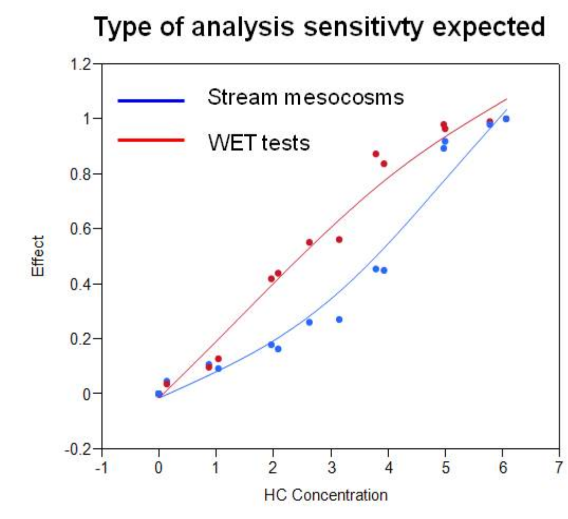
➢ The results *suggest* that biological impact assessments based on data obtained from WEA laboratory bioassays are **likely** to be conservative relative to effects seen in more realistic systems, such as stream mesocosms: **additional data required**

Phase 3: 2013 SETAC Poster



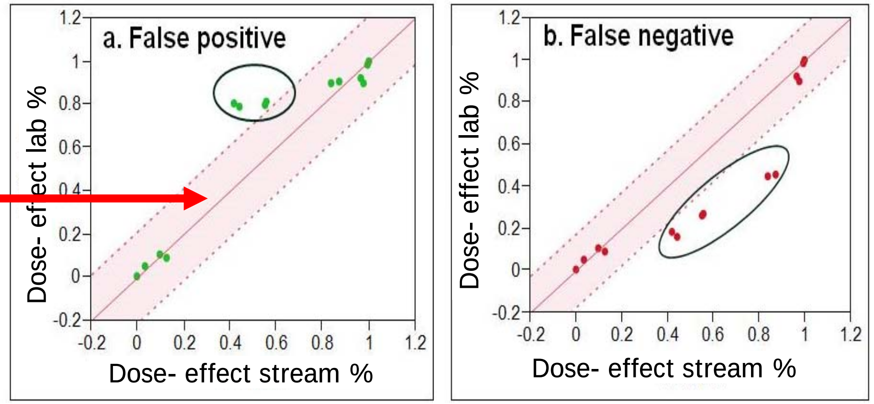
Phase 4: Study objectives

- To provide more dose-response data to support conclusions of phase 3 study i.e. whether results of the WEA bioassays are overly conservative.
- Understand the two types of error that can occur (false positives and false negatives) and their impact when trying to correlate WEA bioassays to the effects observed in the streams
- A dose response experiment conducted in stream mesocosms should help identify false-positive and false negative results when using WEA bioassays



