Fundamentals of biodegradation and biodegradation modeling

Prof. Kathrin Fenner,

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Outline

- Relevance of persistence and transformation product formation for chemical assessment
- Why and how do microbes degrade chemicals?
- Factors influencing biodegradation
- Publically available data sets and models

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Persistent chemicals and transformation products (TPs) in the environment

WATERKEEPER ALLIANCE

Congress Must Stop 6PPD In Its Tracks

By: Waterkeeper Alliance

Blog | Clean Water Defense | News | 6PPD | Take Action

August 27, 2024

Join Our Urgent Call for Congress to Protect Our Waterways from the Highly Toxic 6PPD!

As communities nationwide grapple with the escalating effects of climate change and industrial pollution, there is increasing urgency to address the environmental dangers posed by a lesser-known but highly toxic chemical: 6PPD.

Scientific American, January 2022

POLLUTION

Forever Chemicals Are Widespread in U.S. Drinking Water

ENDOCRINE SOCIETY

PRESS RELEASE

Plastics pose threat to human health

Washington, DC | December 15, 2020

Authoritative report shows plastic, microplastics are pervasive sources of exposure to dangerous endocrine-disrupting chemicals

SONNTAGSZEITUNG

Abo Toxische Schadstoffe

«Auf einem solchen Boden möchte ich keinen Spielplatz haben»

In Lausanne wurden Dioxine in hohen Konzentrationen gefunden. Die Gefahr lauert auch an anderen Orten, sagt Chemiker Markus Zennegg, der die Substanzen seit bald 30 Jahren erforscht.

BBC NEWS

Pharmaceuticals in rivers threaten world health - study

By Jonah Fisher
BBC Environment Correspondent

15 February | Comments

SRF Zu viel Chlorothalonil

Gift im Trinkwasser: Aargau nimmt zwei Wasserfassungen vom Netz

Dienstag, 23.07.2019, 12:17 Uhr

Persistence as hazard criterion in chemical regulation

Persistence	A substance fulfils the persistence criterion (P) in any of the following situations: (a) the degradation half-life in marine water is higher than 60 days; (b) the degradation half-life in fresh or estuarine water is higher than 40 days; (c) the degradation half-life in marine sediment is higher than 180 days; (d) the degradation half-life in fresh or estuarine water sediment is higher than 120 days; (e) the degradation half-life in soil is higher than 120 days.	A substance fulfils the "very persistent" criterion (vP) in any of the following situations: (a) the degradation half-life in marine, fresh or estuarine water is higher than 60 days; (b) the degradation half-life in marine, fresh or estuarine water sediment is higher than 180 days; (c) the degradation half-life in soil is higher than 180 days.

(*REACH, 2017*)

Consideration of TPs in chemical regulations

- Pesticide Directive (91/414/EEC):

  - Identification of relevant transformation products requested
  - Clear guidance on assessing relevance

- Industrial chemicals (REACh):

  - Identification of transformation products requested for products > 100 t/y
  - No guidance on assessment

- Human and veterinary medicines (EMA):

  - Consideration of environmental transformation products subject to expert judgment

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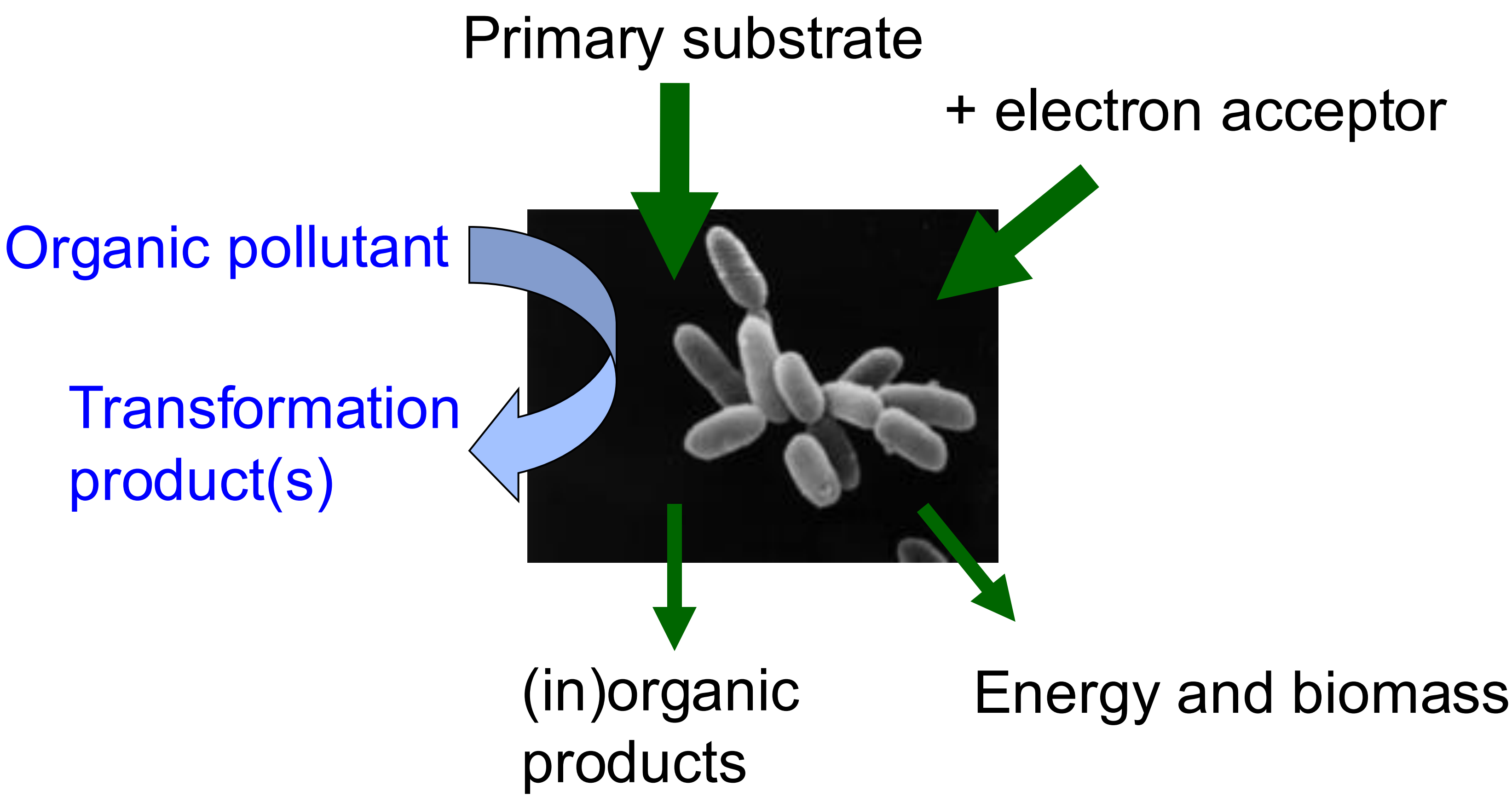
Microorganisms and their role in chemical degradation

