



*“The greatest threat to our planet is the belief that someone else will save it.”<sup>1</sup>*

Robert Swan (1956-), the first person to walk to both the North and South Poles.

*“The future will either be green or not at all.”*

Bob Brown (1944-), former leader of the Australian Greens

Food for Thought ...

# Alternative Methods Go Green! Green Toxicology as a Sustainable Approach for Assessing Chemical Safety and Designing Safer Chemicals

Alexandra Maertens<sup>1</sup>, Thomas Luechtefeld<sup>1,2</sup>, Jean Knight<sup>3</sup> and Thomas Hartung<sup>1,4</sup>

<sup>1</sup>Center for Alternatives to Animal Testing (CAAT), Bloomberg School of Public Health, Johns Hopkins University, Doerenkamp-Zbinden-Chair for Evidence-based Toxicology, Baltimore, MD, USA; <sup>2</sup>Insilica LLC and ToxTrack Inc., Bethesda, MD, USA; <sup>3</sup>Consultant, Half Moon Bay, CA, USA, <sup>4</sup>CAAT-Europe, University of Konstanz, Konstanz, Germany

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Correspondence:  
Thomas Hartung, MD, PhD  
Center for Alternatives to Animal  
Testing (CAAT)  
Johns Hopkins University  
615 N Wolfe St.,  
Baltimore, MD, 21205, USA  
(THartun1@jhu.edu)



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## Abstract

Green toxicology is marching chemistry into the 21<sup>st</sup> century. This emerging framework will transform how chemical safety is evaluated by incorporating evaluation of the hazards, exposures, and risks associated with chemicals into early product development in a way that minimizes adverse impacts on human and environmental health. The goal is to minimize toxic threats across entire supply chains through smarter designs and policies. Traditional animal testing methods are replaced by faster, cutting-edge innovations like organs-on-chips and artificial intelligence predictive models that are also more cost-effective. Core principles of green toxicology include utilizing alternative test methods, applying the precautionary principle, considering lifetime impacts, and emphasizing risk prevention over reaction. This paper provides an overview of these foundational concepts and describes current initiatives and future opportunities to advance the adoption of green toxicology approaches. Challenges and limitations are also discussed. Green shoots are emerging with governments offering carrots like the European Green Deal to nudge industry. Noteworthy, animal rights and environmental groups have different ideas about the needs for testing and their consequences for animal use. Green toxicology represents the way forward to support both these societal needs with sufficient throughput and human relevance for hazard information and minimal animal suffering. Green toxicology thus sets the stage to synergize human health and ecological values. Overall, the integration of green chemistry and toxicology has potential to profoundly shift how chemical risks are evaluated and managed to achieve safety goals in a more ethical, ecologically-conscious manner.

## Plain language summary

Green toxicology aims to make chemicals safer by design. It focuses on preventing toxicity issues early during development instead of testing after products are developed. Green toxicology uses modern non-animal methods like computer models and lab tests with human cells to predict if chemicals could be hazardous. Benefits are faster results, lower costs, and less animal testing. The principles of green toxicology include using alternative tests, applying caution even with uncertain data, considering lifetime impacts across global supply chains, and emphasizing prevention over reaction. The article highlights European and US policy efforts to spur sustainable chemistry innovation which will necessitate greener approaches to assess new materials and drive adoption. Overall, green toxicology seeks to integrate safer design concepts so that human and environmental health are valued equally with functionality and profit. This alignment promises safer, ethical products but faces challenges around validating new methods and overcoming institutional resistance to change.