Area of good agreement, =conservative prediction for WEA test

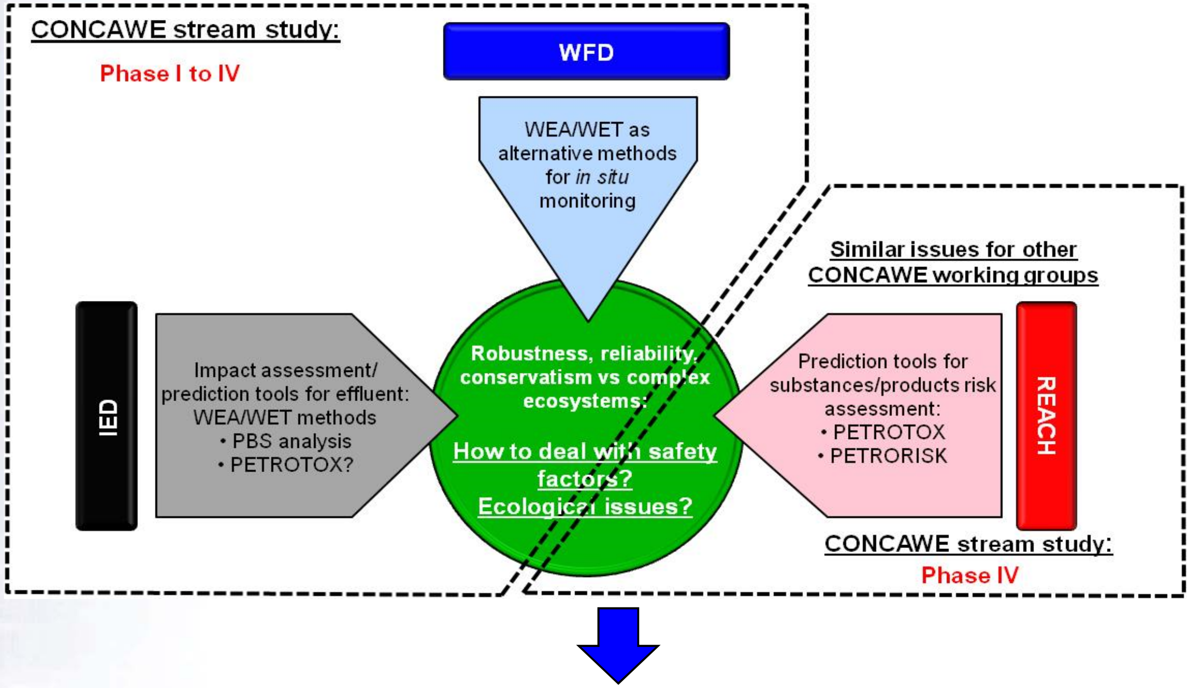
Concordance Analysis



- ➢ Generation of data to support models used in REACH chemical safety assessments

Phase 4: Linking WFD- IED to REACH

- An understanding of effects as a function of effluent composition is required for the results to be used in risk assessment for REACH
- Artificial effluent prepared with single blends (easier to control effluent quality and toxicity)
- Sampling and analysis modified: 2D-GC analysis used to measure effluent composition and confirm contaminant exposure in stream mesocosms



- ➢ Closer working between STF32 and Ecology Group for phase 4
- ➢ Experimental data will be used to validate safety factor used in PETROTOX

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Phase 4: Experimental Design- I

Phase 4: Experimental design- II

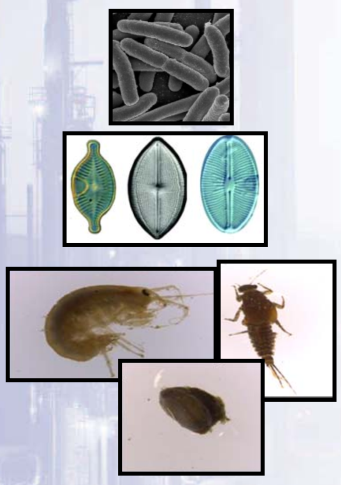
- Artificial effluent: Mixture of 3 single blends (Gasoline (19%), Kerosene (29%), Gasoil (52%))
- Dosing: continuous for 21 days

Phase 4: Bioassays and Chemical Analysis

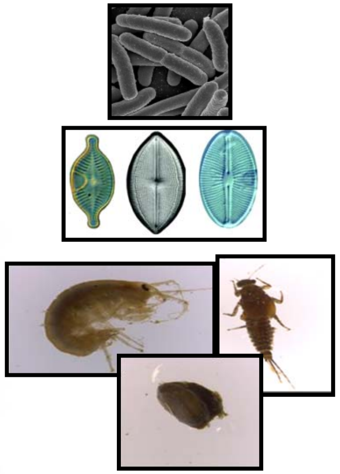
Day -60 | Day 0 | Day 21 | Day 42

Reference → Effluent Dosing (flow through) → Recovery

In the streams



In the streams



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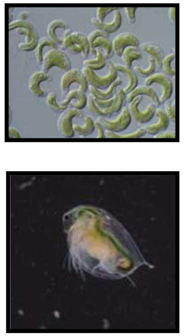
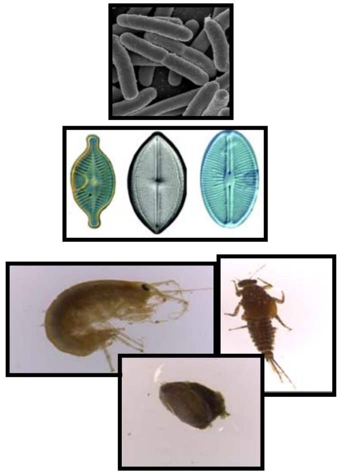
pH, O_2, conductivity