- Microbial communities are assemblies of diverse types of microorganisms:
  - Bacteria (prokaryotes)
  - Archaea (prokaryotes)
  - Fungi (eukaryotes)
  - Protozoa (single/multicellular eukaryotes) etc.
- Microorganisms catalyze degradation of synthetic chemicals through providing enzymes and/or co-substrates
  - Microbial biotransformation is the mass-wise most important degradation process removing synthetic chemicals from the environment (diverse and ubiquitous microorganisms)

Principles of enzymatic transformation of chemicals

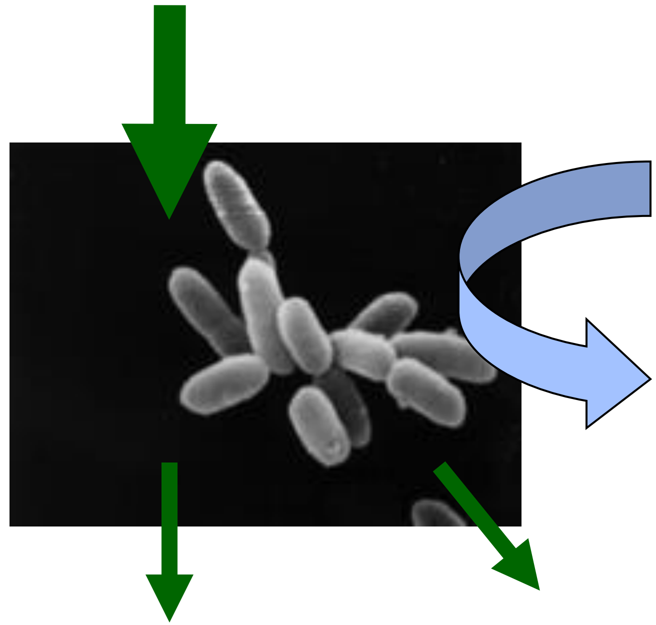
- Enzymes primarily present for synthesis (anabolism) and degradation (catabolism) of biologically essential molecules
  - Synthetic chemicals are "foreign" structures (xenobiotics) and hence enzymes for their degradation not present a priori
- Why are synthetic chemicals still transformed by microbes?
  - Co-metabolism:
    - Imperfect substrate specificity of enzymes
    - All microorganisms carry some unspecific enzymes for defense ("Swiss army knife")
  - Metabolism: Microorganisms can evolve to use synthetic chemicals as source of energy/nutrients

