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DOI: https://doi.org/10.2533/chimia.2023.742

doi:10.2533/chimia.2023.742 Chimia 77 (2023) 742–749

Early Assessment of Biodegradability of Small Molecules to Support the Chemical Design in Agro & Pharma R&D

Claudia Coll[a], Kathrin Fenner[bc], and Claudio Screpanti[a]*

Abstract: The use of agrochemical and pharmaceutical active ingredients is essential in our modern society. Given the increased concern and awareness of the potential risks of some chemicals, there is a growing need to align with 'green chemistry' and 'safe and sustainable by design' principles and thus to evaluate the hazards of agrochemical and pharmaceutical active ingredients in early stages of R&D. We give an overview of the current challenges and opportunities to assess the principle of biodegradability in the environment. Development of new medium/high-throughput methodologies, combining predictive tools and wet-lab experimentation are essential to design biodegradable chemicals early in the active ingredient discovery and selection process.

Keywords: Active pharmaceutical ingredients · Biodegradability · Green chemistry · Plant protection products · Transformation products

Claudia Coll is originally from Mexico, where she studied chemical engineering. She obtained her MSc and PhD degree in Environmental Science from ETH Zurich and Stockholm University with a focus on assessment of environmental persistence of chemicals. She then moved to the group of Prof. Kathrin Fenner at the Swiss Federal Institute of Aquatic Science and Technology (Eawag) to work specifically on the biodegradation of small molecules. After successful collaborations with industry partners, she joined the Soil Health Center team in Syngenta Crop Protection to explore ways to perform early-stage assessment of biodegradability in R&D.

Kathrin Fenner studied chemistry at the University of Zurich and received her Doctoral degree from ETH Zurich in 2001. Today, she is Professor for Environmental Chemistry at the Chemistry Department of the University of Zurich and Group Leader at the Environmental Chemistry Department of the Swiss Federal Institute of Aquatic Science and Technology (Eawag). In her research, she is interested in the principles of microbial biotransformation of chemicals in the environment, with the goal to predict and minimize environmental persistence of chemicals. Kathrin chairs the Section Chemistry and the Environment of the Swiss Chemical Society.

Claudio Screpanti is an agronomist working in Syngenta R&D in Switzerland. He has several years of experience in agricultural research. He obtained his PhD in agronomy from the University of Bologna (IT) in 2003. He carried out additional studies in molecular biology and genetic engineering at the University of Louvain-la-Neuve, Belgium. Later Claudio joined Syngenta, covering different scientific roles always in relation to soil. In 2018 he became a Syngenta Fellow, a company award recognizing outstanding scientific achievements. In his current role, Claudio leads the Soil Health Centre in Stein and acts as Syngenta soil expert looking at the behavior and effects of new small molecules in the soil-crop systems. The aim is to support the discovery and development of new and more sustainable crop protection solutions.

1. Biodegradability as a Principle of Green Chemistry and Benign-by-Design

Paul Anastas and John Warner first proposed the 12 principles of Green Chemistry in their book published in 1998, providing a broad framework to increase the sustainability and reduce the hazards of chemical products during design, production, use and disposal.[1]

The concept of green chemistry has since then circulated over the decades with different names interrelating similar frameworks such as 'benign-by-design', 'clean chemistry' and the latest term coined by the EU 'safe and sustainable by design' (SSbD).[2] All frameworks have commonalities, agreeing that chemicals should have 1) low or controlled hazard towards humans and the environment and 2) optimized sustainability along their life cycle: raw materials, production, use, disposal and recycling/recovery/reuse phases (as an incentive also for circularity).

One of the shared principles in all green chemistry frameworks relates to degradability (*i.e.* principle number 10 in green chemis-

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try): the idea that chemical products should be designed to break down into non-hazardous transformation products (TPs) either through environmental or technical processes such as wastewater treatment plants. Degradability helps to reduce the environmental risk of chemical products by reducing the concentrations of chemical residues in the environment and thus minimizing the exposure to chemicals.

Specifically in the EU guidelines for SSbD published in December 2022, chemical hazard is defined based on the existing Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation (EC) No 1907/2006. This means that hazard should be assessed through the criteria of persistence, mobility, bioaccumulation, and toxicity, which are evaluated through the OECD Guidelines for the testing of chemicals. However, it is specified that this evaluation should be performed during the design phase, before even assessing hazards in production, processing or use. As we discuss in the following sections, there are some challenges to effectively implement the OECD Guidelines in the early phase of discovery and optimization of small molecules like agrochemical or pharmaceutical active ingredients (APAIs).