Effluent tested In the laboratory



In the streams

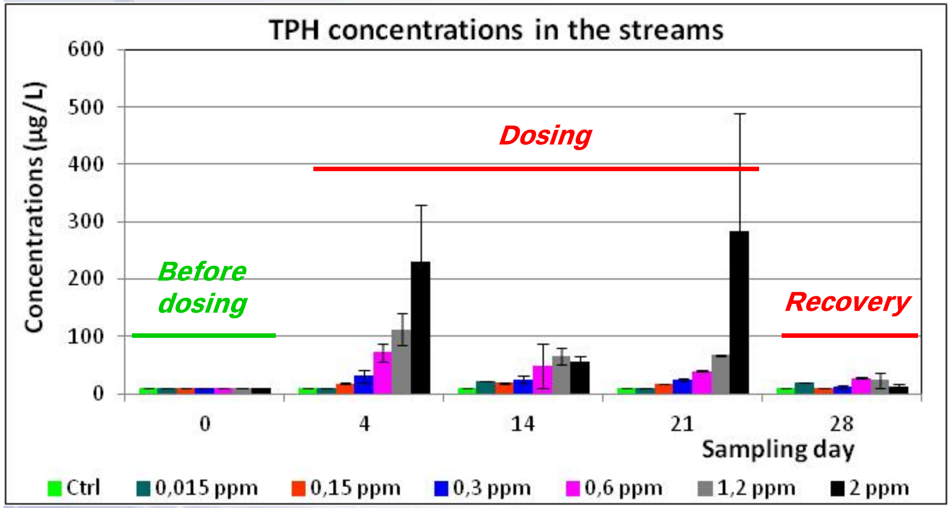


Chemical analysis

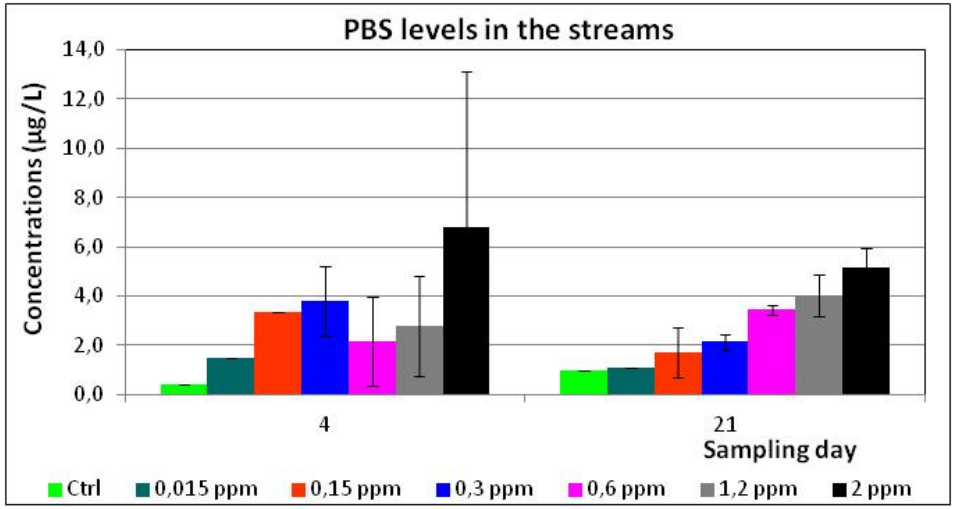
TPH, PBS, 2DGC

TPH, PBS, 2DGC

Phase 4: Preliminary Results- I



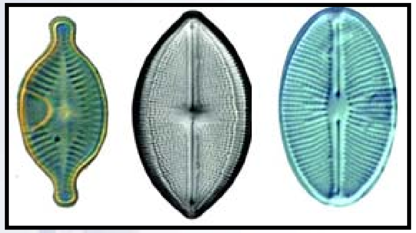
(TPH: Total hydrocarbons)

