Deuterium **I**sotope **P**robing **(DIP):**

a new diagnostic tool for the assessment of **bio**degradation and **N**on-**E**xtractable **R**esidues (**NER**) formation in regulatory testing of organic chemicals

Acronym: **DIP-bio-NER**

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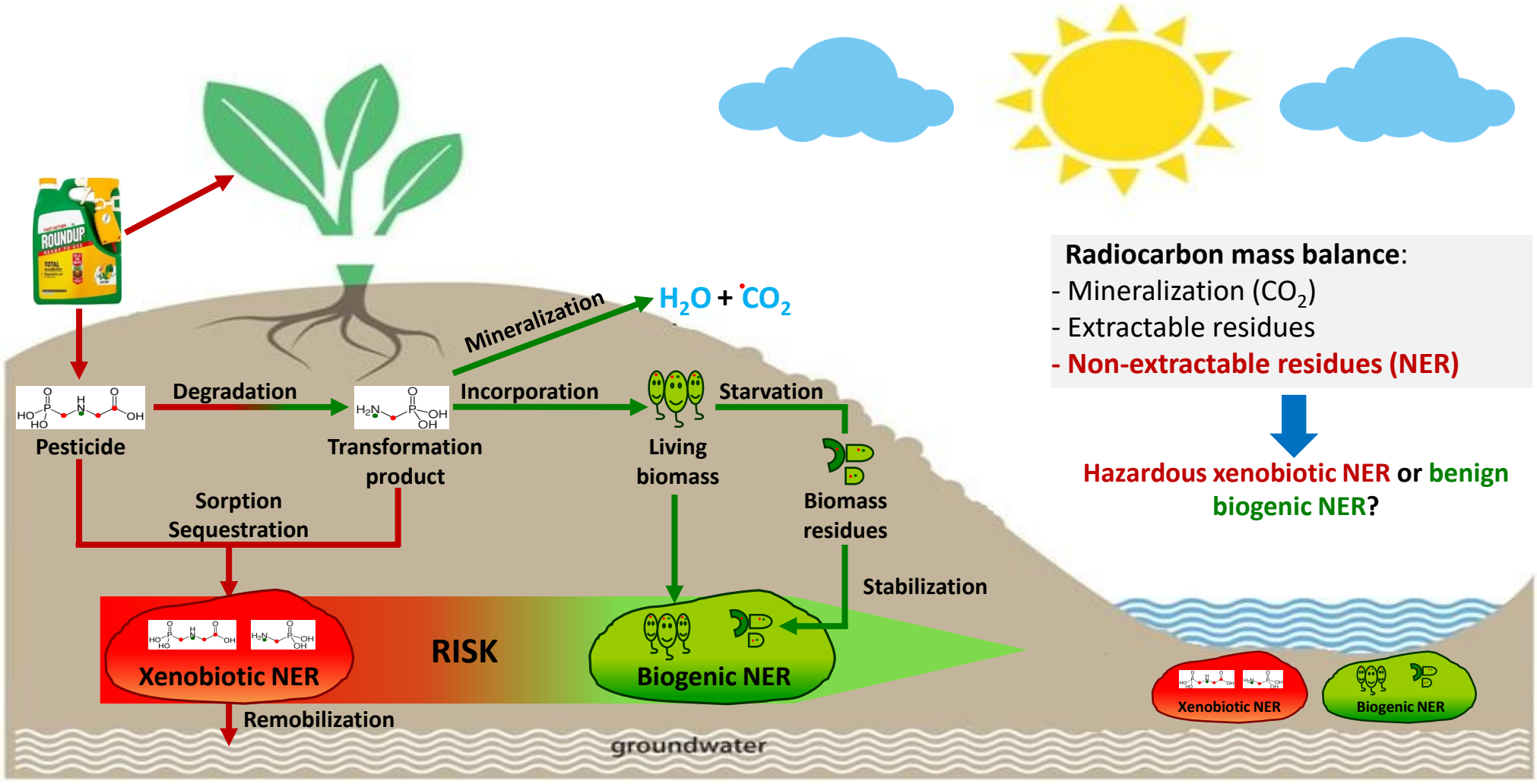
Major hypothesis

Deuterium isotope probing (DIP) is a fast and cost-efficient screening tool to:

- Assess biodegradation in complex environments
- Quantify NER formation
- Differentiate biogenic NER from xenobiotic NER

