Benign by Design: Advancements and Challenges in Developing Readily Biodegradable Pharmaceuticals

Public symposium - Espoo, Finland

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Funded by the European Union

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Environmental biodegradability

Aerobic biodegradability of aromatic molecules

Environmental biodegradability

Definitions

- Metabolic degradation of organic molecules by microorganisms
  - Primary elimination or mineralization to inorganic compounds i.e. CO_2, H_2O, Cl^- etc.
  - Assimilation of organic molecules to build molecules using energy (Anabolism)
  - Degradation to smaller molecules for energy release (Dissimilation)
- Biodegradation pathway can be similar to human metabolism but can also be very different
  - pH, redox potential, O_2 levels (anaerobic or aerobic), microorganisms, time scale, abiotic degradation i.e. photolysis and hydrolysis

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Environmental biodegradability

Assessment of aerobic biodegradability in fresh waters

- Ready biodegradability (OECD 301, 310), inherent biodegradability (OECD 302), simulation tests (OECD 303, 309, 314)
  - All 301 tests are equal however the test outcome can vary highly within these tests
  - Not every API can be assessed with every test due to volatility, solubility, foaming
  - Surface water, activated sludge, secondary effluent are used as inoculum
  - Biodegradability calculated by sum parameters O_2 levels, DOC, CO_2, $^{14}CO_2$ or $^{3}H_2O$

Figure 1: Closed Bottle Test (301D), Manometric Respiratory Test (301F), Zahn-Wellens-Test (302B).

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Environmental biodegradation of APIs

Required stability vs. biodegradability

- Stability of APIs targeted during drug development
- Environmental biodegradability does not imply low stability in humans
  - Environmental biodegradation not specifically targeted during drug development
  - Pseudo-persistence still possible for highly consumed APIs e.g., ibuprofen

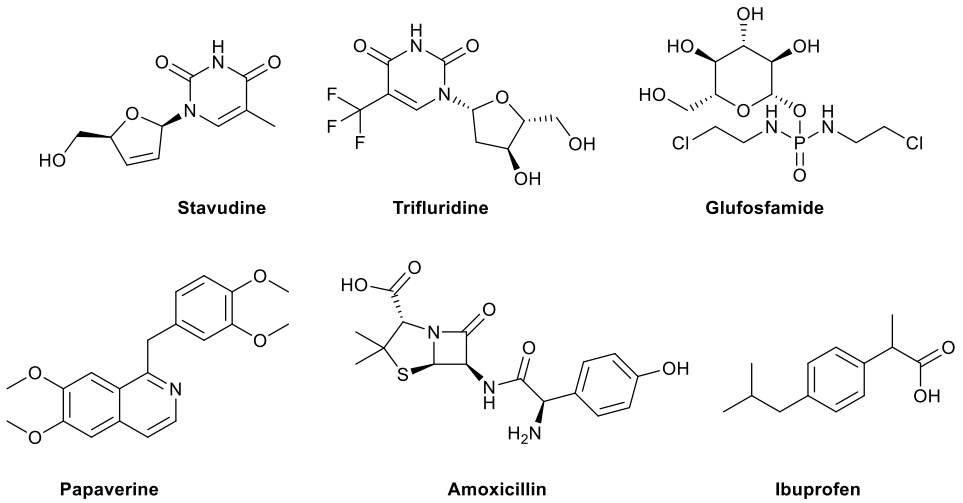

Figure 2: Examples of environmentally biodegradable APIs.