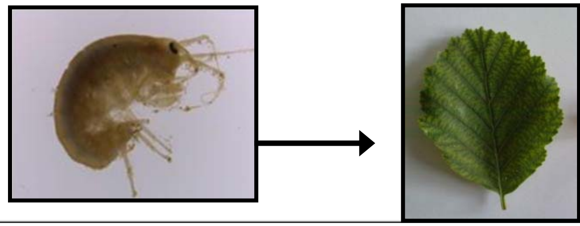


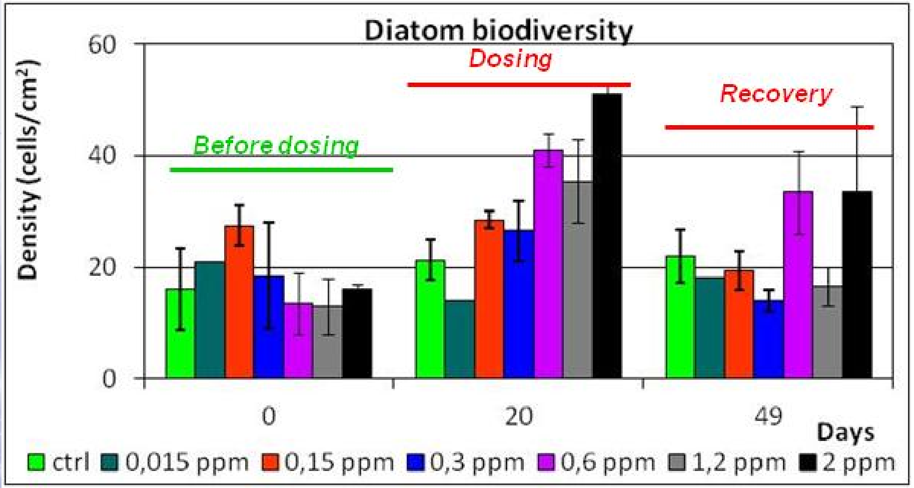
(PBS: potentially bioaccumulable substances)

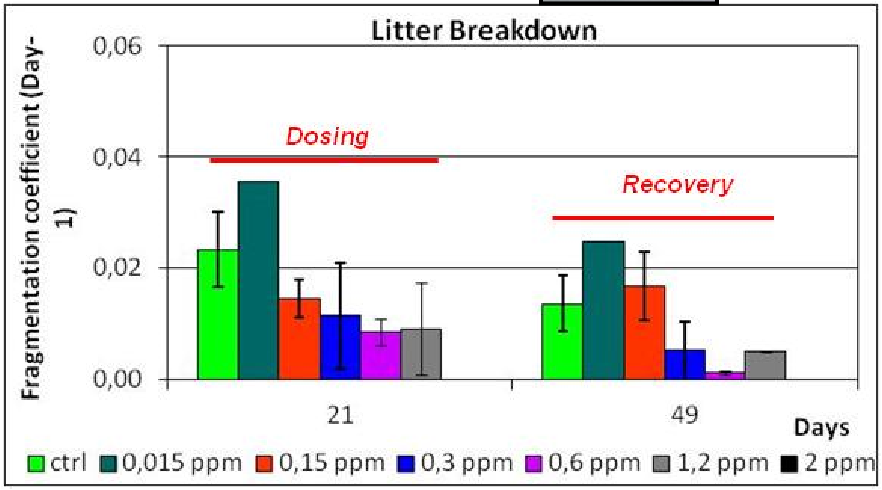
➡ Relatively constant exposure in stream mesocosms