Fate of pesticides in the environment



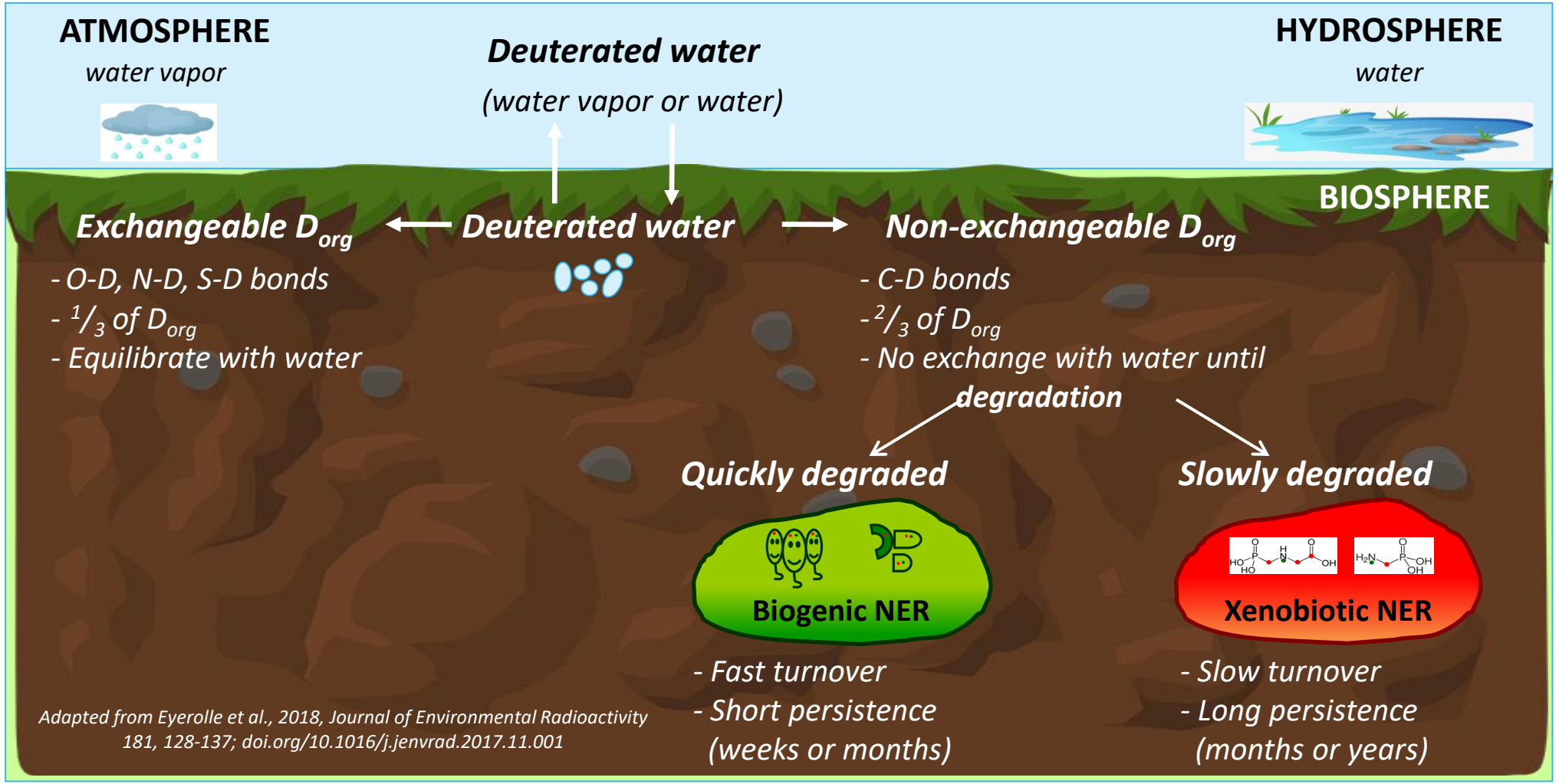
Radiocarbon mass balance:
- Mineralization (CO_2)
- Extractable residues
- **Non-extractable residues (NER)**

Hazardous xenobiotic NER or **benign biogenic NER**?

Deuterium as tracer to assess fate of chemicals

- Low natural abundance (0.015%)
- Cheaper than stable carbon (^{13}C)
- Universal in biomolecules
- C-D bonds generally stable