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Environmental biodegradation of APIs

Contribution of *in silico* tools in the design of biodegradable APIs

- Most *in silico* models for environmental biodegradability are trained on industrial and fine chemicals (MITI data set), but not APIs
- Data quantity, quality, comparability challenging
  - Biodegradation data not required for approval of APIs
  - Balance of biodegradable and non-biodegradable data difficult
  - Biodegradability data often not comparable even within the same test series i.e. OECD 301 e.g., Ibuprofen is persistent in 301D while readily biodegradable in 301F
  - Minor modifications greatly impact biodegradability i.e. isomers, bioisosteres, stereochemistry

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Environmental biodegradation of APIs

Impact of common bioisosteres and isomers on biodegradability

- Bioisosteres are often not tolerated either due to different electronic parameters or interference with biodegradation pathway
- Small modifications can also greatly impact biodegradability

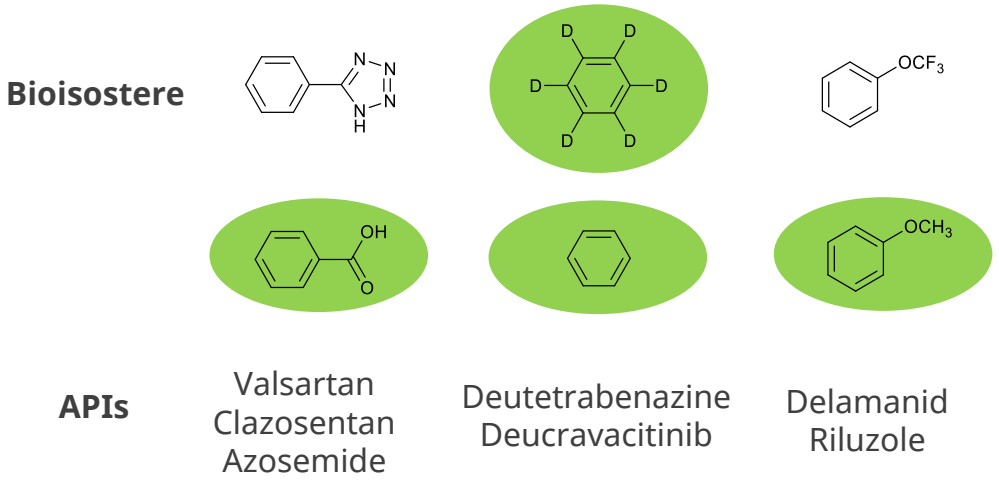

Figure 3: Structures of the investigated bioisosteres (green circle = readily or ultimately biodegradable).

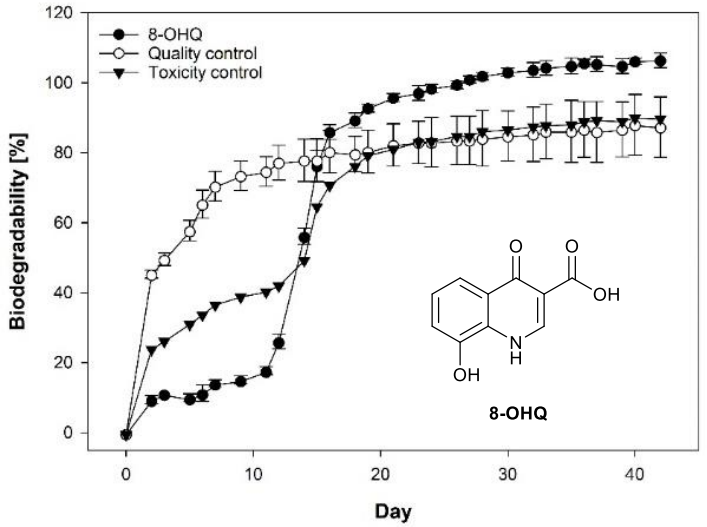

Figure 4: Biodegradation curve of 8-OHQ in the MRT OECD 301F.

