Pulsed-dose studies for algae and macrophytes in practice

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Risk Assessment Challenge

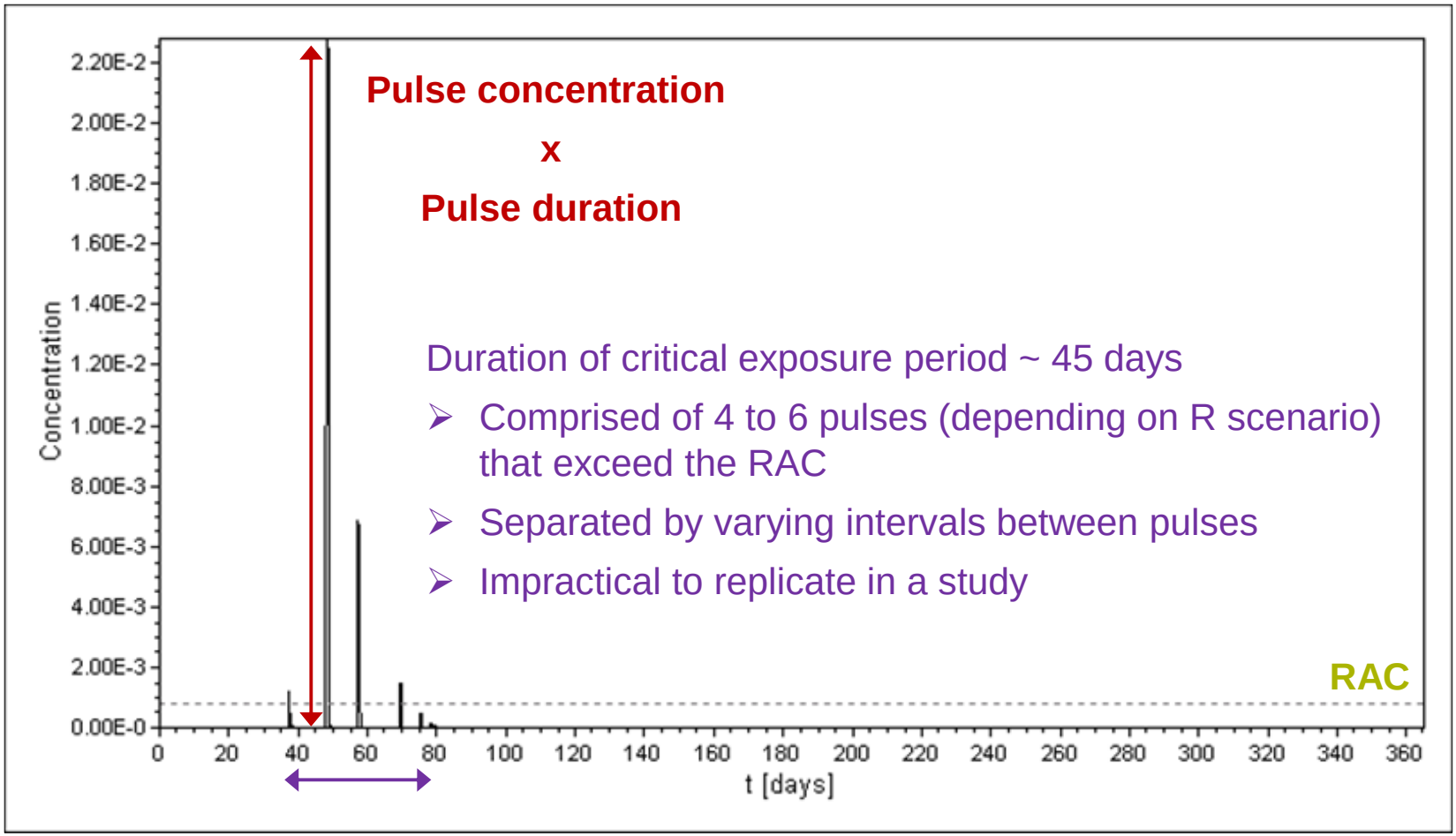
Tier 1 risk assessment

- Maize herbicide
- Highly active against Tier 1 algae and macrophyte species, in standard OECD tests under continuous exposure conditions
  - *Pseudokirchneriella subcapitata* – 72 hours, continuous light
  - *Lemna gibba* – 7 days, continuous light
- Regulatory Acceptable Concentrations (RAC) for both species exceed FOCUS SW Predicted Environmental Concentrations (PEC) from all run-off scenarios (R1 to R4)

Higher-tier risk assessment

- Pulsed-dose studies whereby test species is transferred from treated media to fresh media as required to mimic the FOCUS exposure profile and so, produce a more realistic estimate to toxicity
  - How to translate FOCUS SW exposure profile into a practical test design?
  - Should OECD validity criteria apply to these test designs ?
  - How to use the resulting output in risk assessment ?

