Persistence Assessment of Azo-Dyes Based on Metabolomics Approach

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Regulatory Background

REACH Regulation (EC) No 1907/2006

Annex VII

Information requirement: Screening biodegradation tests: OECD 301, OECD 302

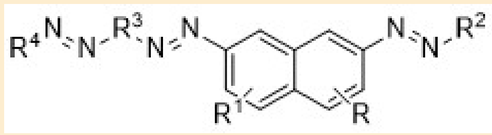
Annex VIII

Information requirement: Screening biodegradation results indicate potential persistence of substances ⟺ Trigger for higher-tier studies required under Annex IX

Annex IX

Information requirement: Simulation testing on ultimate degradation in surface water (OECD 309)

Azo – dyes; Technical challenges



Organic dyes properties: high molecular weight; structural complexity; variable compositions (mono-constituent substances, or multi-constituent, or UVCBs)

OECD 309 limitations

Radiolabelling:
- need to radiolabel all molecular position sensible to cleavage of each substance constituent;
- economically prohibitive;
- technical issues for multi-constituent and UVCB substances

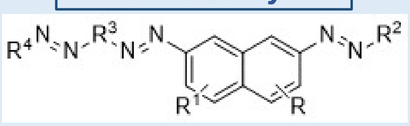
Standard analytical methods:
- targeted approaches: able to identify transformation products already chemically characterised

! *Need of alternative solution*

e-FATE METABOLOMICS FOOTPRINT (EMF): materials and methods

Project scope: investigation of biodegradation pathway in natural surface water amended with sediments of parent compound and transformation products, including their quantification and identification. The standard OECD 309 and EMF study were conducted in parallel in natural surface water and the kinetic results were then compared to evaluate the validity of the Metabolomics approach.

Case study



OECD 309:

Kinetic study with the determination of DT_{50} and DT_{90} of parent compound.

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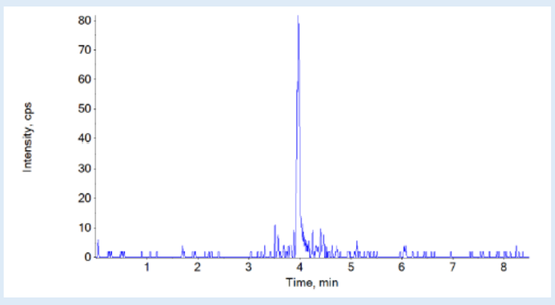
Analytical monitoring of parent compound concentration by LC-MS/MS

EMF adapted to OECD 309

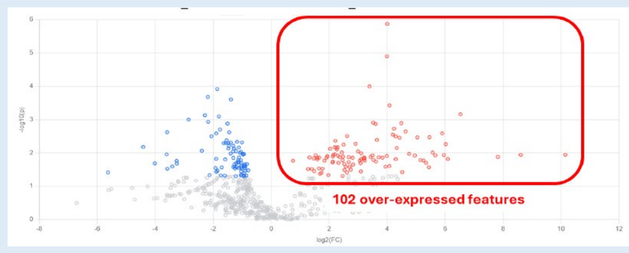
- Kinetic study: determination of DT50 and DT90 of parent compound;
- Monitoring and identification of transformation products: statistical interpretation of analytical chromatograms detected by UHPLC-HRMS
- Quantification of transformation products: determination of relative concentration based on standard curves of test substance.



LC-MS/MS analysis: biodegradation behaviour of parent compound

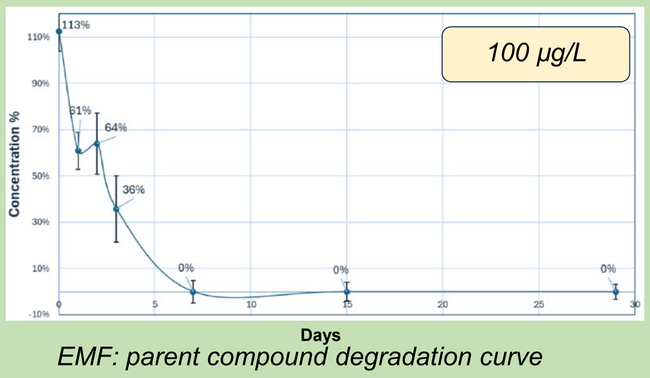
VERSUS

Statistical analysis: monitoring of biodegradation behaviour of parent compound and transformation products (EMF adapted to OECD 309)