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SBR of aromatic molecules

Effects of different EDG and EWG on biodegradation of arenes

- EDGs e.g., OH or weak EWG e.g., COOH tend to improve biodegradability
- EWGs e.g., CF_3 impairs with S_EAr as environmental biodegradation mechanism of aromatic molecules
  - Environmental biodegradability mechanism of arenes similar to CYP_{450}

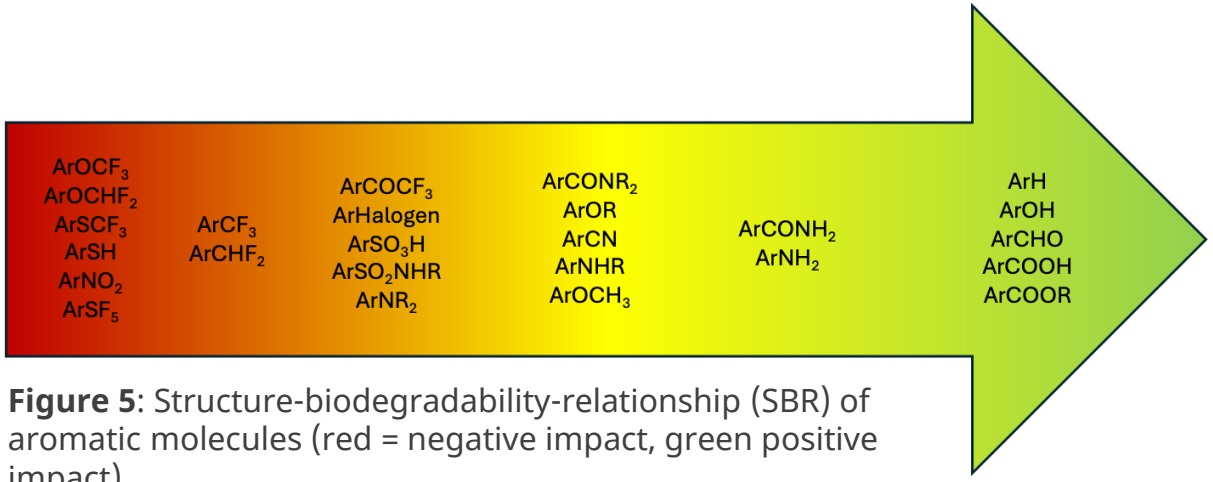

Figure 5: Structure-biodegradability-relationship (SBR) of aromatic molecules (red = negative impact, green positive impact).

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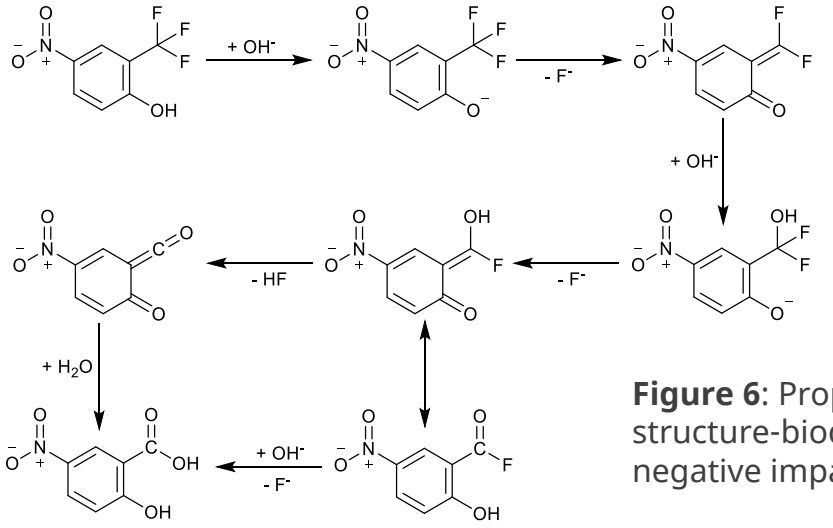
SBR of fluoroaromatic molecules

Effects of fluorinated groups on biodegradation of arenes

- Fluorination has a negative impact on environmental biodegradability, but they are not always persistent
  - Single CF bond with EDG or COOH were often readily or ultimately biodegradable
  - Aromatic CF_3 bonds require hydrolysis to COOH to initiate biodegradation
  - Hydrolysis requires strong EDG in *o*- or *p*-position to CF_3 to generate electrophilic intermediate ➞ potentially cytotoxic