FOCUS SW Exposure Profile from Run-off Scenarios



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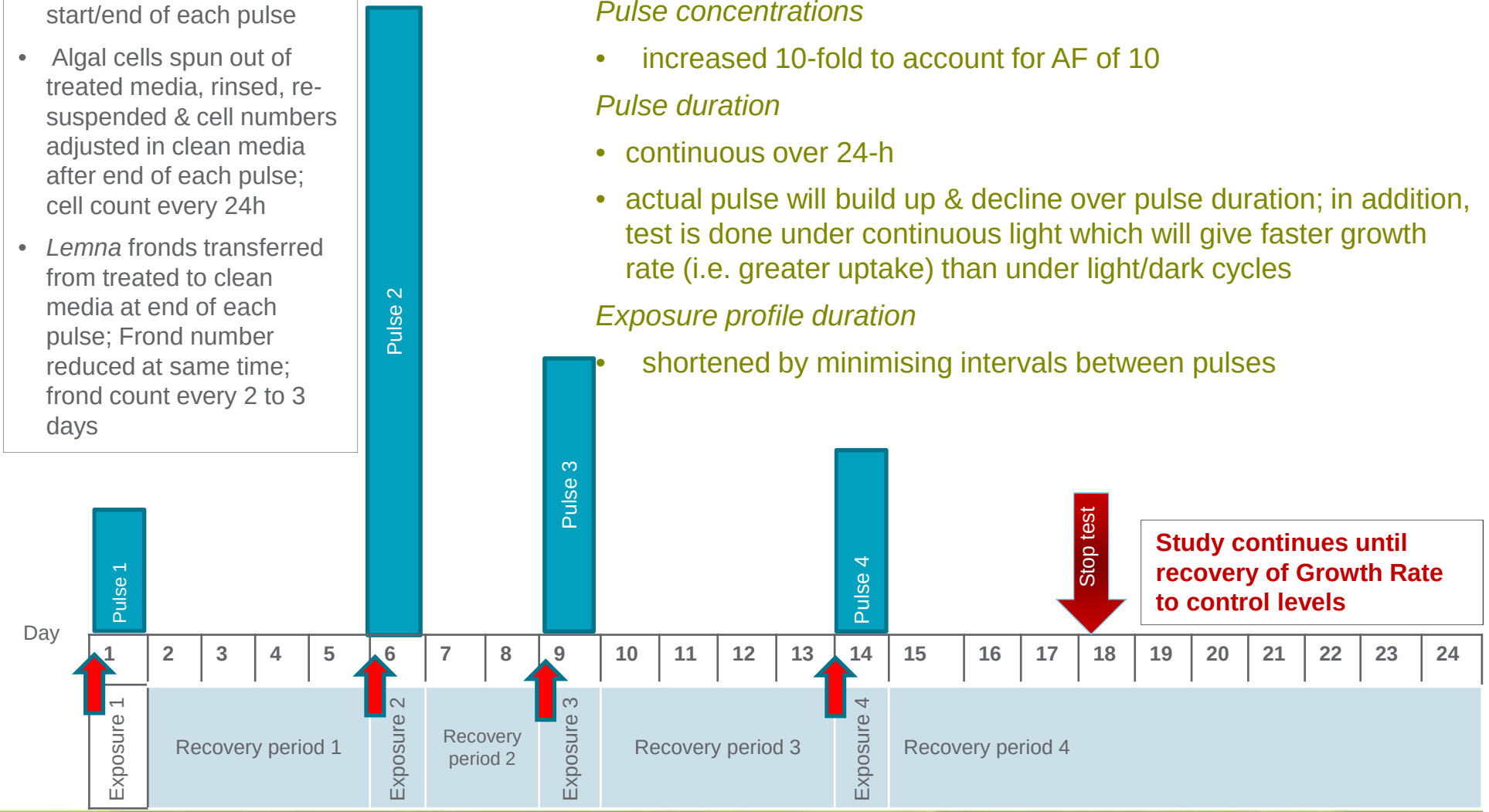
Study Exposure Profile