Co-Metabolism



Synthetic chemical **does not** serve as carbon and energy source

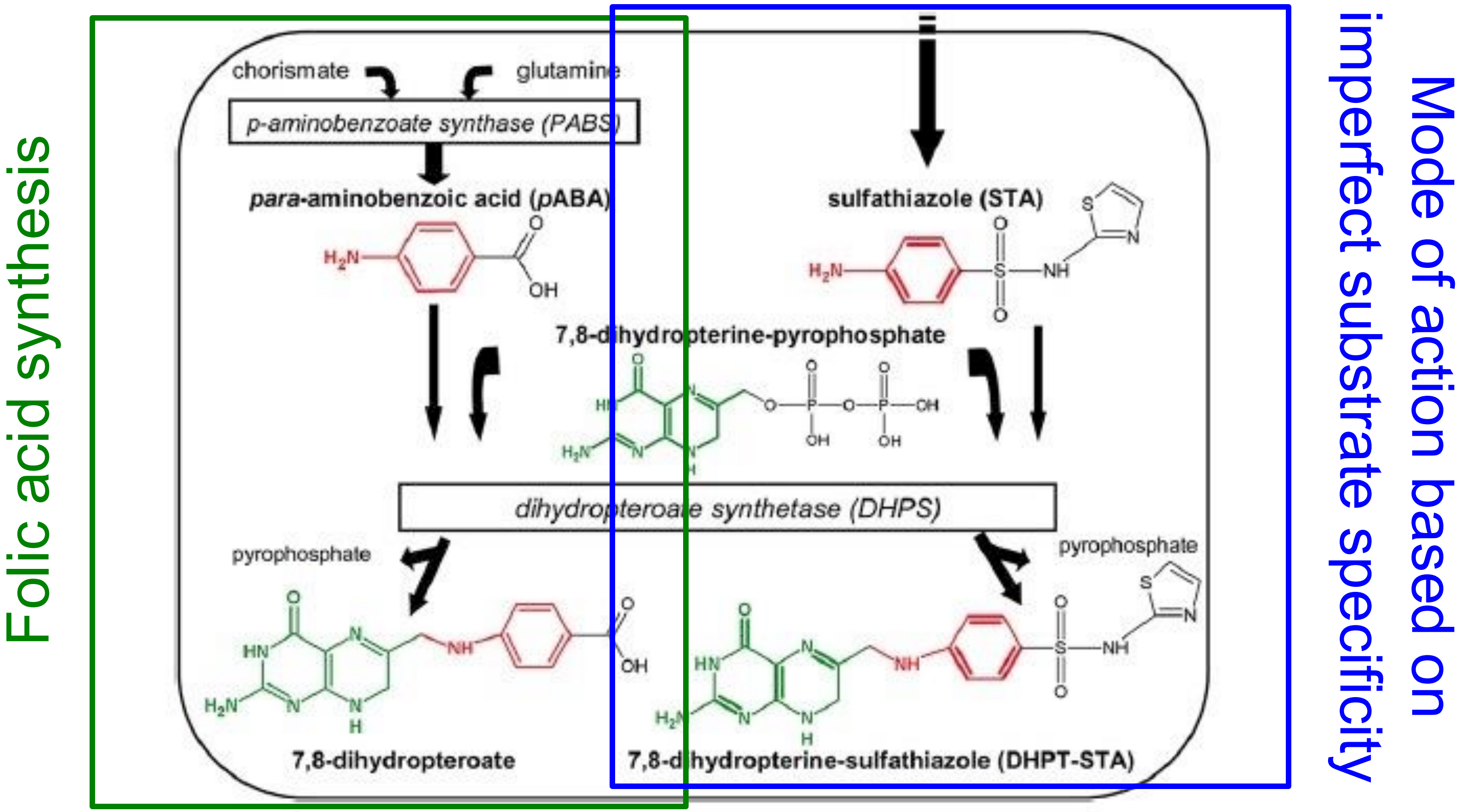
Co-Metabolism



Synthetic chemical **does not** serve as carbon and energy source

Example for co-metabolism – Sulfonamide antibiotics

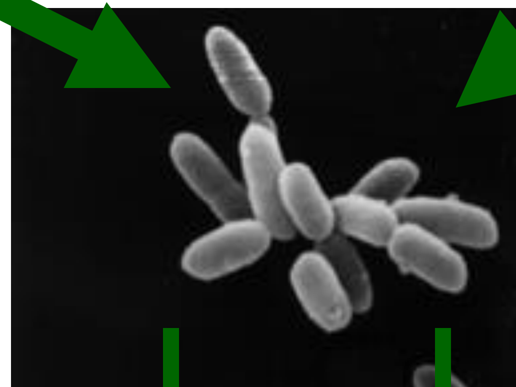
- Imperfect substrate specificity underlies sulfonamide antibiotics biotransformation:



Richter et al., *Environ. Poll.* **172** (2013), 208–215

Metabolism

Organic pollutant + electron acceptor



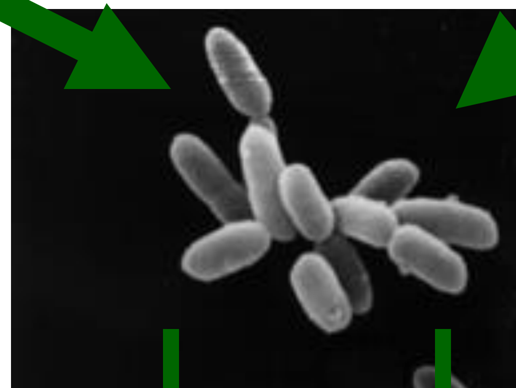
Diverse products
(catabolism)

Energy and biomass
(anabolism)

Synthetic chemical serves as sole carbon and energy source

Mineralization
(Special case of metabolism)

Organic pollutant + electron acceptor



«Mineralization»:

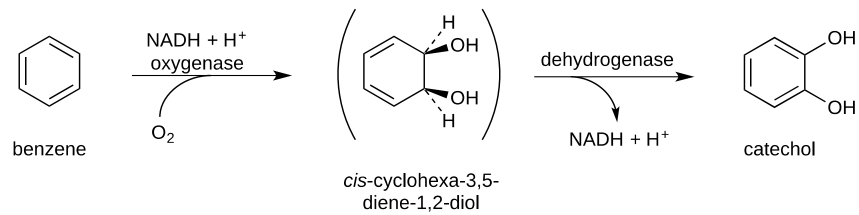
Only inorganic end products (CO_2, Cl^-, H_2O etc.)

Energy and biomass (anabolism)

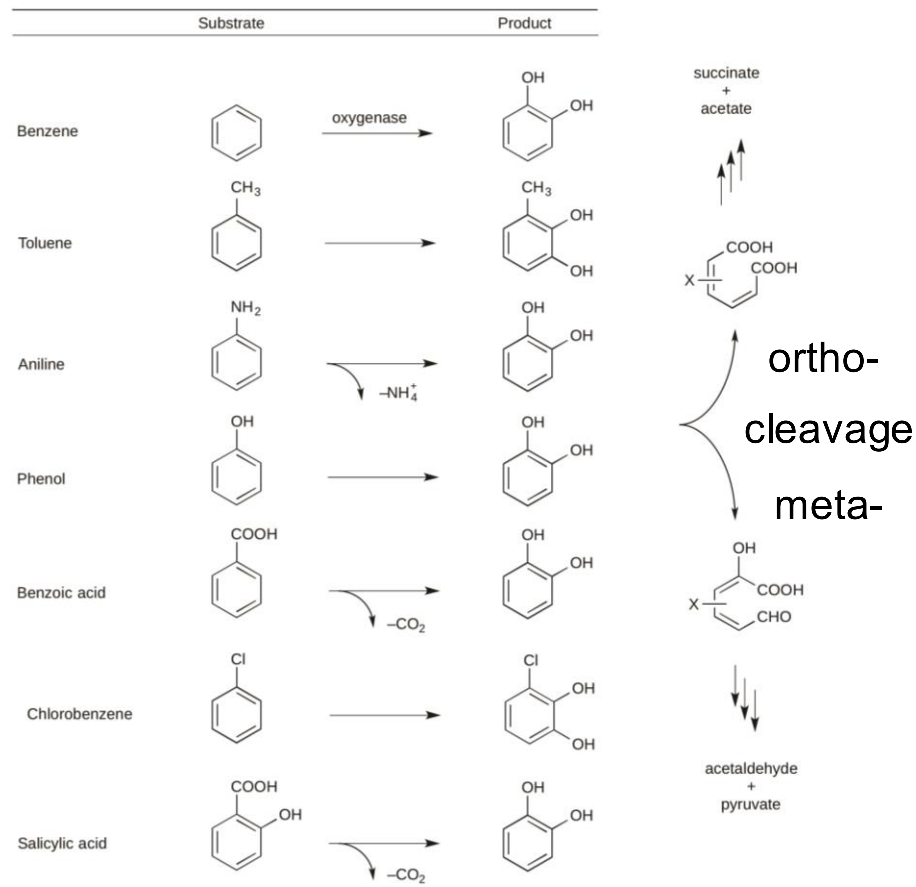
Synthetic chemical serves as sole carbon and energy source

Example for metabolism – Substituted benzenes biodegradation

- Oxidation of substituted benzenes to catechol by oxygenase followed by dehydrogenase



