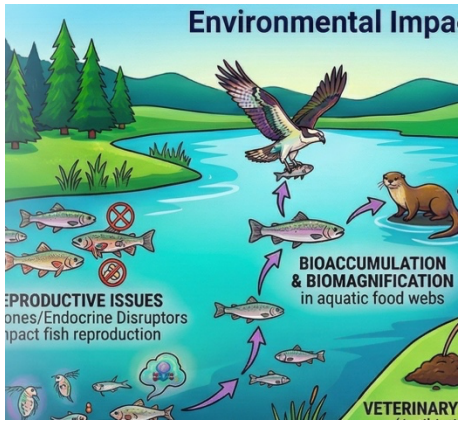
Workshop Summary: In Silico and In Vitro Approaches to Assess Biodegradability



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This workshop, held under the **OneHealthdrugs COST Action**, focused on integrating environmental impact assessments—specifically biodegradability and ecotoxicity—into the early stages of drug discovery. The session bridged the gap between medicinal chemistry (human efficacy) and environmental science (ecological safety).

Key Presentations & Insights

1. Green Drug Score for Compound Ranking

- **Presenter:** Daniele Aiello (University of Modena)
- **Core Concept:** Introduction of the **Green Drug Score (GRECO)**, a scoring function that balances ADME properties, drug-likeness, and environmental toxicity.
- **Case Studies:** Applied to Trypanosomiasis (T-Brucei) and Leishmaniasis projects. Results showed that including environmental metrics rearranged compound rankings by ~30%, effectively de-prioritizing "safe-for-human" hits that were ecologically harmful.

2. In Vivo/In Vitro Evaluation of Biodegradation

- **Presenter:** Prof. Gerald Thouand (University of Nantes)
- **Critical Critique:** Highlighted that standard OECD tests (e.g., 301B/D) are often outdated (60+ years old) and treat microbial inoculums as a "black box."
- **Recommendations:** Proposed using **multi-sensor arrays** (CO2/O2/biomass) and specific microbial fingerprints to predict persistence. He emphasized that researchers should test biodegradability in-house *before* expensive regulatory standard tests.

3. Bioavailability in Persistence Testing

- **Presenter:** Jose Julio Ortega Calvo (CSIC, Seville)
- **Technical Focus:** Discussed how **bioavailability** (the fraction of a chemical accessible to microorganisms) is often ignored in risk assessments.
- **Findings:** Hydrophobic compounds (like many drugs) can "age" in soil, becoming less available for degradation but also less acutely toxic. He proposed integrating ISO-standardized extraction methods into existing biodegradation assays.

4. In Silico Annotation & Knowledge Graphs

- **Presenter:** Regan Karki (Fraunhofer ITMP)
- **Tools:** Demonstrated how to use virtual screening (e.g., Lipinski's Rule of Five) and **Knowledge Graphs** to fetch data from public repositories (ChEMBL, PubChem, EcoTox).
- **Visualization:** Used TSNE plots to show how compounds cluster based on physical-chemical properties versus biological activity.

5. Innovations in Bioassays

- **Presenter:** Sheraz Gul (Fraunhofer ITMP)

- **Practical Kits:** Identified low-cost, miniaturized kits (e.g., **Biotox/Microtox**) that use bioluminescent bacteria to measure toxicity.
- **Throughput:** Noted that while in silico tools are efficient, they can be "oversold" and must be validated by in vitro data. He offered to screen compounds for COST Action members (1–2 mg required).

6. Metabolite Prediction & The GAIA Platform

- **Presenter:** Eleni Chontzopoulou (CloudFarm)
- **Metabolic Impact:** Focused on how Phase I and Phase II metabolites—and gut microbiome transformations—can reactivate drugs or create new toxicities.
- **GAIA Platform:** A tool integrating **GLORIA rule-based software** to predict human metabolites and subsequently calculate their specific ecotoxicity and bioaccumulation scores.