Furthermore, the availability of OECD tests is not evenly distributed among the different sectors of chemical industries. The agrochemical industry has been regulated regarding degradability since the 1970s for commercial authorizations and accordingly, numerous degradation tests have been performed in soil and water-sediment systems, the results of many being publicly available (*e.g.* as assessment reports from the European Food Safety Authority EFSA).[3,4] Meanwhile the regulation for the pharmaceutical industry has prioritized human health benefits of the products. Pharmaceutical ingredients do not necessarily require environmental risk assessments to get commercial authorization, and if performed, an adverse outcome in the risk assessment does not necessarily compromise its authorization.[5,6]

Overall, there is an enormous challenge and area of opportunity for innovative methodologies that enable a balance between screening for degradability/persistence, while also increasing performance and sensitivity of APAIs.

In this paper we aim to examine the current status, challenges and opportunities of incorporating degradability screening within the R&D cascade of active ingredients in agro and pharma industries with the ambition of boosting the development of more SSbD products.

2. Historical Perspective of Biodegradability of Small Molecules

Diffuse contaminations of organic compounds in soils and waters from municipal and industrial waste as well as from some agricultural practices have been raising increasing concerns since the early 1960s. Most of these compounds belonged to the class of chlorinated aromatics such as PCBs (polychlorinated biphenyls), some organochlorine insecticides like DDT and other phenol derivatives with different industrial applications.[7] The broad occurrence of these xenobiotics in the environment triggered concrete actions such as the Stockholm convention with the goal to ban or limit the production and use of these so-called persistent organic pollutants (POPs). The chemical industries had to respond to the challenges by identifying alternative substances with a more environmentally favorable profile, particularly in terms of degradability.

Since then, the environmental awareness of the risk of chemicals has only increased. The most recent advances in analytical chemistry for highly sensitive detection and multiresidue analysis, in combination with a substantial abatement of the operational costs, made it possible to develop extensive monitoring programs that aim to identify chemicals of concern.[8–10]

Given the hundreds of thousands of chemicals in use, assessing the risk of chemicals is inherently complex and major efforts are taking place to attempt to quantify it holistically. In the framework of the planetary boundaries proposed by Rockström,[11] it is believed that we have exceeded the safety boundaries of chemical emission and that chemicals contribute to other global crises like climate change and biodiversity loss.[12,13] Therefore, an International Panel on Chemical Pollution (IPCP) has been formed, to deal with the challenge of assessing and dealing with contaminants risk. Considerable work remains to be accomplished in order to achieve this goal and degradability is now identified as a key aspect to assess the risk of chemicals.[14]

3. Degradation of Small Molecules in Soil: Knowledge and Learning for the Chemical Design

The factors and processes governing the fate of small molecules in the environment are quite complex and they have been extensively studied for many years. Systematic investigations on the matter started to appear in the scientific literature since the early 1960s.[15] A quite rich and detailed literature is available to depict the environmental fate of molecules. The cover of the SSSA publication 'Reaction and Movement of Organic Chemicals in Soils'[16] remains for many environmental chemists one of the most emblematic diagrams to depict these complex processes.

Once the compounds are released in the environment, they undergo different transformation, transport and retention processes. Soil and water can be considered the major environmental compartments into which agrochemical and pharmaceutical ingredients can be emitted. The importance of soil as a major compartment for the degradation of molecules like agrochemicals and other xenobiotics has been recognized for a long time,[17] and soil is essential to remove agrochemicals from the environment once they have accomplished their function. For this reason, it is imperative to integrate the degradability of compounds in soil as key criterion in the chemical design of new synthetic molecules, particularly agrochemicals.

The diagram in Fig. 1 highlights a conceptual framework that can guide the chemical design for degradability. Due to the nature of soil, its matrix composition and the astonishingly high taxonomic and functional biodiversity harbored in it, assessing the degradation of chemicals in soil is a very complex process. There are two major and orthogonal driving components behind the degradability of small molecules.