- Further degradation paths for catechols are microbially ubiquitous



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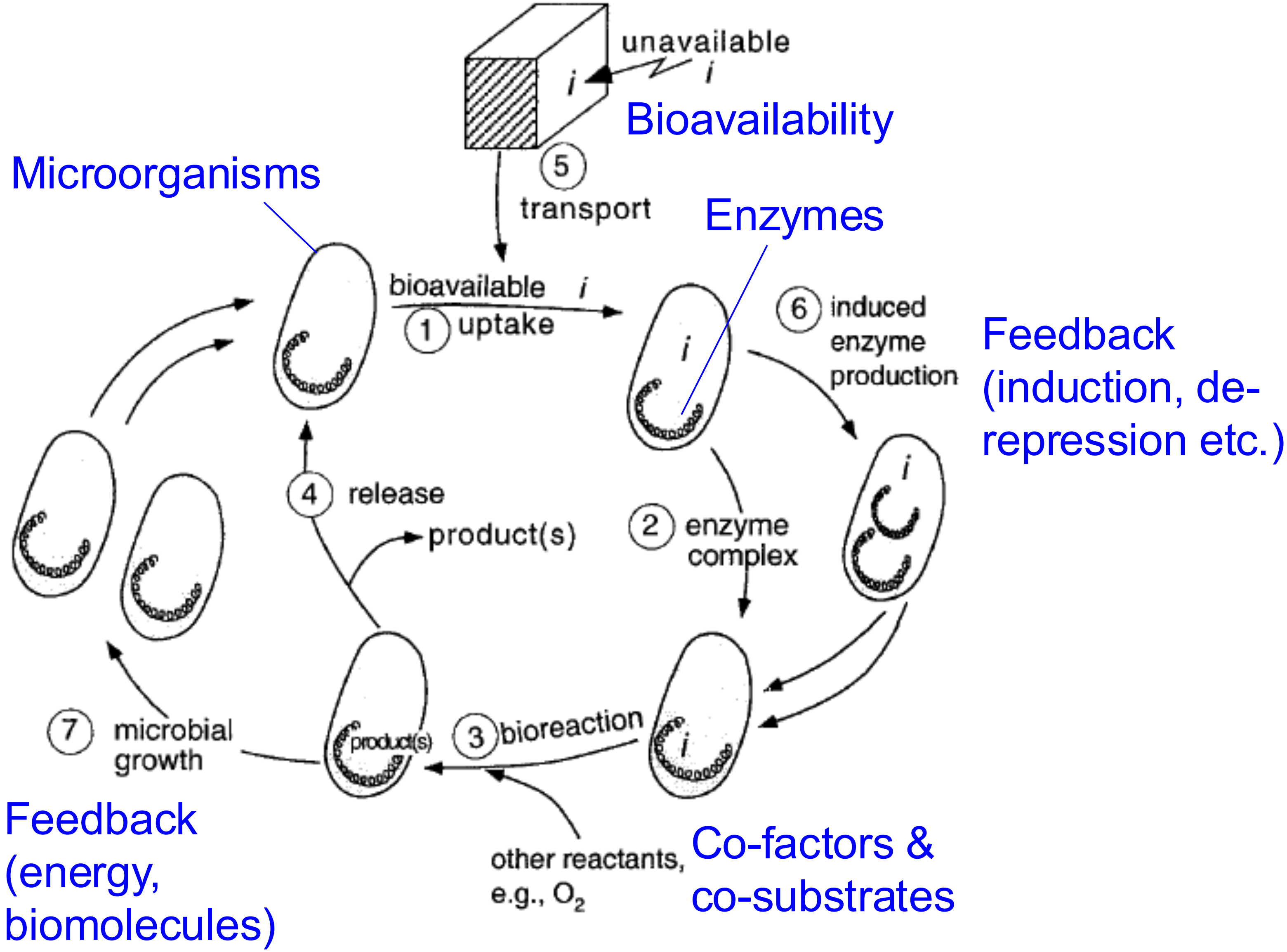
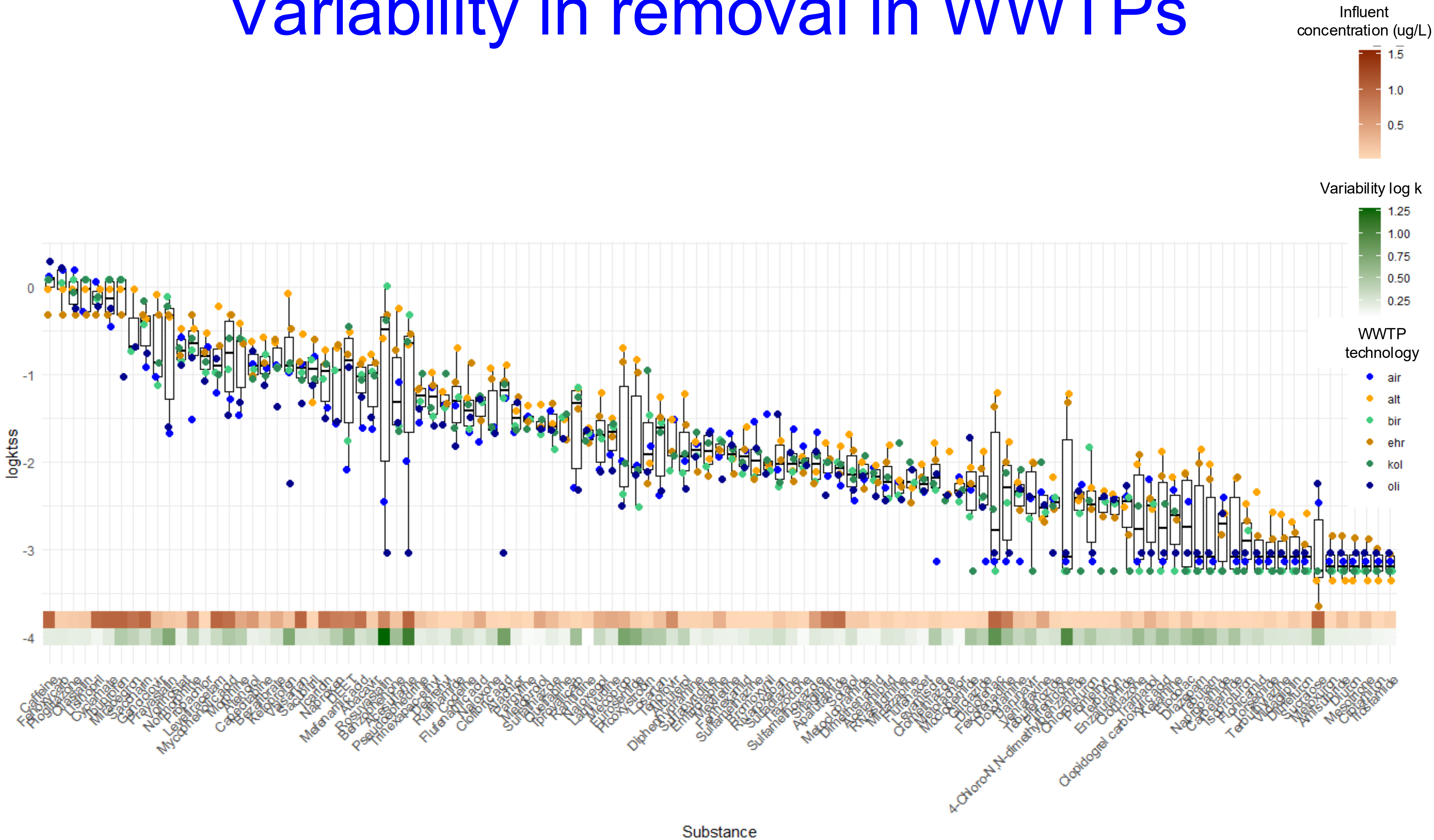
Factors influencing biotransformation processes