<sup>1</sup> <https://www.activesustainability.com/environment/robert-swain-and-our-planet/>



## 1 Introduction

Traditional toxicity testing and chemical risk assessment paradigms have relied heavily on whole animal testing and high-dose experiments (Hartung, 2017). However, there are growing calls within the toxicology community for new approaches that address ethical concerns over animal use and provide more human-relevant safety data to keep pace with the rapid expansion of the chemicals marketplace (Crawford et al., 2017; Schmidt, 2009). Green toxicology has emerged as a promising framework to help guide this transformation. It aims to incorporate key sustainability principles from green chemistry while protecting human and environmental health (Maertens et al., 2014). Janine M. Benyus (1958-), an American natural sciences writer, summarized it, “*Green chemistry is replacing our industrial chemistry with nature’s recipe book. It’s not easy, because life uses only a subset of the elements in the periodic table. And we use all of them, even the toxic ones*”.

Green toxicology is a concept that refers to the application of predictive toxicology in the sustainable development and production of new, less harmful chemicals and materials (Maertens et al., 2014, 2021; Crawford et al., 2017; Krebs and McKeague, 2020). Its goal is to minimize potential toxicity as early in production as possible, by integrating the principles of toxicology into designing safer chemicals. The approach emphasizes the use of 21<sup>st</sup> century toxicology tools as a preventative strategy to “design out” undesired human health and environmental effects, thereby increasing the likelihood of launching a successful, sustainable product. Green toxicology is an emerging discipline that is currently spreading internationally and being refined via an iterative process. Its unique emphasis is on driving innovation by moving safety considerations to the earliest stage in a chemical’s lifecycle, i.e., to molecular design. The concept of green toxicology is closely related to the green chemistry movement, which embraces similar ideas for the development of less toxic products, safer processes, and less waste and exposure (Maertens et al., 2021). Amy Cannon, Executive Director of Beyond Benign, put it this way, “*Green chemistry represents the pillars that hold up our sustainable future. It is imperative to teach the next generation to think about chemistry in a new light*”.

The concern that chemicals contribute to cancer is probably the most common fear among the general public. Bruce Ames (2020) discusses the causes of cancer and the history of cancer research. A third of untreated rodents have cancers at the end of their life, which necessitates the use of large numbers of rodents in cancer studies. In the past, occupational chemical exposures were a source of some cancers. This led to animal testing using very high doses of chemicals. However, high dose testing gives misleading results that should not be extrapolated to typical human exposures. Ames and team set up a carcinogenic potency database analyzing over 6,000 animal cancer tests. Key findings were that over half of all chemicals tested, both synthetic and natural, caused cancer at the high doses used in studies. This suggests the high cancer rates in the animals are an artifact of the testing methods. He argues against the assumptions made by Rachel Carson in *Silent Spring*, such as the idea that “natural” chemicals

are inherently safe. Many naturally occurring pesticides and other chemicals can be carcinogenic or toxic. Rather than focusing on small hypothetical cancer risks from synthetic chemicals, Ames argues that more attention should be paid to known major disease risks like unhealthy diets. Deficiencies in vitamins and minerals trigger mechanisms that accelerate aging and diseases like cancer. Supplementation and improved diet could significantly reduce chronic disease risk.

A key problem is the enormous number of chemicals in consumer products. A study by Phillips et al. (2018) ground up 100 consumer products, including formulations (e.g., shampoos, paints), articles (e.g., upholsteries, shower curtains), and foods (e.g., cereals), and found 4270 unique chemical signatures across the products, with 1602 signatures tentatively identified, of which 1404 were not present in a public database of known consumer product chemicals.

Green toxicology provides solutions to the limitations of traditional toxicology, including a focus on animal welfare, human health protection, ecological impacts across chemical lifecycles, and hazard prevention over reaction (Maertens, 2022). This new paradigm has potential to profoundly shift how chemical risks are evaluated and managed to achieve safety goals in a more ethical, ecologically-conscious manner. However, adopting green toxicology approaches also faces challenges around validating alternative methods, risk communication, and policy reform. Green toxicology differs from traditional toxicology in several ways:

1. *Focus*: Traditional toxicology primarily focuses on assessing the toxicity of existing chemicals and substances, often after they have been released into the environment or used in products. Green toxicology, on the other hand, emphasizes the early integration of toxicology principles into the design and development of new chemicals and materials. The goal is to identify and mitigate potential toxicity risks at the molecular design stage, thus preventing the production of harmful substances in the first place.
2. *Preventative approach*: Green toxicology takes a proactive and preventative approach to minimize potential toxicity. It utilizes 21<sup>st</sup> century toxicology tools and predictive models to “design out” undesired human health and environmental effects. By identifying and addressing potential toxicity issues early in the development process, green toxicology aims to increase the likelihood of launching safe and sustainable products.
3. *Sustainability*: Green toxicology is closely aligned with the principles of sustainability. It seeks to promote the development and production of new, less harmful chemicals and materials that have reduced environmental impacts. This aligns with the broader goals of the green chemistry movement, which aims to create safer products, processes, and reduce waste and exposure.
4. *Integration*: Green toxicology integrates toxicology principles with other disciplines, such as chemistry, materials science, and engineering. This interdisciplinary approach allows for a more holistic understanding of the potential hazards and risks associated with chemicals and materials, enabling the development of safer alternatives.

**Tab. 1: Green chemistry's 12 principles** (emphasis added to show links to toxicity)

1. Prevent waste: Design chemical syntheses to prevent waste. Leave no waste to treat or clean up.
2. Maximize atom economy: Design syntheses so that the final product contains the maximum proportion of the starting materials. Waste few or no atoms.
<b>3. Design less hazardous chemical syntheses: Design syntheses to use and generate substances with little or no toxicity to either humans or the environment.</b>
<b>4. Design safer chemicals and products: Design chemical products that are fully effective yet have little or no toxicity.</b>
5. Use <b>safer solvents</b> and reaction conditions: Avoid using solvents, separation agents, or other auxiliary chemicals. If you must use these chemicals, use safer ones.
6. Increase energy efficiency: Run chemical reactions at room temperature and pressure whenever possible.
7. Use renewable feedstocks: Use starting materials (also known as feedstocks) that are renewable rather than depletable. The source of renewable feedstocks is often agricultural products or the wastes of other processes; depletable feedstocks are often fossil fuels (petroleum, natural gas, or coal) or mining operations.
8. Avoid chemical derivatives: Avoid using blocking or protecting groups or any temporary modifications if possible. Derivatives use additional reagents and generate waste.
9. Use catalysts, not stoichiometric reagents: Minimize waste by using catalytic reactions. Catalysts are effective in small amounts and can carry out a single reaction many times. They are preferable to stoichiometric reagents, which are used in excess and carry out a reaction only once.
10. Design chemicals and products to degrade after use: Design chemical products to break down to innocuous substances after use so that they <b>do not accumulate in the environment</b> .
11. Analyze in real time to <b>prevent pollution</b> : Include in-process, real-time monitoring and control during syntheses to minimize or eliminate the formation of byproducts.
12. Minimize the potential for accidents: Design chemicals and their physical forms (solid, liquid, or gas) to minimize the potential for chemical accidents including explosions, fires, and <b>releases to the environment</b> .

5. *Innovation*: Green toxicology drives innovation by placing safety considerations at the forefront of the design process. By integrating toxicology early on, researchers and developers can identify potential toxicity issues and make informed decisions to modify or eliminate harmful chemical structures. This promotes the development of novel, safer, and more sustainable products.

6. *Avoiding regrettable substitutions*: Some instances have come to light, where problematic substances have simply been replaced by less tested ones, not necessarily less problematic ones (Maertens et al., 2021).

In summary, green toxicology differs from traditional toxicology by emphasizing early integration of toxicology principles into the design and development of new chemicals and materials, taking a preventative approach, promoting sustainability, integrating multiple disciplines, and driving innovation.