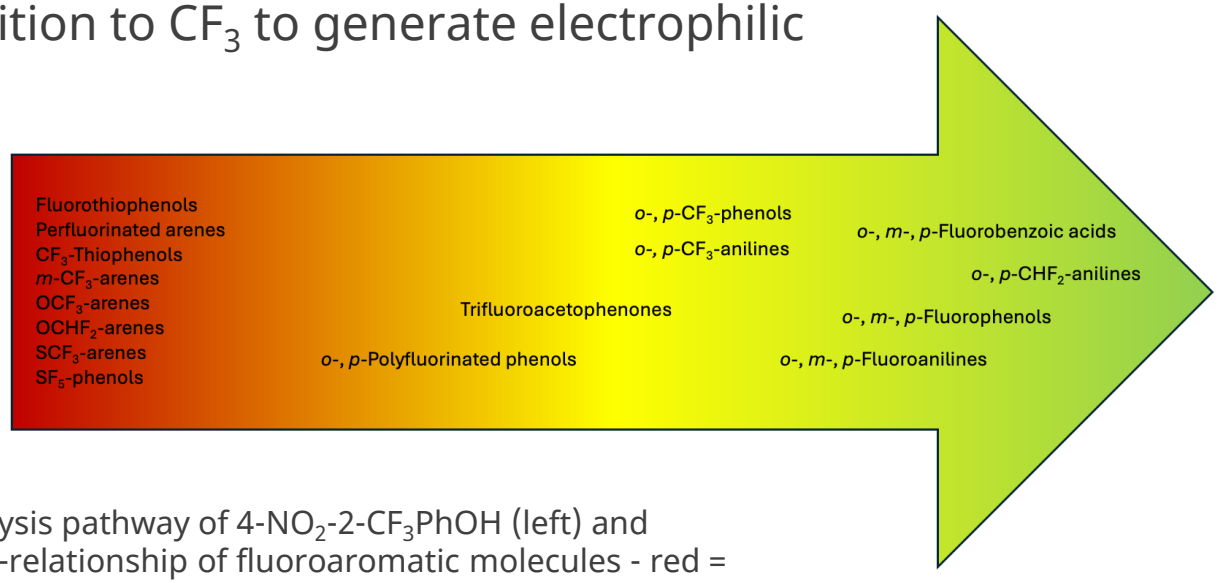


Figure 6: Proposed hydrolysis pathway of 4-NO_2-2-CF_3PhOH (left) and structure-biodegradability-relationship of fluoroaromatic molecules - red = negative impact, green positive impact (right).

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Current Methodological Challenges and Knowledge Gaps

Different needs at different stages

Prioritisation vs. Environmental Risk Assessment (ERA)

Environmental protection

Pharmaceutical R&D

Early drug design and discovery

Prioritisation
('legacy' pharmaceuticals)

- **High throughput**
- **Information of degradation products (and pathways)**

Definitive ERA
(e.g., for future EPR exemption)

Marketing authorisation

- **Standardised test guidances**
- **Comparable endpoints**

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Selection of the test method

From among the standardized tests (OECD) required by authorities

Commonly used tests measure the consumption of dissolved oxygen (O_2) by microorganisms as they break down the test substance, including

- **OECD 301 D (Closed Bottle) – Uses secondary effluent (from WWTP)**
  - ***Low concentration of the test substance*** → Good compatibility with early R&D (and substances that might inhibit microbial activity at high doses)
  - ***Low concentration of microorganisms*** → Most stringent, but prone to false negatives
- **OECD 301 F (Manometric Respirometry) – Uses activated sludge (from WWTP)**
  - ***Higher concentration of microbes*** → Biodegradation more easily detected, but requires specialty laboratory safety procedures
  - ***Also higher concentration of the test substance needed***

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Current Test Methods vs. Available Data