Schwarzenbach et al., Environmental Organic Chemistry, 2003

Variability in removal in WWTPs



Kalt et al., 2025: Manuscript in preparation

Further environmental factors that influence biotransformation rates

- Physical factors (T, pH, etc.)
- Nutrients (mainly nitrogen, phosphorous)
- Presence of other organic substrates
- Redox conditions (aerobic, anaerobic)
- Microorganisms specifically adapted to extreme environments:
  - pH: acidophiles
  - Salinity: halophiles
  - Temperature: psychrophiles, mesophiles, thermophiles

Summary so far

- Half-lives and transformation product formation are important to know for regulatory decision making
- Metabolic and co-metabolic biotransformation of chemicals possible
- Structure of chemical, available enzymes and concentration matters
- Experimental/environmental conditions are key to understand variability in biotransformation

- But: Do we have sufficient biotransformation data to assess chemicals in commerce and/or can we predict?

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- Why and how do microbes degrade chemicals?
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- Publically available data sets and models
  - Ready biodegradability
  - Compartiment-specific half-lives

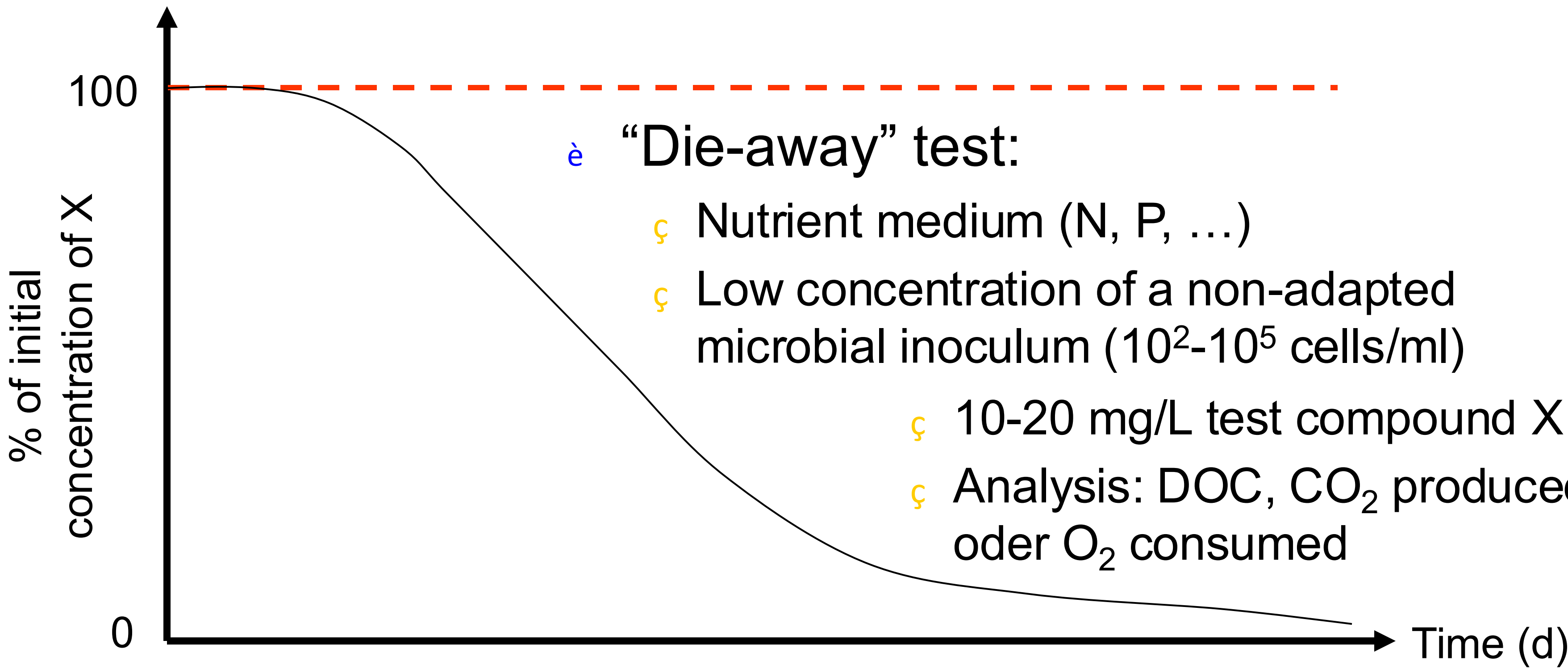
Persistence data – Testing for ready biodegradability

Indication of P and vP properties	(a) Results from tests on ready biodegradation in accordance with Section 9.2.1.1 of Annex VII; (b) Results from other screening tests (e.g. enhanced ready test, tests on inherent biodegradability); (c) Results obtained from biodegradation (Q)SAR models in accordance with Section 1.3 of Annex XI; (d) Other information provided that its suitability and reliability can be reasonable demonstrated.