Total pool of deuterium in the environment

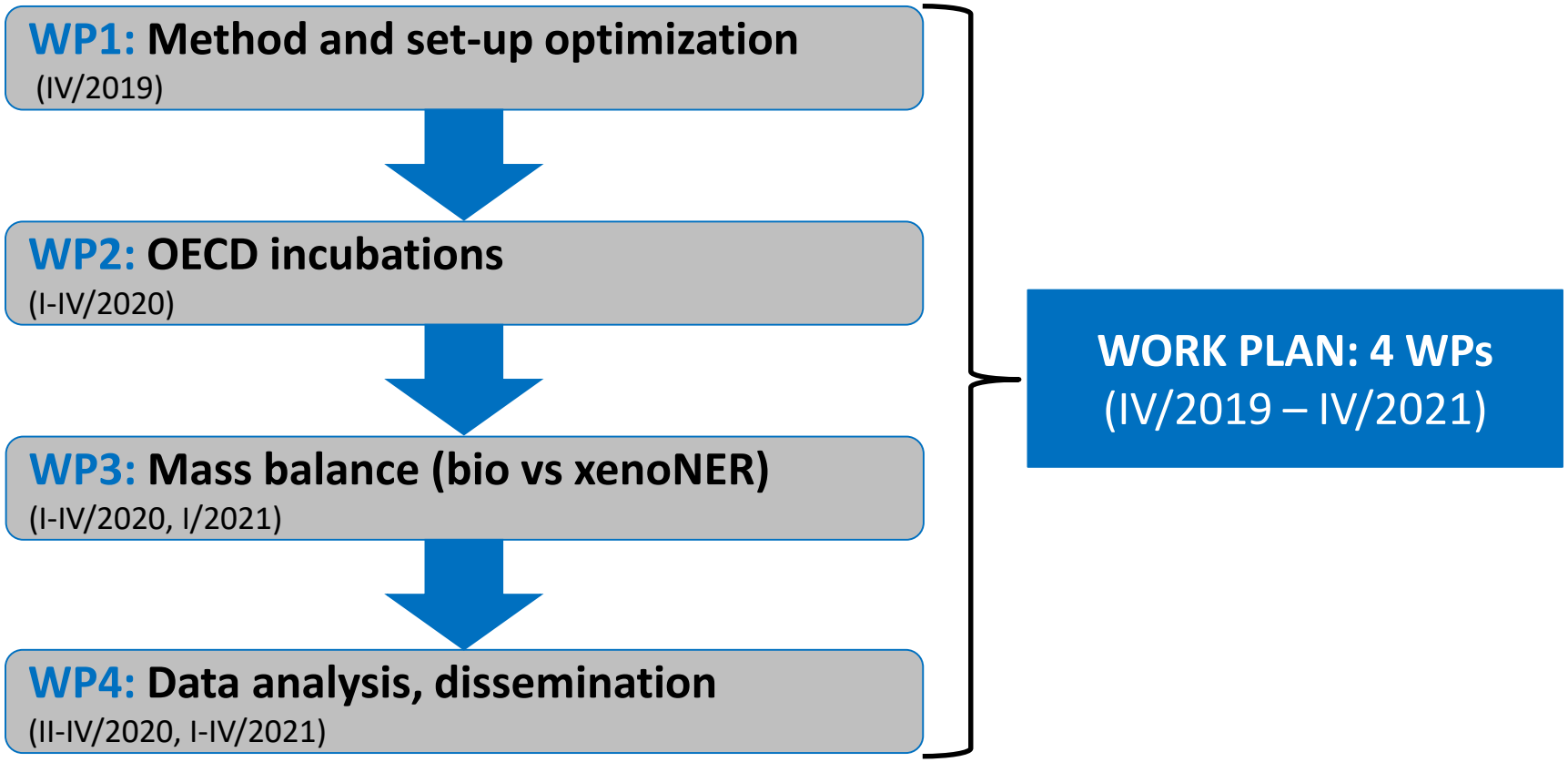


Adapted from Eyerolle et al., 2018, Journal of Environmental Radioactivity 181, 128-137; doi.org/10.1016/j.jenvrad.2017.11.001