Test methods developed primarily for Environmental Risk Assessment (ERA)

- The primary aim in ERA (PBT assessment) is to understand if the test substance is persistent or not
- Standardised OECD test guidances inform of ‘elimination half-life’

Example: OECD 301D (ready biodegradability)

- Ready biodegradability is defined as ≥60% degradation within a 10-day window during 28-day period
- Degradation % determined based on an indirect endpoint (oxygen consumption)

✓ **Standardised**

❑ **But time-consuming**

✓ **A clear endpoint:**
Yes/No

❑ **But determining the degradation products and pathways:**
Requires additional (HRMS) analysis
Not requested by authorities
Not routinely produced by CROs

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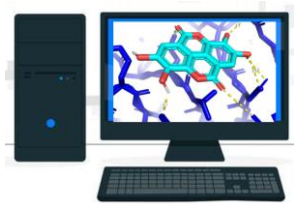
Incorporating Biodegradation in BbD Principles

Aligned with EU's Strategic Approach to Pharmaceuticals in the Environment

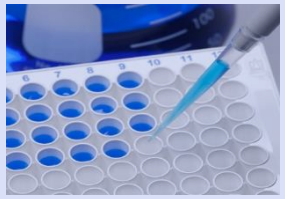
Action 5.2 *Support the development of pharmaceuticals intrinsically less harmful for the environment (and promote greener manufacturing)'*



Structure-Biodegradation Predictions

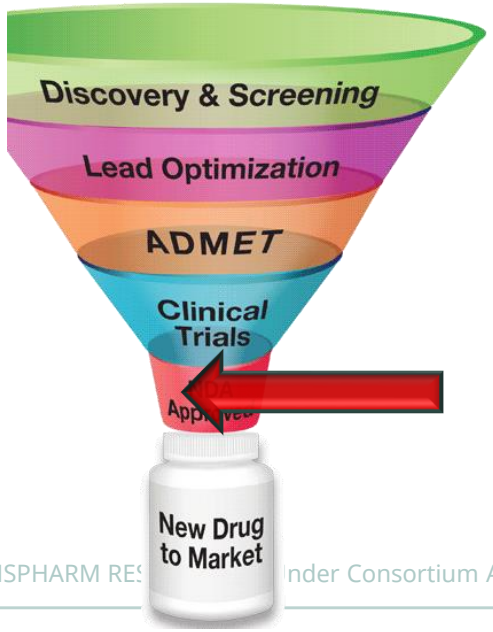
- Current model maturity limited

Current focus point



Experimental verification

Training datasets



Currently

PBT assessment

- Persistent or not?
- No SAR-type data

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Recent Advancements

Toward Accelerated Empirical Testing of the Biodegradability of Pharmaceuticals

Selected examples of progressive developments

Aligned with OECD TG 301 (adopted for use back in 1992) and beyond

- **Adapting noninvasive O_2 sensing methods to replace traditional Clarke electrode**
  - Optical fiber based sensing from closed bottles → e.g. Friedrich et al., *Soil Air Water* 41, 251 (2013)
  - Improved precision and accuracy, but still, low throughput

- **Miniaturization toward R&D scale**
  - Well plate-based assays → e.g. Cregut et al. *Environ Sci Pollut Res* 21, 9545 (2014); François et al., *Sci Pollut Res* 23, 18684 (2016)
  - Higher throughput, but limited accuracy (not necessarily oxygen-tight)

- **Use of alternative endpoints on well plate-based assays (beyond standardised assays)**
  - Monitoring cellular activity → e.g. Cregut et al. *Environ Sci Pollut Res* 21, 9545 (2014)
  - Monitoring bacterial growth → e.g., Wennberg et al., *Environ. Toxicol. Chem.* 45, 15 (2026)
  - Determining first-order rate constants (k_{deg}) for biotransformation → e.g., Partanen et al., *Environ. Sci. Technol. Lett.* 12, 1561 (2025)
  - High throughput testing (~48h) and thus, improved R&D compatibility but still, no information of degradation products