(*REACH, 2017*)

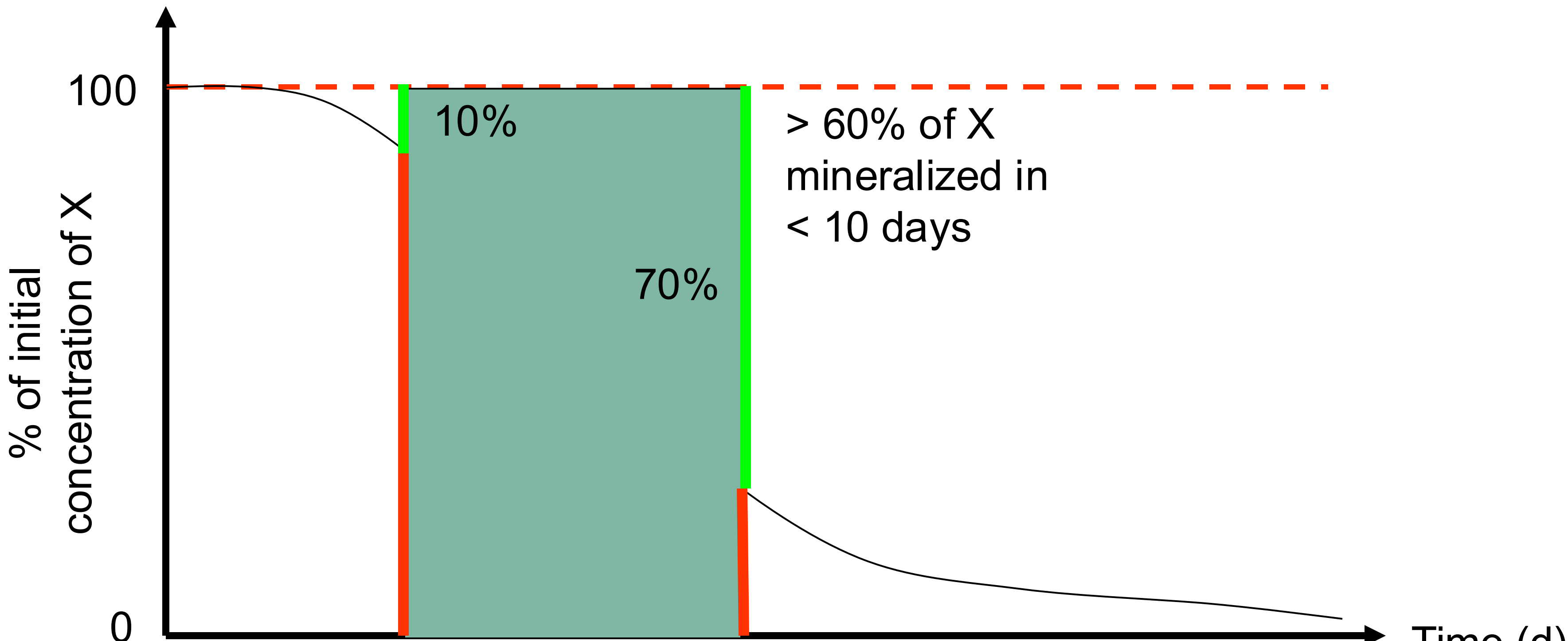
- Ready and screening tests: Rather stringent *mineralization* tests (high substrate concentrations, low inoculum density, no ^{14}C-labeling required) → Pass/fail "ready biodegradability" (RBT)

Ready biodegradability test (OECD 301)



- “Die-away” test:
  - Nutrient medium (N, P, …)
  - Low concentration of a non-adapted microbial inoculum (10^2-10^5 cells/ml)
    - 10-20 mg/L test compound X
    - Analysis: DOC, CO_2 produced, oder O_2 consumed

Ready biodegradability test (OECD 301)



Readily biodegradable compounds usually readily disappear from the environment
BUT: Small, persistent TPs like trifluoroacetic acid might evade attention

RBT data sets and models – Overview

Model	Dataset size	Balanced accuracy	Sensitivity	Specificity
Howard *et al.* (1992)[10] (non-linear)	264			
Test set	7.4%	88.8%*	—	—
Boethling *et al.* (1994)[1] (non-linear)	295			
Training set	—	93.2%*	BIOWIN 1 & 2	
Loonen *et al.* (1999)[11] (with fragment interactions)	894			
Test set	25%	89%*	—	—
Tunkel *et al.* (2000)[12] (linear)	884			
Validation set	33.3%	74.9%*	BIOWIN 5 & 6	
Cheng *et al.* (2012)[13]	1440			
Test set (GASVM-kNN)	11.4%	81.9%	72.6%	91.2
External test set (GASVM-kNN)	27	53.8%	25.0%	82.6%
External test set (consensus model)	27	100%	100%	100%
Mansouri *et al.* (2013)[14] (consensus II)	1055			
Test set	20%	91%†	89%†	94%†
External test set	670	87%‡	81%‡	94%‡
Zhan *et al.* (2017)[18] (NBC)	1055			
Test set	20%	83.8%	86.1%	81.5%
External test set	670	82.6%	79.6%	85.6%
Lunghini *et al.* (2020)[19]	3146			
Test set	30%	81%*	—	—
External test	362	75%*	65%	85%
Huang and Zhang (2022)[20]	6139			
Test set	20%	84.9%	89.0%	80.9%
Test set with chemical speciation	20%	87.6%	87.8%	87.4%

Körner et al., *Environ. Sci.: Process. Impacts A*, **26**, 1780, 2024

Available models to predict RBT – BIOWIN

- BIOWIN™

Model	Endpoint	Source data	Regression model	Comment
BIOWIN 1	RB	RB expert classification (y/n)	linear	
BIOWIN 2			non-linear	
BIOWIN 3	Ultimate biodegradation	Expert classification (semi-quantitative ratings)	linear	Scoring system: 5 = hours 4 = days 3 = weeks 2 = months 1 = recalcitrant
BIOWIN 4	Primary biodegradation (PSM model)		linear	
BIOWIN 5	RB probability	RB tests on 884 substances	linear	Also called MITI models
BIOWIN 6			non-linear	
BIOWIN 7	Anaerobic RB	RB tests on 169 substances (y/n)	linear	RB under methanogenic, anaerobic conditions
RB prediction	RB	Combination of BIOWIN 3 and 5	-	

Available models to predict RBT – Beyond BIOWIN

Tool	Availability	Endpoints	Comment	Link
BIOWIN in EPISuite	Standalone, Windows only	RB	Most popular tool	https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411
OPERA	Standalone, Windows and Linux	RB	Based on machine learning; Pre-calculated predictions for >1M compounds	Access to pre-computed values through CompTOX dashboard: https://comptox.epa.gov/dashboard/
Huang and Zhang, 2022	Jupyter Notebooks in SI	RB and % degradation		Online tool disfunctional
Körner et al., 2025	Online	RB	Re-curated data set from Huang and Zhang, 2022	https://biodegradability-prediction-app.streamlit.app

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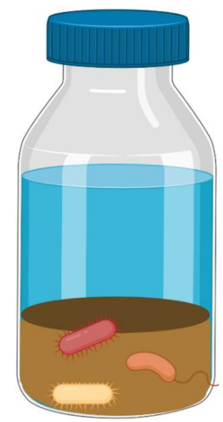
Persistence data – OECD suite of simulation studies to provide half-lives

Assessment of P or vP properties	(a) Results from simulation testing on degradation in surface water; (b) Results from simulation testing on degradation in soil; (c) Results from simulation testing on degradation in sediment; (d) Other information, such as information from field studies or monitoring studies, provided that its suitability and reliability can be reasonably demonstrated.