This paper provides an overview of core concepts, current initiatives, and future opportunities and challenges associated with adopting green toxicology approaches. First, green chemistry principles are briefly summarized to provide context. Then, key pillars of green toxicology are described including use of alternative test methods, application of the precautionary principle, consideration of lifetime impacts and focusing on prevention. Ongoing efforts to advance green toxicology adoption are highlighted. Finally, remaining barriers and limitations are discussed.

## 2 Green chemistry principles

Green chemistry is defined as “*the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances*” (Anastas and Eghbali, 2010). This is achieved through application of 12 guiding principles (Tab. 1) that aim to reduce risk and hazards across the entire lifecycle of a chemical product from design, manufacture, use, and disposal (Anastas and Warner, 1998).

Since emerging in the 1990s, adoption of green chemistry practices has increased substantially by industries, academia, and government entities. This has driven innovation in cleaner production technologies and increased availability of safer, more sustainable consumer products. Some examples include the use of supercritical CO<sub>2</sub> as a green solvent, aqueous-phase catalysis, and atom economy approaches that maximize incorporation of all materials used in the synthesis of a chemical product (Clark et al., 2008). Lackmann et al. (2021) showed how to incorporate toxicological assessments into chemical developmental processes to achieve a sustainable and safe production of catalysts.

However, incorporation of sustainability concepts in chemical hazard and risk assessment has lagged behind technological advances in green synthesis and manufacturing processes (Maertens et al., 2014). Green toxicology aims to address this gap by evaluating potential impacts of chemicals through a “green” lens.



While green chemistry focuses on solutions for less hazardous molecular design, green toxicology expands this approach along the entire supply chain to inform safer development, use, regulation, and disposal (Matus et al., 2012).

### 3 The fail-early-fail-cheap paradigm

The pharmaceutical industry has embraced the mantra of “fail early, fail cheap” in drug development (Keserü and Makara, 2006; McKim, 2010). This means terminating unpromising drug candidates as early as possible before investing in lengthier and more expensive clinical trials. To enable this, the industry relies heavily on frontloading toxicology assessments in the discovery pipeline.

Frontloading toxicology refers to conducting safety testing prior to candidate selection during the hit-to-lead and lead optimization stages. This approach is aimed at identifying and addressing potential safety concerns before a candidate is selected for further development (Beilmann et al., 2019). The goal is to improve the likelihood of success by optimizing the safety profile of drug candidates as early as possible. This contrasts with traditional sequential approaches where in-depth toxicology testing occurred later, after a clinical candidate had been chosen. Frontloading toxicology enables rapid screening and triaging to exchange compounds with safety liabilities early.

Numerous frontloaded toxicology strategies have emerged including:

- High-throughput *in vitro* assays for parameters like genotoxicity and hERG channel inhibition (Bowes et al., 2012).
- *In silico* models to predict toxicity based on structure-activity relationships (Greene, 2002).
- Screens for chemical structural alerts associated with adverse effects (Limban et al., 2018).
- Techniques like 3D organoids and microphysiological organs-on-chips to evaluate tissue-specific toxicity (Ewart et al., 2018; Roth et al., 2021; Marx et al., 2016, 2020).
- Assessment of physicochemical properties like lipophilicity and phospholipidosis potential (Leeson and Springthorpe, 2007).
- Microdosing studies in animals and humans to gauge bioavailability and clearance (Sewell et al., 2016).

These approaches provide rapid insight on absorption, distribution, metabolism, excretion (ADME), and toxicity processes to inform early go/no-go decisions. This prevents investing in poorly behaving candidates unlikely to become safe medicines, saving costs and resources.