Major Themes & Discussion

- **Timing of Testing:** A debate occurred between Prof. Thouand and Paul Selzer regarding *when* to test. While medicinal chemists often wait for lead optimization to avoid wasting resources, environmental scientists argued that waiting too long risks developing a "dead-end" molecule that will fail regulatory environmental standards.
- **The "Metabolite Gap":** There is a significant need for better infrastructure to identify and test metabolites, as the parent drug is rarely the only substance entering the environment.
- **Data Scarcity:** A recurring point was the lack of experimental ecotoxicity data needed to train more reliable AI/ML models.

Conclusion

The workshop concluded that a **"Green-by-Design"** approach is essential. By utilizing early-stage in silico filters (like GDS) and miniaturized in vitro kits (like Biotox), researchers can prioritize chemotypes that are both therapeutically effective and environmentally degradable.

Next Steps: Integration of these tools into the COST Action database and continued cross-border collaboration for compound screening at Fraunhofer.

Details about the presentations

The workshop was less a dry technical meeting and more an urgent call to arms for the "Green-by-Design" movement. Spanning over three hours, the atmosphere was one of high-stakes problem-solving, as medicinal chemists and environmental microbiologists attempted to bridge a historical divide: the gap between creating a life-saving drug and ensuring that same drug doesn't devastate an ecosystem once it leaves the human body.

The Opening: A Shift in Perspective

The session opened with a sobering reminder from **Daniele Aiello** and **Paola Costi**. They didn't just talk about data; they spoke of "environmental failure." By showing how common drugs like Diclofenac have brought bird species to the brink of extinction, they set a moral tone for the technical work. Aiello's introduction of the **GDS (Green Drug Score)** felt like a turning point—a mathematical attempt to force scientists to look at a molecule's "ecological footprint" at the same moment they look at its "healing potential."

The Scientific "Black Box"

One of the most narrative-driven segments came from **Prof. Gerald Thouand**, who challenged the status quo with the energy of a reformer. He painted a picture of current regulatory tests as "relics" from sixty years ago—clunky, expensive, and often wrong. He described the microbial world not as a simple tool, but as a complex, cooperative "black box." His message was clear: we are currently throwing expensive chemicals at microscopic communities we don't fully understand, often resulting

in "false negatives" that kill potentially great drugs or "false positives" that let persistent pollutants slide through.

The Invisible Shield: Bioavailability

The narrative shifted to the soil with **Jose Julio Ortega Calvo**. He introduced a hidden character in the story of pollution: **Time**. He described how a drug doesn't just sit in the water; it hides. It "ages" into the microscopic nooks and crannies of soil and sediment, becoming an invisible ghost—less toxic to some creatures, but impossible for bacteria to find and destroy. This "hide-and-seek" dynamic, he argued, is why our current persistence tests often fail to reflect the reality of the Earth's crust.

The Digital Twin and the Laboratory

As the meeting moved into the afternoon, **Regan Karki** and **Sheraz Gul** represented the modern digital-meets-physical laboratory. Karki showcased "Knowledge Graphs"—vibrant, interconnected webs of data that look like digital constellations—mapping out every known interaction of a molecule.

However, a brief but pointed debate broke out when **Paul Selzer** challenged the timing of these tests. He spoke for the "traditional" chemist, arguing that testing for environmental safety too early might stifle innovation and "kill" good hits before they can be optimized. This created a palpable tension: **How do we balance the desperate need for new medicines with the desperate need for a clean planet?**

The Final Frontier: Metabolites

The workshop closed with **Eleni Chontzopoulou** highlighting a final, often overlooked complication: **Transformation.** She reminded the group that the drug we swallow is rarely the drug the fish encounter. Through the human liver and the "reactivations" caused by gut bacteria, drugs are constantly changing shape. Her work with the **GAIA platform** felt like the final piece of the puzzle—a way to predict these "shapeshifting" chemicals before they ever reach a wastewater treatment plant.

The Closing Sentiment

By the time Paola Costi rejoined to close the session, the takeaway was clear. The "One Health" approach isn't just a buzzword; it's a difficult, interdisciplinary puzzle. The workshop ended not with a simple "mission accomplished," but with an open invitation for collaboration—a recognition that no single chemist, biologist, or data scientist can solve the problem of environmental toxicity alone.