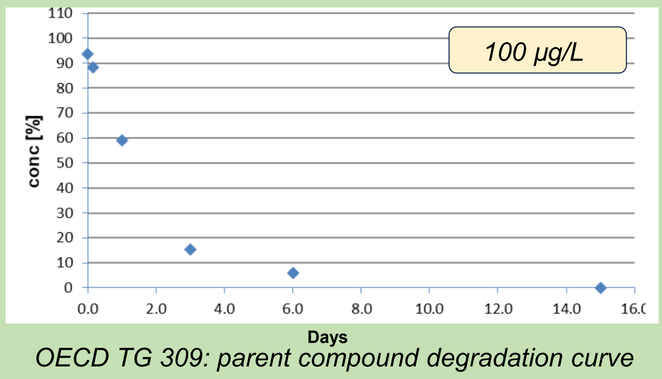


Results and discussions

Parent compound: biodegradation kinetics



EMF: parent compound degradation curve



OECD TG 309: parent compound degradation curve

Test method	*Test period (days)*	*DT50*	*DT90*
OECD TG 309	15	1.36	4.5
EMF	29	2.30	4.06

EMF vs OECD TG 309 → Comparable ***biodegradation kinetics*** → 100% biodegradation of parent compound

Validity of EMF approach as alternative solution

Transformation products

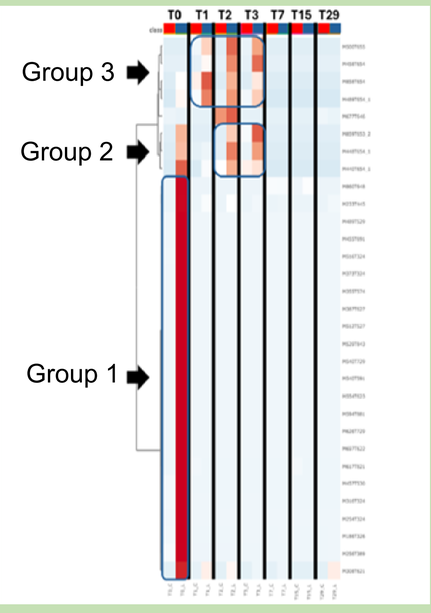
Degradation pathways of transformation products

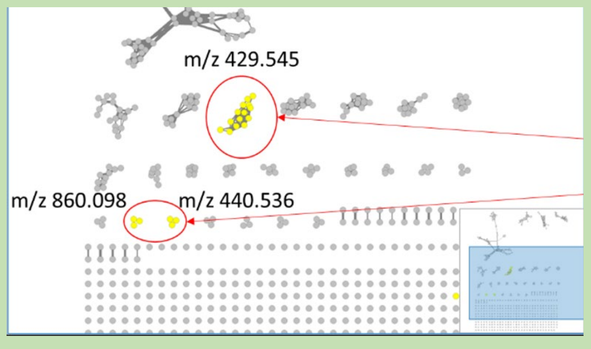
Mass signals associated to transformation products are grouped in the Heatmap based on similarity between their degradation pathways.

Group 1 → ○ Appereance day 0 ○ Disappereance day 0 *parent compound*

Group 2 → ○ Appereance day 2 ○ Disappereance day 3 *transformation products*

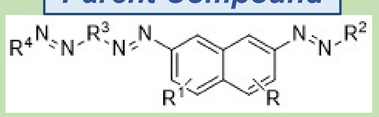
Group 3 → ○ Appereance day 1 ○ Disappereance day 3 *transformation products*



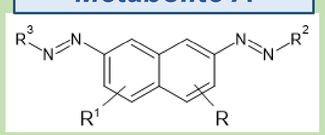


Development of molecular network:
identification of molecular clusters based on mass spectra similarity of transformation products.

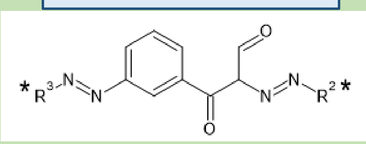
Parent Compound



Metabolite A



Metabolite B



**: transformated groups*

Conclusions: OECD 309 vs EMF

The EMF is a novel approach designed to investigate the fate of complex substances in environmental compartments, thereby enabling its application across diverse regulatory frameworks, including biocides and plant protection products.

EMF approach: key achievements

- Biodegradation behaviour of parent compound consistent with OECD 309
- Monitoring of kinetic pathways of transformation products
- Identification and quantification of transformation products

EMF: robust alternative for persistence assessment of organic dyes