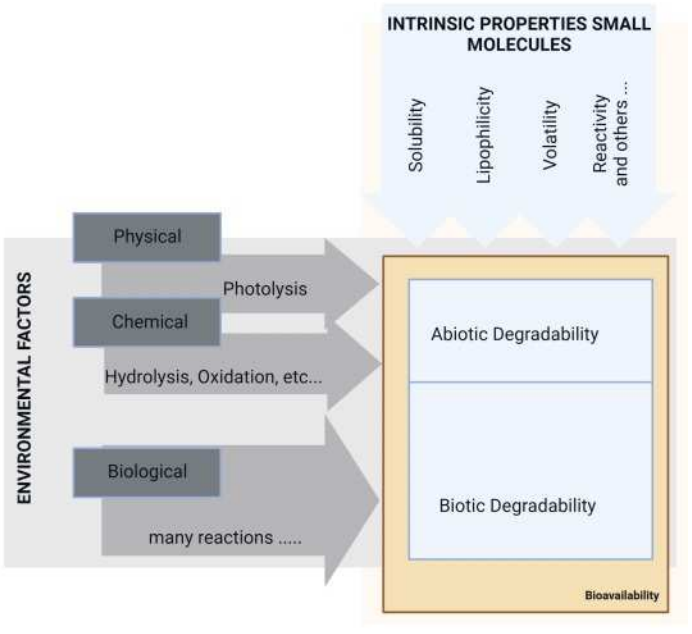

Fig. 1. Conceptual framework depicting the two orthogonal components governing the degradability of small molecules in soil.

The first component is linked to the intrinsic properties of the molecules like solubility, lipophilicity, volatility, and other physicochemical properties which make the molecules more or less available and prone to react with the environment. The other component is related to the medium where the degradation occurs and encompasses three major transformation processes: physical, chemical and biological. The main physical driving forces behind degradability of compounds are radiation, which promote photolysis, and heat. The pH and redox potential are among the main chemical driving forces which promote hydrolysis, oxidation, intramolecular rearrangement, hydrolytic dehalogenation, dialdehyde formation and reduction.[18] However, biodegradation remains the major transformation process. Traditionally, the biodegradation of a chemical refers to an 'ultimate' biodegradation. This means a full mineralization of a compound to CO_2 which would usually encompass several reactions and intermediate transformation products. However, in practice, since full mineralization of many compounds would take a long time, in some cases only the biodegradation or disappearance of the parent compound is followed.

Biodegradation processes are led by the diverse and abundant soil microbial communities encompassing three major domains: bacteria, archaea and fungi.[19,20] A remarkable and broad range of biotransformation processes has been identified in soil, which can be grouped into oxidative reactions (from frequent hydroxylations and oxidations to N-dealkylation); hydrolytic or oxidative scission of ether bonds, and various other hydrolysis reactions, including cleavage of heterocyclic rings.[21–23]

During the optimization of active ingredients, a large number of compounds are evaluated. Many share a core scaffold that holds the active site of the molecule and which is substituted with different moieties during the search for the most optimal APAI. In pursuance of green chemistry principles, synthetic chemical efforts are focused on including soft metabolic spots in the molecules of APAIs for biodegradability without compromising the desired technical efficacy against the main biological targets. Most common functional groups adopted include, for example, esters or nitrile groups, which easily undergo hydrolysis and thus improve the biodegradability of the parent APAI. Table 1 shows some of the less common substitutions in the scaffold of the chemophores leading to an improved biodegradability.

Given the wide structural diversity of APAIs in general but the close structural similarity when assessing substitutions in the chemical scaffold, ensuring the biodegradability of an APAIs is no easy task. At present, our understanding of biodegradation processes in soil is insufficient to provide a definitive *a priori* strategy to ensure biodegradability during early stages of R&D.

Moiety-1	Moiety-2	Improvement in biodegradability
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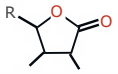
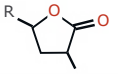
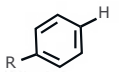
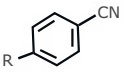
		+ + +
		+ + +
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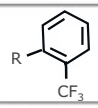
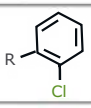
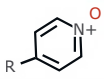
		+
		+
		+

Table 1. Overview of impact of changes in specific moiety on the chemical scaffold leading to an improvement of biodegradability. Symbols qualitatively indicate the degree of improvement of biodegradation when moiety group 2 is replacing moiety 1.

4. Challenges of the Current Testing Approaches for Biodegradability

Pharmaceutical and agrochemical R&D organizations have close similarities in the way they discover and optimize small molecules. The identification and registration of the final compound reaching the market is the result of a long selection process lasting ten years or more and with several stages and gate decisions. Fig. 2 shows the analogies between the two industries.

