BioSINC project: Integrating Experimental and *In Silico* Approaches to Assess the Biodegradability of Complex Natural Substances – The Case of Essential Oils

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Background & regulatory context

The revision of the CLP regulation 2024/2865, part of the EU Green Deal's Chemical Strategy for Sustainability (CSS), aims to ensure a toxic-free environment by updating classification rules for substances containing more than one constituent, such as Natural Complex Substances. This rule implies assessing substances with more than one constituent on the basis of their constituents for certain health hazards or properties such as biodegradation, persistence, mobility and bioaccumulation within environmental hazard classes (aquatic toxicity, PBT, vPvB, PMT, vPvM).

Article 5 paragraph 6 ensures this new rule does not apply to substances containing more than one constituent which are extracted from plants or plant parts that are not chemically modified. EU Parliament and Council have agreed on a specific derogation for plant extracts, including essential oils, with a review of the scientific evidence by the Commission in December 2029.

Natural Complex Substances (NCS), including essential oils (EOs), typically contain a large and variable number of constituents, and in some cases a high concentration of unknown constituents.

In addition, standardized OECD tests for biodegradation are difficult to interpret for multi-constituent substances and especially NCS. Individual constituent assessment may not reflect real-world mixture behavior (co-metabolism, cocktail effects).

In the absence of biodegradation information on constituents, from a regulatory perspective, a substance will be considered non-biodegradable.

In the current regulatory context, it is important to understand if there is a scientific basis for assessing the hazards of essential oils (EOs) on the basis of data on its constituents rather than on the basis of data on the substance itself. The outcome will be used to confirm the scientific basis for the CLP derogation before end of 2029.

Scientific hypothesis & objectives

Core hypothesis: Constituents within an essential oil undergo a degradation process different from that observed when tested in isolation — notably through co-metabolism.

Primary objective: Investigate, explain and predict biodegradation mechanisms of EOs to confirm the scientific basis for the CLP derogation.

Secondary objective: Develop an *in silico* predictive model for the biodegradability of multi-constituent mixtures.

Methodology

This project is based on 3 lines of research which are carried out in parallel

Axis 1 — GLP experimental testing (CRO)

Modified ready biodegradability tests (OECD 301F) performed under Good Laboratory Practice on:

5 single constituents | **5 artificial mixtures** | **5 essential oils**

A serie of modified studies of biodegradation will be carried out on isolated constituents, artificial mixtures and essential oils containing constituents considered to be partially or not biodegradable.

Steps to be followed :

1. Choice of 5 partially/not biodegradable constituents present in 5 essential oils produced in France
2. Performing enhanced OECD301F on the partially/not biodegradable constituent in order to confirm the biodegradability status
3. For constituents confirmed to be partially/not biodegradable performing enhanced OECD301F on an mixture of the partially/not biodegradable constituent » and a biodegradable constituent as well as on the essential oil
4. Genomic and transcriptomic analysis to explore the potential for co-metabolism between two or more constituents, i.e. the capacity of the microorganisms present in the community to produce enzymes that allow the metabolization of recalcitrant substances in the presence of more rapidly biodegradable substances and to facilitate their degradation.
5. Analytical confirmation of constituent concentrations throughout the test.

Axis 2 — Academic laboratory (Nantes Université / GEPEA)

On the same constituents/mixtures and essential oils :

- Investigate the experimental conditions, in particular the choice of inoculum, enabling the best possible biodegradation of complex substances during an OECD 301F test
- Characterize the genetic and functional microbial diversity of the inoculums to determine the initial enzymatic potential for biodegradation of oils and constituents.

Optimised experimental strategy :

Toxicity screen (MINITOX / OECD 209)

Biodegradation (TOBIE / OECD 301F)

Key innovation: patented automated platforms (MINITOX, TOBIE — 48 parallel tests) enabling biodegradability screening adapted for poorly water-soluble substances

Multi-inoculum screening

Inoculum characterisation: cell density, genetic diversity, functional diversity, and phenotypic profiling

For mono-constituents

Carbon balance (UTOC : Ultimately Transformed Organic Carbon)

Novel carbon mass balance approach distinguishing mineralisation, biomass incorporation and residual carbon — applicable to constituents not reaching 60% pass level.

For NCS

specific chemical analyses

To determine the fate of the individual components present

Axis 3 — *In silico* modelling

Predict the overall biodegradability potential of an essential oil using an *in silico* model based on a machine learning approach.

Predictions to be compared with the experimental results from the previous phases and with relevant data recovered from various sources for other mixtures.

1. QSAR / ASRIT model

Predict microbial toxicity (activated sludge respiration inhibition, OECD 209) to limit number of *in vitro* toxicity tests required in Axes 1 and 2.

2. Multi-constituent biodegradability model

Built on a mono-constituent base model capable of differentiating: readily biodegradable (10-day window); readily biodegradable (no window); inherently biodegradable; potentially persistent.

3. Validation & calibration

Model predictions compared to experimental results from Axes 1 & 2 for artificial mixtures and EOs, as well as for substances that are not readily biodegradable but are not persistent (i.e., substances falling into the categories of "readily biodegradable without the 10-day window" and ">20–60% biodegradable within 60 days") ; recalibration where needed to capture co-metabolic effects.

Methodology overview

Three parallel, complementary approaches

GLP / CRO
Regulatory-grade data

Academic
Mechanistic insight

In silico
Prediction & scaling

All studies run in parallel across axes to allow iterative cross-validation between experimental and predicted data.

Essential oils and constituents to be studied (French production)

Essential oil	Constituent with biodegradable status to be confirmed	Readily biodegradable constituent
Pepper mint oil	Mentyl acetate	L-menthol
Lavander oil	Beta-farnesene	Beta-myrcene or linalool
Clary sage oil	Germacrene D	Sclareol
Pine scotch oil	Camphene	Alpha-pinene
Cade oil	Delta-cadinene	Beta-caryophyllene

Expected outcomes

- ❑ Robust experimental dataset on biodegradability of essential oils (EOs) and their constituents, generated under optimized and GLP conditions
- ❑ Mechanistic evidence of co-metabolism between constituents in complex natural substances
- ❑ Validated *in silico* model capable of predicting biodegradability of multi-constituent substances and natural complex substances for regulatory use
- ❑ Scientific support for the CLP derogation allowing substance-level assessment of plant extracts
- ❑ Sector relevance: results will support fair and representative classification of essential oils under CLP, with direct implications for growers, distillers and downstream users across Europe.

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