To describe this workshop with scientific completeness, we must analyze the **mechanistic intersection** of medicinal chemistry, environmental microbiology, and computational toxicology. The central challenge addressed was the **Persistence, Bioaccumulation, and Toxicity (PBT)** assessment of pharmaceuticals.

The following is a high-level scientific synthesis of the three-hour proceedings:

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1. The Chemocentric View: Molecular Prioritization

The workshop opened with a shift from classical **Structure-Activity Relationship (SAR)**—which focuses solely on human therapeutic targets—to **e-SAR (Environmental SAR)**.

- **The GDS Scoring Algorithm:** Daniele Aiello presented a multi-parametric scoring function. Scientifically, this is a **weighted non-linear aggregation** of disparate endpoints. It integrates (lipophilicity), (bioconcentration factor), and (lethal concentration for 50% of a population) across multiple trophic levels (algae, daphnia, fish).
- **The De-prioritization Phenomenon:** The "narrative" of the data showed that high human efficacy often correlates with high environmental persistence. This is because "sturdy" molecules designed to survive the acidic environment of the human stomach and first-pass metabolism in the liver are, by definition, resistant to microbial enzymatic cleavage in the wild.

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2. The Biocentric View: Kinetics of Mineralization

Prof. Gerald Thouand's contribution moved the discussion into **enzyme kinetics and microbial ecology**.

- **Ready vs. Inherent Biodegradability:** A "Ready" test (OECD 301) requires a compound to reach 60% mineralization (measured as —Theoretical Oxygen Demand or —Theoretical Carbon Dioxide production) within a 10-day window of a 28-day study.
- **The Inoculum Effect:** Thouand argued that the standard "Activated Sludge" used in these tests is genetically drift-prone. He proposed using **metagenomic profiling** to characterize the "biodegradative potential" of the bacterial community. If the specific oxygenases or hydrolases required to break a specific drug's amide or ester bonds aren't expressed in the test batch, the drug is labeled "persistent" (a false negative), when in reality, the environment simply lacked the "primed" biomass.

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3. The Geospatial View: Bioavailability and Sequestration

Jose Julio Ortega Calvo introduced the **mass-transfer limitations** of biodegradation.

- **Desorption-Limited Degradation:** The rate-limiting step for drug removal in soil is often not the microbial metabolic rate (), but the **desorption rate** from the soil matrix.
- **The Hysteresis of Aging:** As organic molecules reside in soil, they undergo "aging," moving from easily exchangeable sites to internal micropores or becoming encapsulated in humic polymers.
- **Tenax Extraction ():** Scientifically, they utilized Tenax-TA beads as an "infinite sink" to measure the **bioaccessible fraction**. This distinguishes between a molecule that is *chemically* persistent and one that is merely *physically* sequestered.

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4. The Toxicological View: The Metabolic Continuum

The final segment addressed the **Metabolite-Parent Relationship**.

- **Phase I and II Biotransformation:** Eleni Chontzopoulou's description of the **GAIA platform** utilized rule-based systems to predict **Phase I (functionalization)** and **Phase II (conjugation)** metabolites.
- **The "Pro-Ecotoxicant" Risk:** A critical scientific point raised was **de-conjugation**. Many drugs are excreted as glucuronides (biologically inactive). However, environmental bacteria possess **-glucuronidase enzymes** that can cleave the sugar moiety, effectively "re-activating" the drug in the aquatic environment.
- **Predictive Toxicology:** The use of **Knowledge Graphs** (Karki) and **High Content Imaging** (Gul) allows for "Phenotypic Profiling." By observing "Cell Painting" patterns, researchers can identify **Mode of Action (MoA)** for ecotoxicity—such as mitochondrial disruption or oxidative stress—before conducting expensive animal testing.

Summary of the "Scientific Conflict"

The workshop concluded on a technical impasse regarding **Lead Optimization**. If a medicinal chemist adds a halogen (like Fluorine or Chlorine) to a molecule to increase its half-life in a patient, they simultaneously increase its **electronegativity and metabolic stability**, often making it "recalcitrant" (non-biodegradable).

The goal for the "One Health" project is to find the **"Isokinetic Window"**: a chemical space where the molecule is stable enough for human therapy (of hours) but unstable enough for microbial degradation in a wastewater plant (of days).