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Recent examples of progressive developments

Machine-learning (ML) models

- **ML models to predict soil primary biotransformation half-lives**
  - Salz, M., et al., *Environ. Sci. Technol.* 60, 11077 (2026) https://github.com/FennerLabs/pepper

- **Further research necessary to be able to predict the transformation products and pathways**
  - A substantial knowledge gap with a view to ERA as well as the BbD ambition

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Examples of the work done in TransPharm

Aligned with the Closed Bottle Test (OECD 301D) and beyond

- Accelerating ready biodegradability testing through an integrated approach:

✓ **Miniaturization using closed bottles**
→ Accurate, leakage-free O_2 measurements

✓ **Automation of the standard endpoint (O_2 consumption) detection**
→ Aligned with regulatory needs

Miniaturization of the Closed Bottle Test

- Performance comparable to standard (≥100 mL) bottles was achieved down to ca. 20 mL test volume

With a commercial O_2 sensing platform

- Compatible with 24-well plate format
- **Or equal size closed containers**

O_2 sensor spots at the bottom of the sample vials

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Examples of the work done in TransPharm

Aligned with the Closed Bottle Test (OECD 301D) and beyond

- Within TransPharm, we aimed at accelerating ready biodegradability testing through an integrated approach:

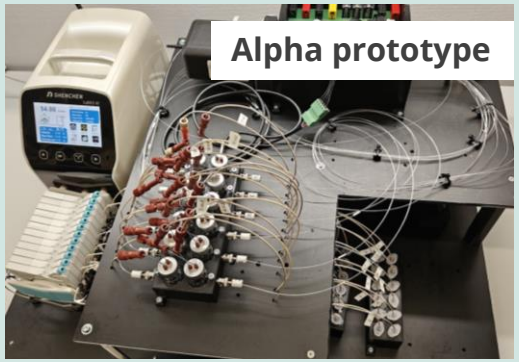
✓ **Miniaturization using closed bottles**
→ Accurate, leakage-free O_2 measurements

✓ **Automation of the standard endpoint (O_2 consumption) detection**
→ Aligned with regulatory needs

✓ **Automation of sample taking over the course of the biodegradation assay**
→ Biodegradation products/pathways
→ Aligned with pharma R&D needs (BbD)

Using a prototype instrument developed within TransPharm

- Compatible with practically any bottle size
- Automated multichannel O_2 measurement
- Automated, multichannel, minimally intrusive sampling for further (HRMS) analysis



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Certain fundamental challenges remain…

How low can we go in terms of miniaturization?

Miniaturisation (reduced test volume) – Two sides of the coin

- Sample consumption ↓ (aligned with early R&D needs)
- Number/natural diversity of microorganisms ↓ (risk of false negatives)

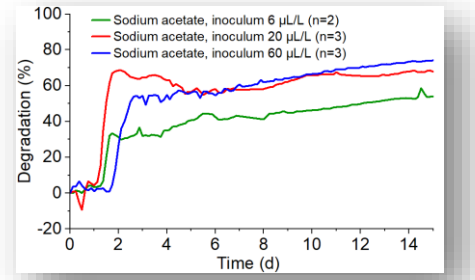
Overcome reduced diversity necessitates increased concentration of microorganisms