Method

- Analytical samples taken start/end of each pulse
- Algal cells spun out of treated media, rinsed, re-suspended & cell numbers adjusted in clean media after end of each pulse; cell count every 24h
- *Lemna* fronds transferred from treated to clean media at end of each pulse; Frond number reduced at same time; frond count every 2 to 3 days



How to derive an exposure profile for testing based on worst-case assumptions :

Number of pulses

- consider patterns across all profiles (R1 to R4)

Pulse concentrations

- increased 10-fold to account for AF of 10

Pulse duration

- continuous over 24-h
- actual pulse will build up & decline over pulse duration; in addition, test is done under continuous light which will give faster growth rate (i.e. greater uptake) than under light/dark cycles

Exposure profile duration

- shortened by minimising intervals between pulses

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OECD 201 validity criteria

Method

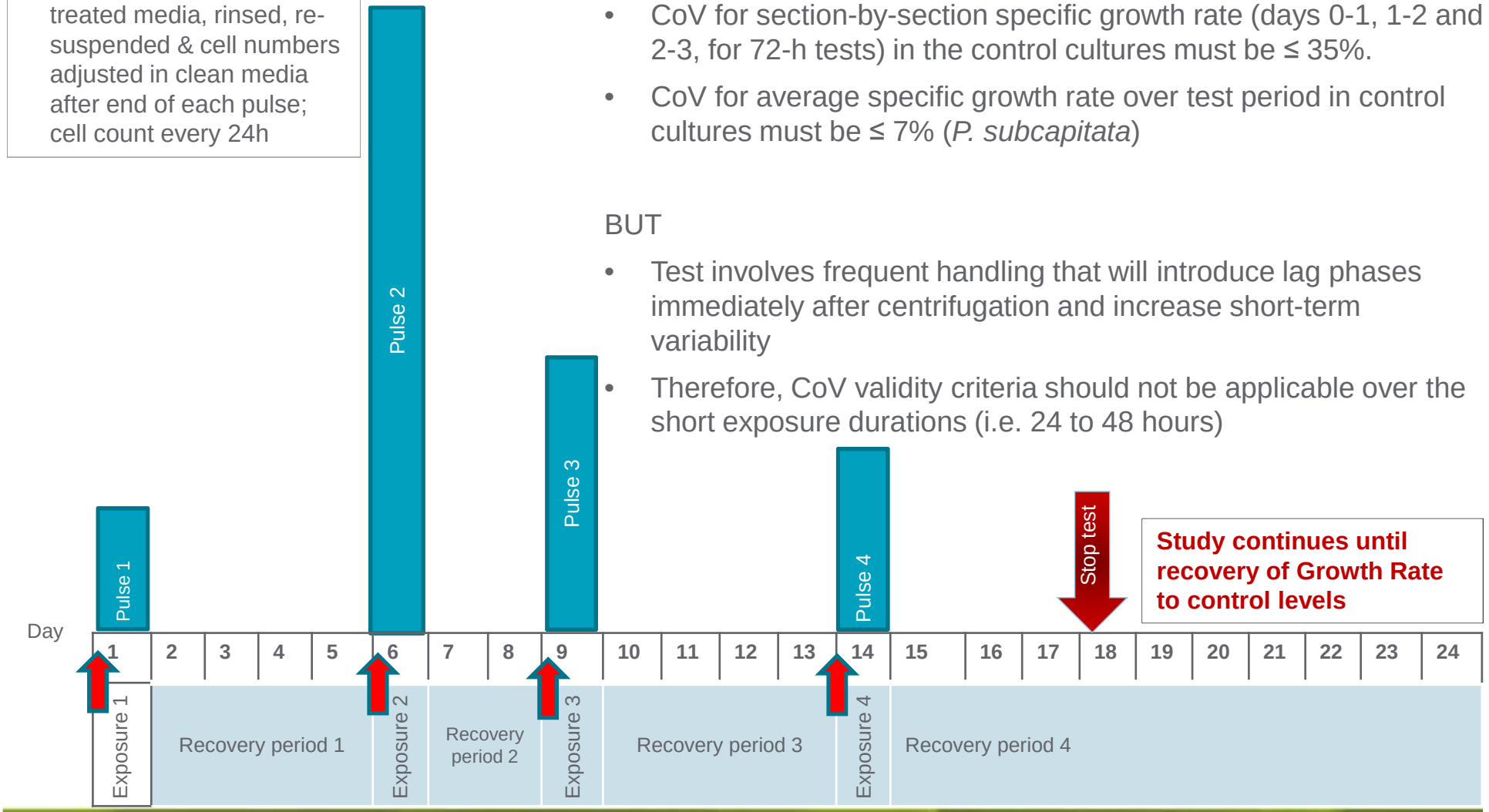
- Algal cells spun out of treated media, rinsed, re-suspended & cell numbers adjusted in clean media after end of each pulse; cell count every 24h