The innovation process hinges on the so-called Design-Synthesis-Testing-Analysis (DSTA) cycle. The identification of the best chemical lead for development from hundreds of thousands of chemicals is the result of a fine multi-criteria optimization process. It is in these early stages that the biodegradability should be included as key criterion in the rational chemical design. A broad catalog of potential microbial mediated reactions exists and can be considered to drive the chemical design in the early phase.[23,24]

The challenges are now exacerbated by an increasing concern about the hazard of transformation products. As mentioned before, most of the current efforts and regulations have solely focused on the degradation of a parent active ingredient. However, it has been shown that in some cases the transformation products could be more mobile, toxic or persistent than the parent compound. Such is the case for trifluoroacetic acid (TFA). Currently, the EU is working on a strict regulation proposal that applies to per- and polyfluorinated substances as a group, which could also include transformation products such as TFA. TFA is of high concern because of its high mobility and persistence, properties that facilitate the transport in water but make it difficult to remove from water. A German study in 2018 showed that TFA can be found in drinking water sources at concentrations up to 2.5 µg/L.[25] TFA is used to produce various industrial fluorinated compounds but can also be a result of the degradation of halogenated refrigerants and

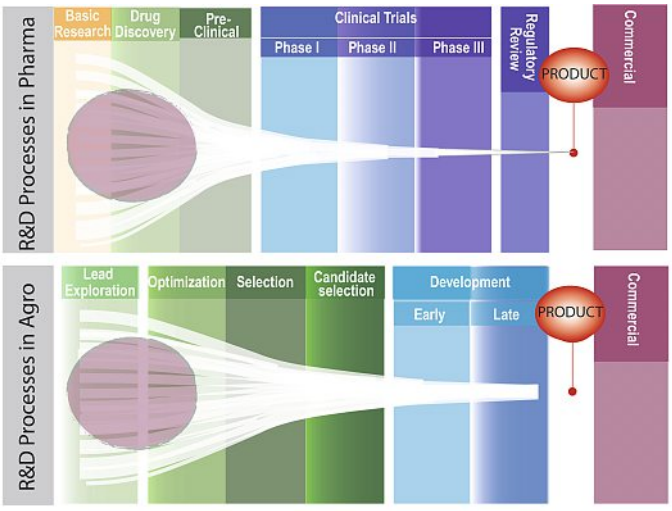


Fig. 2. R&D chemical innovation processes in pharma and agro-industries. The circles indicate where higher impact on the rational design for biodegradability can be expected.

blowing agents, plant protection products, pharmaceuticals, and biocides containing a C–CF_3 moiety.

There are currently six biocidals, 45 plant protection products, eight veterinary pharmaceutical ingredients and at least 39 human pharmaceuticals approved for use in the EU that could form TFA.[26] If the approved regulation would establish that TFA and other substances containing short-chain per- and polyfluorinated moieties are listed as substances of high concern under REACH, the commercialization of parent chemicals could be affected, as they could be banned or have severely restricted use. It is therefore foreseeable that in the future, the biodegradability will need to be assessed generally for the parent chemicals but also some of its transformation products. Considering these constraints, predicting the transformation products being formed is just as important as predicting the half-life of a parent active ingredient.

The currently available screening for biodegradability such as the enhanced ready biodegradability tests and complex simulation tests cannot be used in a cost-effective way in these early stages. More details around those tests will be provided in the following section. The ideal early-stage screening tests to assess biodegradability should:

- Provide reliable estimates of mineralization, half-life and/or transformation pathway;
- Be able to predict differences in biodegradability between analogs with high structural similarity;
- Be medium- or high- throughput (at least 1000 compounds per year) and short in duration to enable agile DSTA cycles;
- Use less than 5 mg of active ingredient, as only small amounts of each compound are synthesized in early stages.

4.1 *Main Approaches to Evaluate the Biodegradability of Small Molecules in Soil. OECD Guidelines and Other Methods*

For many regulatory frameworks worldwide, the assessment of persistence and/or biodegradability relies on the OECD screening and simulation guidelines for testing chemicals. Under the EU-REACH regulation the first tier assessment is performed based on laboratory studies according to the OECD 301,[27] OECD 302[28] and/or OECD 310[29] to determine if chemical is either 'not persistent' (*i.e.* readily or inherently biodegradable) or 'potentially persistent' (Fig. 3). In these tests, activated sludge or wastewater are used as a source of microbial inoculum. The dissolved organic carbon (DOC) declines, the O_2 consumption or CO_2 production are monitored for 28 to 60 days and compared to a theoretical chemical oxygen demand or inorganic carbon production. Since the endpoint is the degree of mineralization of a compound, it is assumed that a chemical that degrades well in this type of test will also degrade well in the environment, but not much can be concluded if the chemical is not readily or inherently biodegradable under the test criteria. Although the first-tier tests produce a reasonably useful endpoint and the equipment/measurements can be done with easily accessible technology (*e.g.* like OxiTop® , Xylem Analytics Germany), the duration of the test and the fact that only one compound can be screened in each batch would limit its implementation in R&D of APAIs.