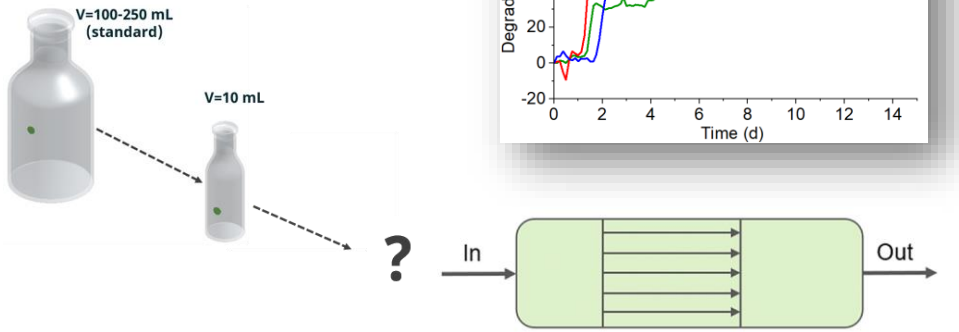
- Accelerates biodegradation processes
- But also rapid loss of dissolved O_2 due to inherent bacterial growth

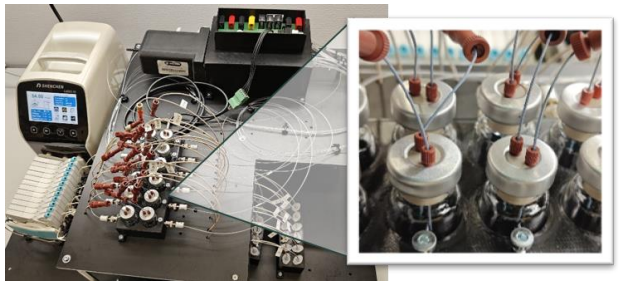
Flow-through biodegradation assays - A novel, nonstandard concept tested within TransPharm

- Introducing controlled influx of oxygen to otherwise closed containers
  - High enough to compensate for bacterial O_2 consumption
  - But small enough to not overrule for API degradation

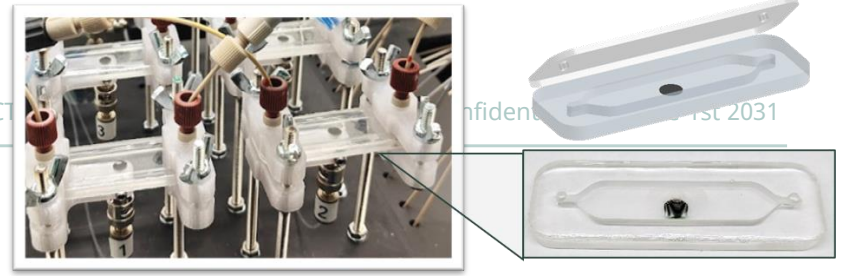
Biodegradation of sodium acetate in 100 mL (no flow)







Haapala, M., Amorim, J., Sikanen, T., Yet Unpublished (2026).



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Is there a window of opportunity
for designing NCEs that are
metabolically stable (in human)
but readily biodegradable (in the environment)?

Certain fundamental challenges remain…

Ready biodegradability is not the only key to happiness

New concepts are necessary for balancing environmental fate (and hazards) with human safety and efficacy

- Physiological stability and environmental biodegradability can be coupled
- No trade-offs can be made between biodegradation and therapeutic efficacy

Integrated Environmental Assessment and Management, 2026, **00**, 1–14
https://doi.org/10.1093/inteam/vjag062
Published: April 16, 2026
Original Article | Health & Ecological Risk Assessment

An interdisciplinary comparative framework for antimicrobial efficacy and environmental risk assessment of antibiotics integrating human and environmental health: A case study from patient to river

Qiyun Zhang[1,*], Pieter-Jan De Sutter[2], Jéssica F. Amorim[3], Päivi Järvinen[3], Caroline T.A. Moermond[4,5], Morten Suk[6], Klaus Kümmerer[6], Kristof Demeestere[7], Tiina M. Sikanen[3,8], and Karel A.C. De Schamphelaere[1]