Phase 4: Preliminary Results- II



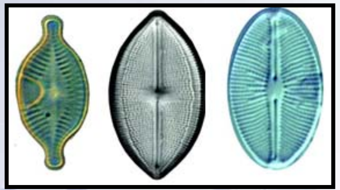


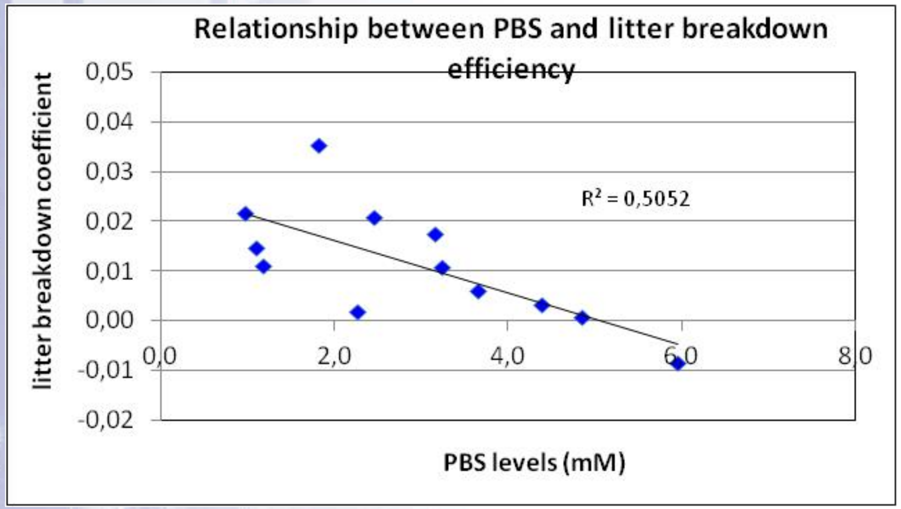


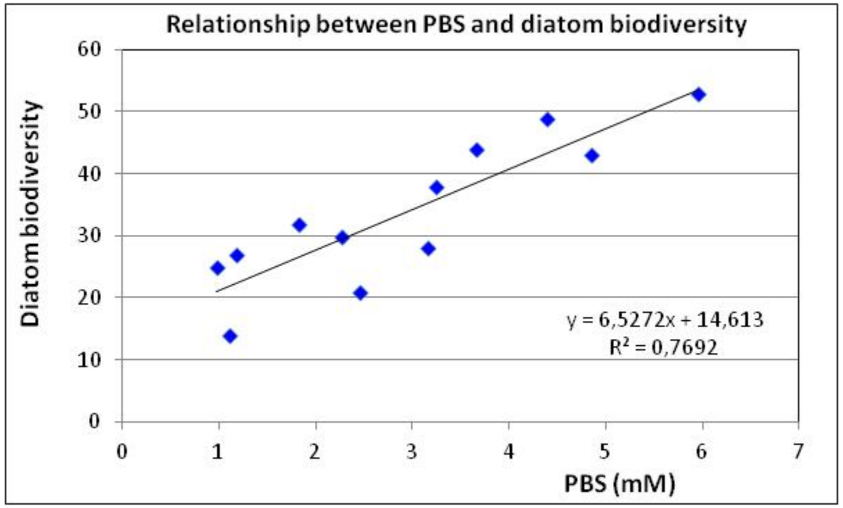


➡ First observation of artificial refinery effluent dose response in stream mesocosms

Phase 4: Preliminary Results- III







- Good relationship between biological effect measured in the stream mesocosms and PBS (potentially bioaccumulative substances)

➢ May provide evidence for validation of the Target Lipid model and the Hydrocarbon block method (work in progress)

Phase 4: Status and Preliminary Conclusions

- Analysis still in progress: expected to be completed end of March/ beginning of April

- Promising preliminary results:
  - Dose response measured in the streams
  - Good relationship between PBS and biological effect measured in the streams
  - Comparison between stream results and bioassays to be performed (April)

- Petrotox simulation still to be performed

Potential Next Steps

- The results from phase IV have provided insight into the toxicity-response for a model refinery effluent derived from a gasoline/ diesel/ kerosene blend
- The next step would be to derive the toxicity-response relationship for a range of effluent composition, using data from the Concawe effluent speciation project.
- Wider conclusions could then be drawn regarding the conservatism of WEA tests and the probability of false negative and false positive results
- The toxicity- response as a function of hydrocarbon block profile could be used to validate the models used for REACH risk assessment for a wider range of substances