If contradictory or negative results are obtained in the first tier, a higher tier assessment using OECD simulation tests (OECD 307,[30] OECD 308[31] and/or OECD309[32]) is performed. The OECD simulation tests are performed with either environmental soil or freshwater sediment and radiolabeled chemicals. The AI concentration is followed over a maximum of 100–120 days (for OECD 308 and 307 respectively), usually using LC-MS measurements. Degradation half-lives in soil, the overall water-sediment system or for the water and/or sediment compartment separately are obtained from these tests, along with major and minor transformation products that can be identified due to the radiolabeling. The obtained DT50s apply only to the parent compound but are the golden standard for comparison with threshold regulatory criteria for persistence. There is ample scientific debate on whether DT50s from simulation tests can be extrapolated to the environment and whether these are good benchmarks for persistence assessment, which should definitely be discussed further for regulatory purposes.[33–35] However, these tests are not applicable for the purpose of early screening of hundreds of thousands of chemical candidates in the R&D discovery process, due to their extreme time and resource demand.

Less stringent non-standardized tests in soil and water-sediment systems can improve the applicability of the simulation tests. Academic and industrial organizations recurrently perform tests based on OECD 307 or 308 but deviating from the standard by using non-radioactive compounds, often in mixes of 5–20. Although these non-standardized tests cannot be used for registration purposes, this flexibility alleviates some of the costs associated with the OECD test and still yields valuable DT50 information to assess biodegradability and to incorporate into DSTA cycles in advanced stages of product development.

4.2 *In silico Prediction*

Within the green chemistry goals, obtaining reliable *in silico* predictions of biodegradability would enable the R&D chain to speed up the DSTA cycles and to screen unlimited chemical variants with very little to no cost. To this end, many platforms have

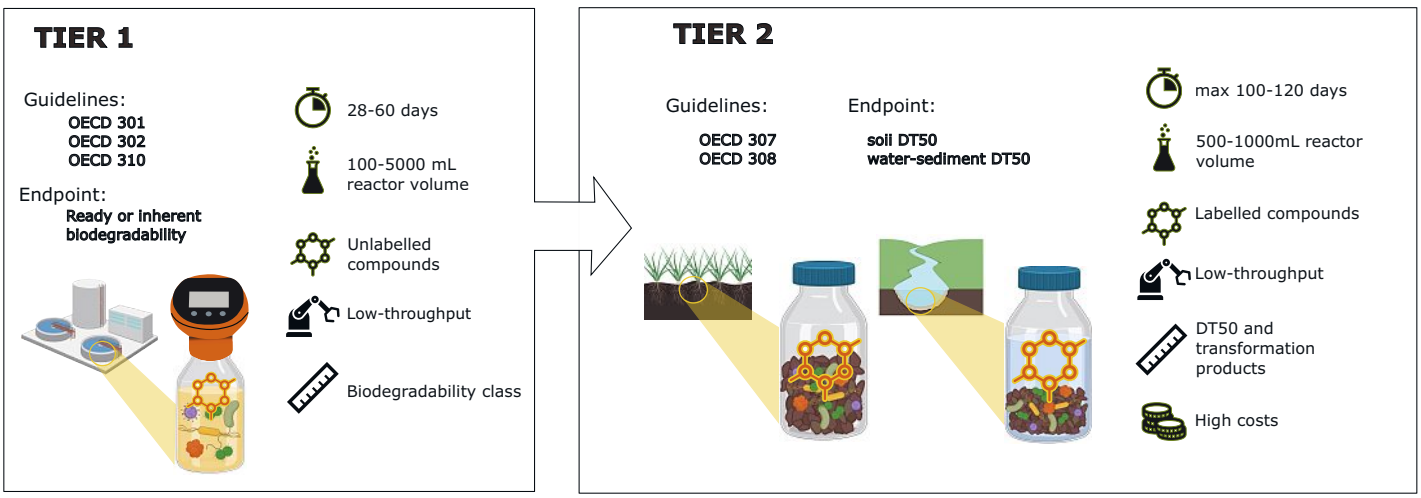


Fig. 3. Overview of the main standard test of OECD to assess chemical persistence and biodegradability in regulatory frameworks. DT50 refers to the degradation half-life of the parent active ingredient.

been developed by different organizations to facilitate estimation of different endpoints of biodegradability. In Table 2 we have compiled a list of the most notable efforts related to estimating environmental biodegradability or biodegradation pathways.