OECD TG 201 validity criteria for a 96-h test with continuous exposure:

- biomass in the control cultures should increase 16-fold
- CoV for section-by-section specific growth rate (days 0-1, 1-2 and 2-3, for 72-h tests) in the control cultures must be ≤ 35%.
- CoV for average specific growth rate over test period in control cultures must be ≤ 7% (*P. subcapitata*)

BUT

- Test involves frequent handling that will introduce lag phases immediately after centrifugation and increase short-term variability
- Therefore, CoV validity criteria should not be applicable over the short exposure durations (i.e. 24 to 48 hours)



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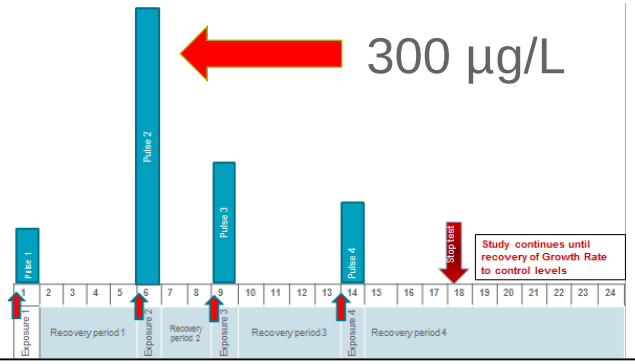
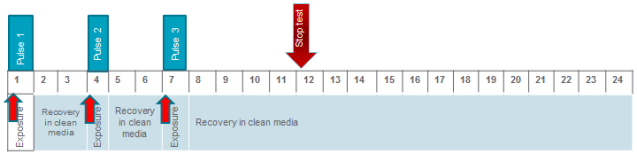
Multiple exposure profiles

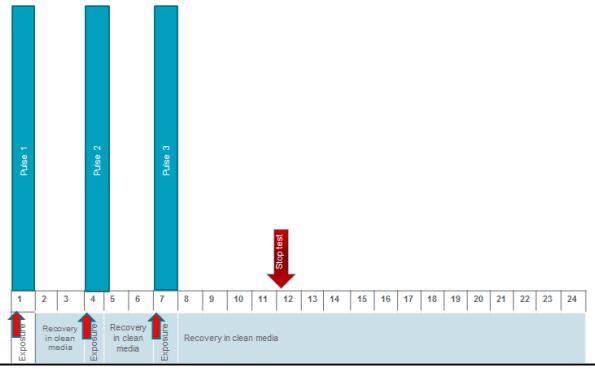
FOCUS R4 (10m buffer+60% RO)



3 pulses at 150 µg/L (2d interval)



3 pulses at 600 µg/L(2d interval)



Output from each exposure profile

- time taken for recovery of growth rate after last pulse
- compare all exposure profiles

➢ No Adverse Effect Exposure Profile

➢ Consider use of ecological modelling to bridge to other profiles

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Points for discussion

1. Is the proposed test design suitable to address the risk assessment issue?

2. OECD validity criteria (i.e. control Coefficients of Variation) should not apply when test design is significantly different from standard test design (eg algal study where frequently handling processes will increase variability)

3. Is the proposed derivation of a “No Adverse Effect Exposure Profile” which incorporates 10-fold AF into pulse concentrations suitable for use in risk assessment?