OECD 309:
Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test

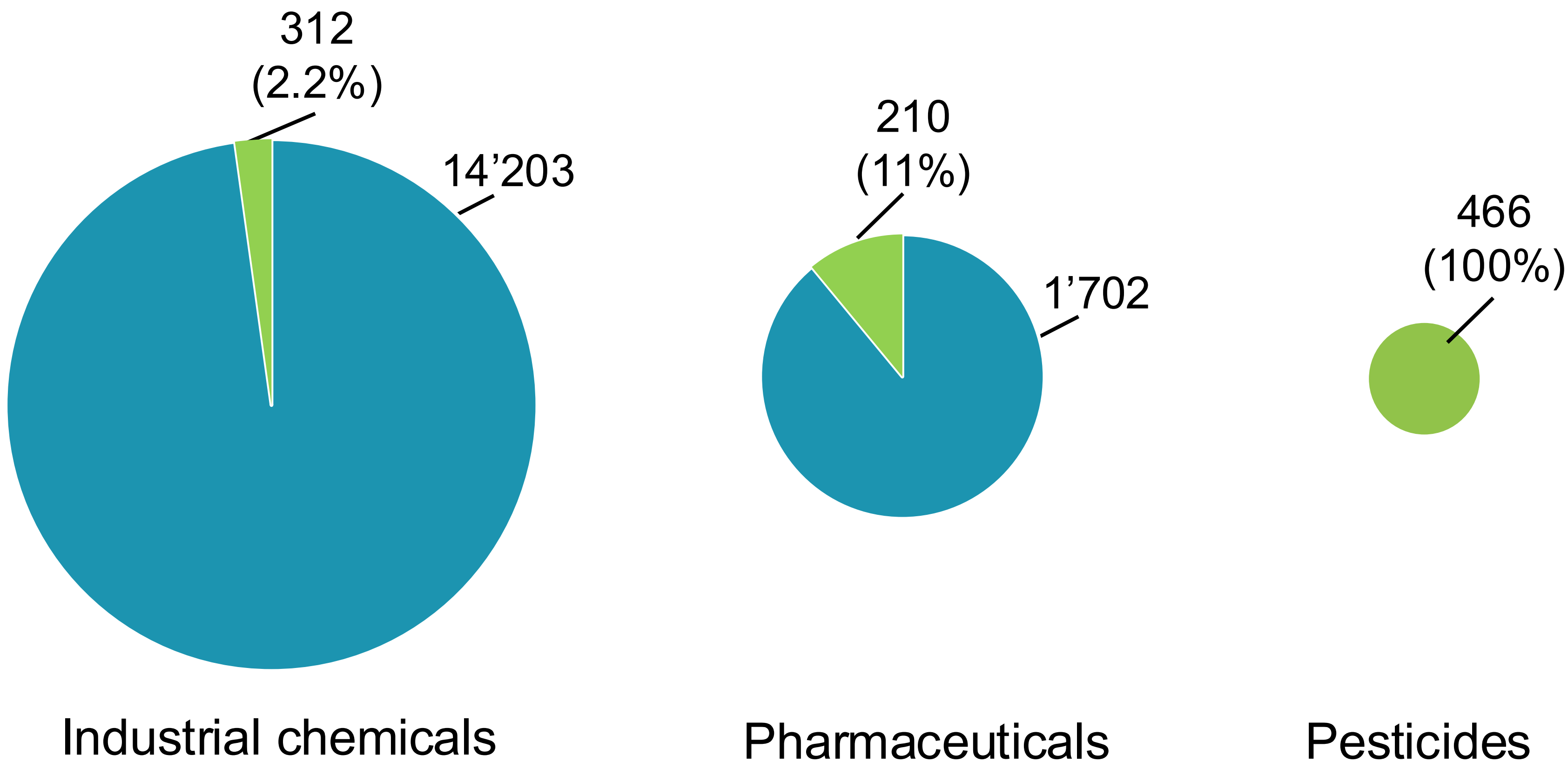
OECD 307:
Aerobic and Anaerobic Transformation in Soil