Beyond the algorithm used, the training data plays a crucial role in the prediction power and applicability domain of the *in silico* models. We highlight that one of the most used sources for training the models are the OECD or similar regulatory outcomes (used in *e.g.* BIOWIN, CATALOGIC, enviPath, OPERA, VEGA). While this ensures a relatively high quality of the training data, it restricts the chemistry coverage to mainly plant protection products and particular industrial chemicals. This results in poor prediction performance when the models are applied to chemical classes not included in the training data, such as pharmaceutical ingredients, ionized or very lipophilic compounds.

Although the real-world applicability of these models into the optimization of new AI in industry is still hampered by the low prediction power of the biodegradability of novel chemical classes, there is consistent improvement over time. We have observed that the OPERA model performs significantly better than its predecessor EPI-Suite to predict the organic carbon-water partition coefficient (Koc) of chemicals. Also, given new ideas to combine artificial intelligence models with computational chemistry parameters to overcome applicability domain limitations, particularly in low data regimes, there is a real possibility that these efforts will yield more reliable predictions of the environmental fate of chemicals over time.[36,37]

Name (Organisation)	Process	Output	Training data	Compound classes	Ref.
BioTransformer (TMIC)	Environmental microbial degradation	Transformation products (Phase I and II)	MetXBioDB	APAIs, toxins, phytochemicals, sterol lipids	[38]
BIOWIN (US-EPA)	Water and soil aerobic / anaerobic biodegradability	Biodegradability class, biodegradation half-lives	OECD biodegradability data	Organic chemicals	[39]
CATALOGIC (Oasis LMC)	Soil	Pathway-transformation products, estimated quantity per mol of parent	Literature and public data (EFSA database, CSCL-Japan)	Unclear	[40]
Chemical Transformation Simulator (US-EPA)	Unclear	Pathway-transformation products, estimated physicochemical properties for parent and/or transformation products	ChemAxon Metabolizer library	Organic chemicals	[41]
EAWAG-BBD (eawag)	Environmental microbial degradation (EAWAG-BBD)	Pathway-transformation products	Biotransformation rules (formerly UM-BBD) enzymatic reactions	Organic chemicals	[42]
EnviPath (EnviPath & University of Zurich)	Environmental, soil- or sludge-specific microbial degradation	Pathway-transformation products	EAWAG-BBD, EFSA DARs dossiers with soil degradation pathways	APAIs and other organic pollutants	[23, 43]
MetabolExpert (CompuDrug)	Human, animal, or plant metabolization	Pathway-transformation products	Literature?	Unclear	[44]
OPERA (US-EPA)	Environmental degradability in OECD data	Biodegradability half-life	OECD biodegradability data	Organic chemicals	[45]
PathPred (KEGG)	Environmental microbial degradation, plant metabolization	Pathway-transformation products	KEGG RPAIR	Xenobiotics	[46]
VEGA (ECHA)	Environmental degradability in REACH data	Biodegradability half-life	REACH biodegradability data (ready, inherent biodegradability and simulation tests)	Organic chemicals	[47]

Table 2. Overview of the most common platforms to predict biodegradability and biotransformation products.

5. New Approaches to Estimate Biodegradability in R&D of AIs

In light of the current need to screen the biodegradability of large numbers of chemicals in the discovery phase of active ingredients for agrochemical and pharmaceutical uses, new methodologies with medium or high throughput must be developed. We review here three published methodologies that would have potential to be embedded in chemistry design workflows.

5.1 *Read-across from Activated Sludge*

Based on multiple experiments using activated sludge,[48–50] we propose a different way to use degradation in activated sludge as a proxy for degradation in the environment. The read-across assay consists of small batch reactors of 50 ml of activated sludge, incubated for 48–72 h. A benchmark set of 20 chemicals and test sets of up to 50 chemicals are spiked as a mix into the reactors at environmentally relevant concentrations (*i.e.* < 20 µg/L), therefore using < 2 mg of each active ingredient. The concentrations of the spiked chemicals are monitored at multiple timepoints using LC-MS. Regression and classification models are built using the known DT50s (from standard OECD307 or OECD308), the experimentally observed half-life in activated sludge (DT50 sludge) of the benchmark compounds (Fig. 4), as well as their experimental or predicted Koc. These models can then be applied to the compounds in the test set to predict their OECD 307 or 308 DT50s or a biodegradability class (persistent, not persistent) based on their experimentally observed half-lives in the activated sludge assay.