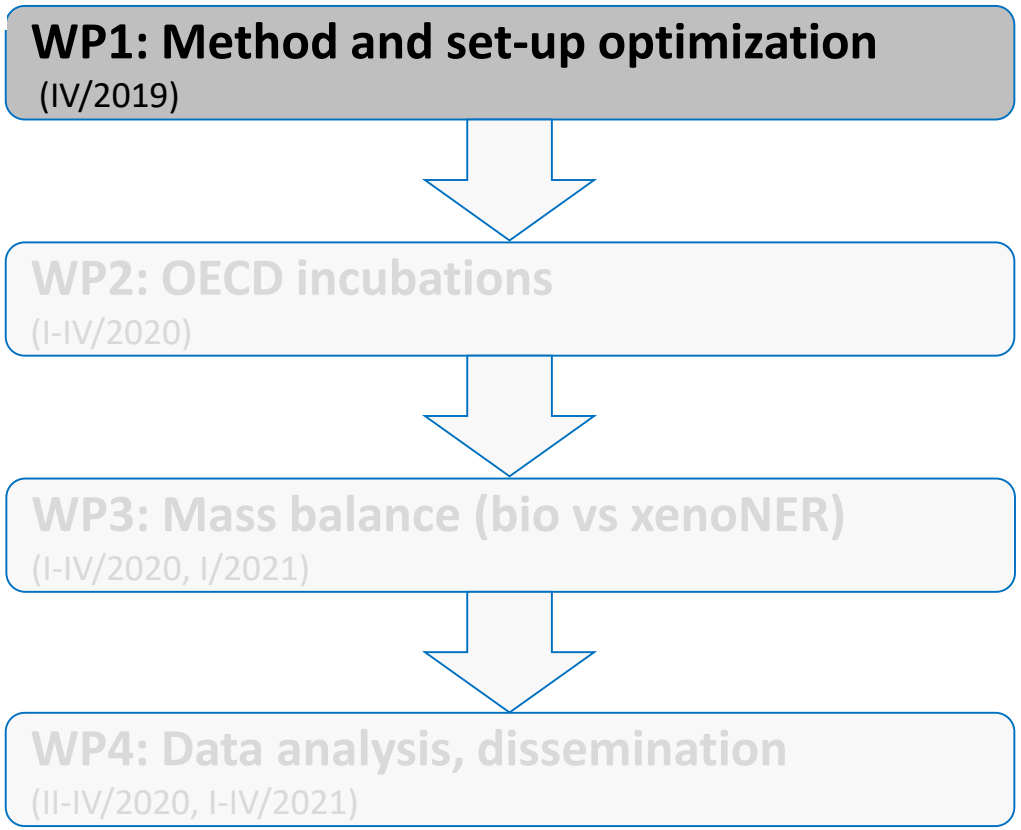
Goals of DIP-bio-NER

- To determine and to compare mass balances of pesticides in soil and water-sediment
  - carbon (^{14}C, ^{13}C), nitrogen (^{15}N) and hydrogen (D, *optimally* ^{3}H)
- To establish a simplified extraction scheme using deuterium
  - bioNER vs xenoNER, stability of xenoNER
- To quantify the incorporation of isotope label into microbial biomass
  - phospolipid fatty acids, *other bioNER (optimally)*

Work plan of DIP-bio-NER



WP1: Method and set-up optimization



Model pesticide

- Stability of C-D bonds?
- Initial concentration?
- Desiccant (D-water)?

WP2: OECD incubations

WP1: Method and set-up optimization
(IV/2019)

WP2: OECD incubations
(I-IV/2020)

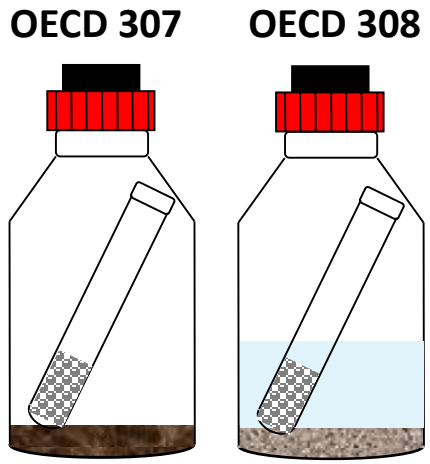
WP3: Mass balance (bio vs xenoNER)
(I-IV/2020, I/2021)

WP4: Data analysis, dissemination
(II-IV/2020, I-IV/2021)

Degradation kinetics (^{14}C, ^{13}C, ^{15}N and D)

Model pesticides

- 2,4-D
- Glyphosate
- Bentazone



Mineralization
Deuterated water
$^{14}CO_2$, $^{13}CO_2$

WP3: Mass balance and NER speciation

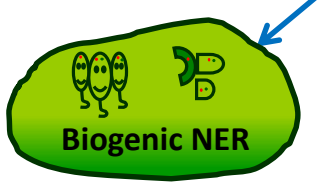
WP1: Method and set-up optimization
(IV/2019)

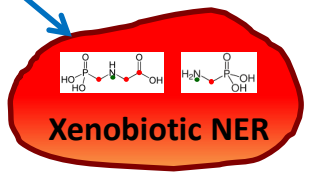
WP2: OECD incubations
(I-IV/2020)

WP3: Mass balance (bio vs xenoNER)
(I-IV/2020, I/2021)

WP4: Data analysis, dissemination
(II-IV/2020, I-IV/2021)

Step 1: 'Screening' extraction
Cleavage of C-D bonds?
General mass balance





Step 2a:
Bligh-Dyer extraction
Phospholipid fatty acids

Step 2b:
Remobilization tests
Climatic conditions
Agricultural practices

Step 3 (optimally):
Other bioNER

Step 4 (optimally):
Tritium (^3H): biodegradation, bioNER vs xenoNER

Benefits of DIP-bio-NER for science and society

- Cheap and rapid test → biodegradation + bioNER
- More tests with less effort → models
- Labeling of a molecule at a specific position → cheaper synthesis
- Tritium in addition to ^{14}C → bioNER vs xenoNER

Less long-lived ^{14}C waste

Lower radiation exposure

Thank you!

Cefic-LRI Programme

European Chemical Industry Council - Cefic aisbl

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