OECD 308:
Aerobic and Anaerobic Transformation in Aquatic Sediment Systems

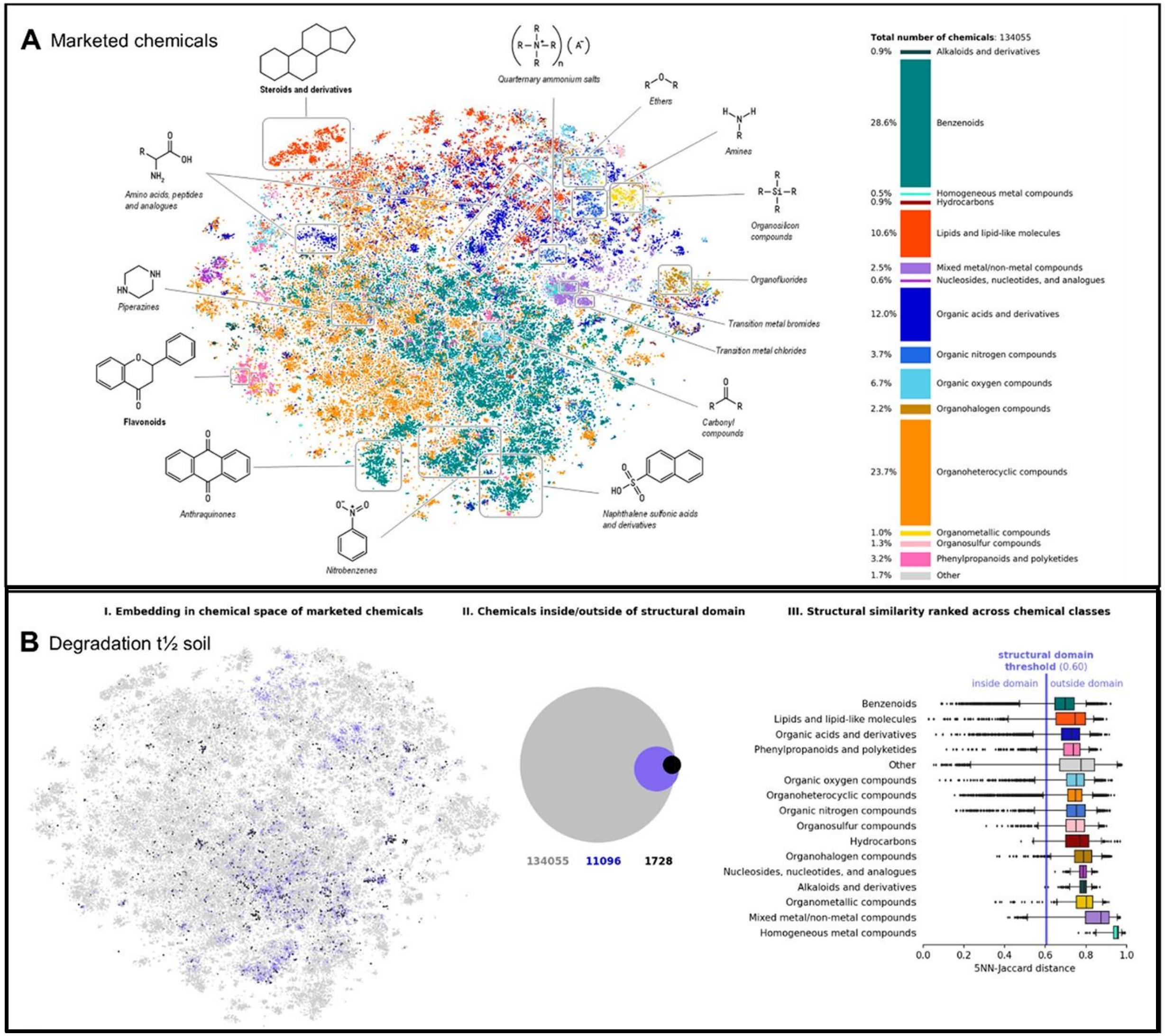
Availability of half-life data sets

- Availability of half-life information



Burns et al., *J. Toxicol. Environ. Health Part B*, **21**, 115, 2018
Arp and Hale, *ACS Environ. A*, **2**, *482–509, 2022*

Can we hope to build models with these data?



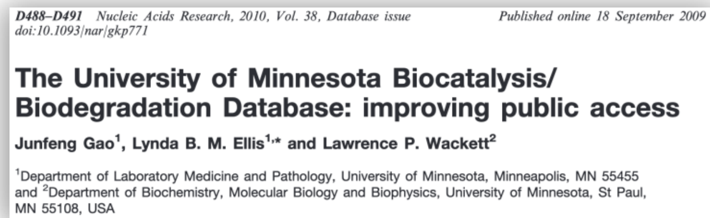
Von Borries et al., *Environ. Sci. Technol., 57, 18259–18270,* **2023**

Available models to predict half-lives

Tool	**Availability**	**Endpoints**	**Comment**	**Link**
BIOWIN in EPISuite	Standalone, Windows only	Semi-quantitative half-life estimates	Most popular tool	https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411
OPERA	Standalone, Windows and Linux	Half-lives for hydrocarbons only	Based on machine learning	Access to pre-computed values through CompTOX dashboard: https://comptox.epa.gov/dashboard/
VEGA	Part of OECD QSAR Toolbox, Standalone	Semi-quantitative half-life estimates	Low reliability for complex chemicals such as pharmaceuticals & pesticides	https://qsartoolbox.org

Available TP data sets and models

- Data: See presentation on enviPath data packages
- Models: A short history of enviPath



D488–D491 Nucleic Acids Research, 2010, Vol. 38, Database issue Published online 18 September 2009
doi:10.1093/nar/gkp771

The University of Minnesota Biocatalysis/ Biodegradation Database: improving public access

Junfeng Gao[1], Lynda B. M. Ellis[1,*] and Lawrence P. Wackett[2]

[1]Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455 and [2]Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, St Paul, MN 55108, USA

Eawag-BBD
UM-BBD taken over by Eawag
Kathrin Fenner & Emanuel Schmid

2010

2012

Since 1995

UM-BBD/PPS
University of Minnesota
Biotransformation/Bio-catalysis
Database and
Pathway Prediction System
Linda Ellis & Larry Wackett

D502–D508 Nucleic Acids Research, 2016, Vol. 44, Database issue Published online 17 November 2015
doi: 10.1093/nar/gkv1229

enviPath – The environmental contaminant biotransformation pathway resource

Jörg Wicker[1,*], Tim Lorsbach[1], Martin Gütlein[1], Emanuel Schmid[2], Diogo Latino[3], Stefan Kramer[1] and Kathrin Fenner[3,4]

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2016

enviPath
Redesign and reimplement-ation of Eawag-BBD
Jörg Wicker & Kathrin Fenner

Available models to predict TPs

Tool	Availability	Endpoints	Environmental context	Comment
EAWAG-PPS	Online	TPs	Environmental, aerobic/anaerobic biotransformation	https://eawag-bbd.ethz.ch/predict/
enviPath	Online	TPs	Environmental, aerobic/anaerobic biotransformation	https://envipath.org Successor of EAWAG-PPS
Chemical Transformation Simulator (CTS)	Online	TPs	Abiotic processes (hydrolysis, photolysis), aerobic/anaerobic biotransformation	https://qed.epa.gov/cts/ Environmental biotransformation rules from enviPath
BioTransformer	Online	TPs	Mammalian metabolism and environmental biotransformation	https://biotransformer.ca Environmental biotransformation rules from enviPath

Lessons learned

- Microorganisms transform chemicals for different reasons and in different ways
  - Often TPs are formed
- Good availability of RB data and models
- Insufficient availability of half-life data and models
  - Metadata on experimental and environmental conditions key for good predictions
- Readily accessible tools for environmental TP predictions mostly rooted in Eawag-BBDPPS/enviPath data and biotransformation rules