We have observed consistency in the regression models calibrated with the benchmark models along experiments with activated sludge performed between 2018 and 2023 (Fig. 4), all reaching correlation $R^2 > 70\%$ and low errors (RMSE < 0.5). When applying the models to predict the biodegradability in soil (OECD 307) of an independent test set of 26 plant protection products, we obtained correlations of 70% between measured and soil DT50s predicted with the regression model and a robust sensitivity (>80%) to predict non-persistent behavior according to a DT50 threshold of 70 days in the classification models. When applying the approach to predict the biodegradability in water-sediment systems (OECD 308) of an additional test set of 46 pharmaceutical ingredients with a wide structural diversity, we found that the prediction performance of regression models decreased (>65%). The classification sensitivity also decreased but still represent a valuable starting point for exploring their application to these types of active ingredients.

The small reactors with activated sludge can easily be adapted to emulate different environmental conditions such as pH, temperature, organic matter or nutrient content. Sterilized and unspiked controls can be added to complement the understanding of the abiotic behavior of the compounds. Even with a small set of reactors, the most basic version of the assay would be able to produce information on 100 compounds per month, which can provide a new medium-throughput way to screen for persistent chemicals at an early stage.

The read-across approach can also be used for screening for transformation products if combined with measurements in LC-HRMS,[50] and thus also provide mechanistic insights on the biotransformation. These data in turn can be used as training data for developing *in silico* models and can be produced in higher throughput than OECD tests to help improve model performance.

There is evidence of the overlap between biotransformation functions in activated sludge, soil and aquatic ecosystems. Some functions might have originated in wastewater but are now ubiquitous in the environment, like the degradation of the artificial sweetener acesulfame-K;[51] whereas some functions such as the hydrolytic dichlorination of triazine-rings (*e.g.* present in atrazine or terbuthylazine), were first observed in the environment,[52] but were found later in activated sludge.[53] Therefore, we should find a way to systematically explore the functional overlap between activated sludge and environmental microbiomes to maximize the information that can be taken from activated sludge. Overall, this approach has the potential to be integrated into the broader DSTA framework not only to screen for biodegradability, but to improve existing *in silico* methods for prediction of biodegradability and biotransformation pathways.

5.2 *Small-scale Assays*

The microplate-based assays of the ProbaBio[54,55] type also present an attractive alternative for a more 'compact' screening of the biodegradability of APAIs in R&D. This specific approach focuses on predicting the output of the ready biodegradability test from OECD 301 by performing analogous measurements of dissolved oxygen consumption (through a non-invasive optical sensor) over 28 days. Several environmental conditions such as temperature, substrate addition of glucose and inoculum originating from activated sludge, freshwater, soil, and seawater have been tested. In line with OECD 301, the theoretical oxygen demand can be calculated from the amount of substance added and compared to the measured oxygen demand (calculated with the dissolved oxygen measurements).