ACS Sustainable Chemistry & Engineering
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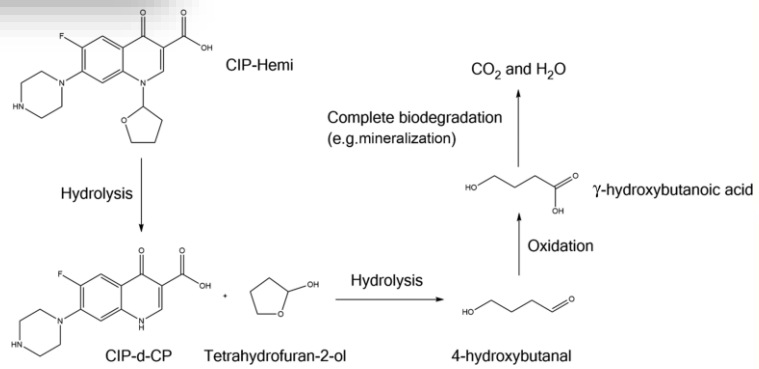
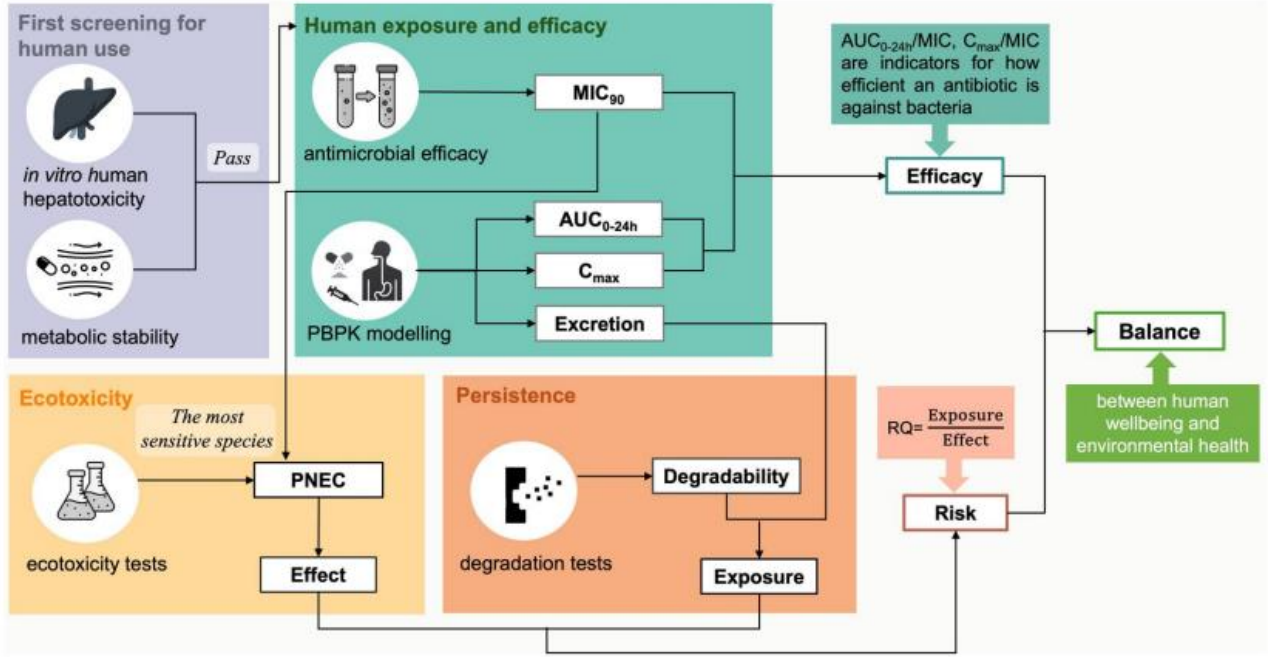
Reducing Environmental Pollution by Antibiotics through Design for Environmental Degradation

Christoph Leder, Morten Suk, Stefanie Lorenz, Tushar Rastogi, Christian Peifer, Manfred Kietzmann, Daniel Jonas, Marion Buck, Axel Pahl, and Klaus Kümmerer*

Cite This: https://doi.org/10.1021/acssuschemeng.1c02243 Read Online





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