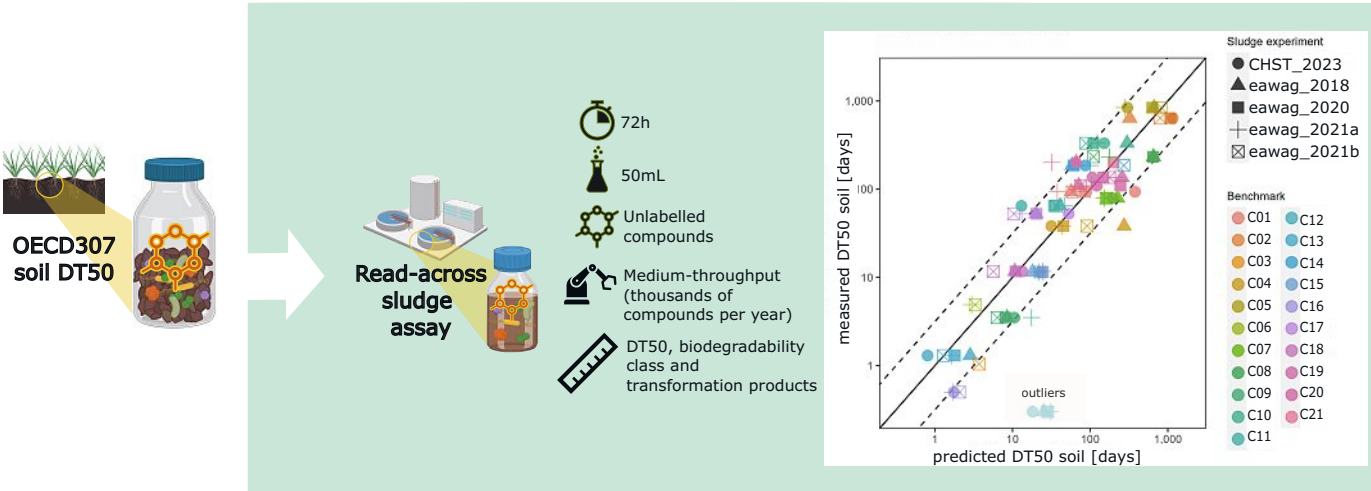


Fig. 4. Regression models produced in the read-across approach with benchmark compounds in five independent activated sludge assays from 2018 to 2023. The models were fitted with the respective DT50 soil from OECD 307, DT50sludge of each experiment and the partition coefficient logKoc from OECD 106.

A potential limitation of the microplate approach is that only relatively simple chemical classes have been tested (*i.e.* only six chemicals were screened throughout one year: sodium benzoate, 4-nitrophenol, diethylene glycol, 2,4,5-trichlorophenol, atrazine, and glyphosate). Although it is important to test the performance of these type of approaches with more diverse structures, this method could lead to promising prospects for future practical applications.

5.3 *Modified Zahn-Wellens Test*

The Zahn-Wellens test described in the OECD 302B to determine inherent biodegradability has been adapted at the University of Applied Sciences and Arts Northwestern Switzerland to enhance the functionality and applicability. This alternative inherent degradation test[56] (AIA in German) uses DOC measurements and has been validated with activated sludge from many wastewater treatment plants across Switzerland. The AIA test has given comparable results to the standard OECD 302B but the duration of the test was significantly shortened to 7 days. It requires a lower concentration of the active ingredient but still uses a rather large reactor volume (1000 mL). It has so far been applied to track the overall biodegradability of input wastewaters in wastewater treatment plants. If applied to individual or mixes of active ingredients, it could provide information on the mineralization of research compounds that could be taken into account during their optimization.

5.4 *Framework for New Methodologies*

The technological and knowledge advances in disciplines related to biodegradability make the time right to explore new concepts and embrace the safe and sustainable by design concept. The combination of new *ad-hoc* design testing concepts like the read-across activated sludge assay in combination with the emerging field of *in silico* prediction (as depicted in Fig. 5) can represent the basis of a new standard to be adopted by the chemical industry. The ultimate goal would be to enable rational design of environmentally friendly molecules and a better alignment with the green chemistry principles.

6. Conclusions

Biodegradability is an important feature for small molecules with practical application in agriculture and/or animal/human health. We observe and can foresee further increasing regulatory requirements for biodegradability of APAIs. The implementation of the safe and sustainable by design principles has become critical and will help the chemistry industry to play an active role in innovation and offering new solutions to address the challenges of the 21st century.

New approaches and mindsets are available today and they can offer new opportunities to support the chemical design. A combination of predictive tools and wet-lab experimentation is essential to achieve these goals. We hope this contribution promotes the discussion on how the chemical industry can embrace these new challenges and be part of the solution.

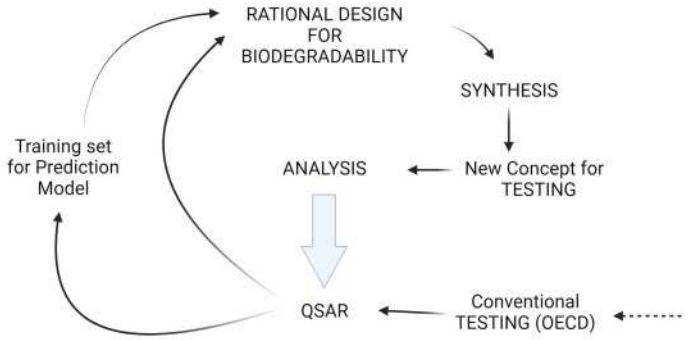


Fig. 5. A new vision for a rational design for biodegradability.

Received: September 15, 2023

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