While frontloaded toxicology has transformed drug discovery, challenges remain around predictive validity, throughput constraints, and risk communication for early screens. Nevertheless, the fail-early-fail-cheap paradigm has greatly increased pharmaceutical R&D productivity, demonstrating the value of upfront toxicology assessment. The lessons learned are applicable to other chemical industries like consumer products and pesticides. There are a few key differences between the fail-early-fail-cheap paradigm in pharmaceuticals and green toxicology for industrial chemicals:

1. *Time of testing*: Fail-early-fail-cheap focuses toxicity testing early in pharmaceutical discovery before candidate selection (Hughes et al., 2011). Green toxicology emphasizes evaluating sustainability and safety during molecular design of new chemicals.
2. *Purpose*: Fail-early-fail-cheap aims to terminate unpromising drug candidates to avoid costly late-stage failures. Green toxicology seeks to avoid toxicity risks entirely rather than just manage failures later.
3. *Test methods*: Pharma relies heavily on rapid *in vitro* assays, high-throughput screens, and QSAR models. Green toxicology utilizes these plus newer approaches like AI-based computational tools (Hartung, 2023a,b; Kleinstreuer and Hartung, in press), high-content imaging, organs-on-chips, and omics tools tailored for industrial chemicals.
4. *Exposure focus*: Fail-early-fail-cheap emphasizes bioavailability, clearance rates, and human therapeutic doses. Green toxicology considers exposures across global supply chains from manufacture through end-of-life.
5. *Holistic scope*: Fail-early-fail-cheap examines human health risks from patient use. Green toxicology evaluates sustainability, ecological impacts, and social benefits alongside human health.

In summary, both share a priority on early testing, but green toxicology takes a more proactive, prevention-focused approach customized for the different use contexts, volumes produced, and complex exposures seen with industrial chemicals. It is a more expansive framework, assessing broader lifecycle considerations beyond human health hazards.

## 4 Pillars of green toxicology

### 4.1 Alternative test methods

A core principle of green toxicology is utilization of test methods that reduce, refine, and replace animal use whenever possible. Alternative tests may include *in silico* methods like quantitative structure-activity relationships (QSAR) and read-across or *in vitro* techniques (Pamies and Hartung, 2017) using human cells or cell lines or bacterial assays, organ-on-chip microphysiological systems, and various omics technologies (Hartung, 2017). Benefits include more human-relevant data, lower costs, and higher throughput screening potential. Challenges remain around validating newer approaches against traditional animal-based methods (Hartung, 2010a; Judson et al., 2013) and developing integrated testing strategies to combine results from different tests into predictions (Hartung et al., 2013; Rovida et al., 2015; Caloni et al., 2022). But their expanded use aligns with green toxicology goals around ethical animal treatment and efficiency.

### 4.2 Precautionary principle

The precautionary principle states that “when an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause-and-effect relationships are not fully established scientifically” (Kriebel et al., 2001). This contrasts with traditional risk assessments, where reg-





ulatory action is only taken when there is evidence that a chemical poses harm. Applying the precautionary principle aligns with the hazard prevention focus of green toxicology by supporting prudent actions in face of uncertainty. However, implementing precautionary approaches risks trade-offs against social and economic benefits and requires clear risk communication.

### 4.3 Lifetime impacts

Green toxicology advocates evaluating chemical threats across their entire lifecycle from raw material extraction to manufacture, use, and disposal (Maertens et al., 2014). This contrasts with typical risk assessments that often focus only on intended use applications. Considering lifetime impacts is critical for identifying unintended hazards associated with byproducts, wastes, and degradation across global supply chains. It also evaluates holistic impacts to ecosystems and communities, expanding the scope beyond just human health. However, collecting lifecycle data is extremely challenging and requires new assessment tools.

### 4.4 Prevention over reaction

Central to green science philosophy is “*preventing waste rather than cleaning up after the fact*” (Anastas and Beach, 2007). In practice, green toxicology aims to eliminate hazards before products enter the market rather than trying to regulate risky chemicals after problems arise. Key initiatives include design of inherently less hazardous molecules and materials as well as development of alternative assessment frameworks to identify safer substitutes to toxic chemicals (Tickner et al., 2015). Preventative approaches can stimulate innovation but require cross-sector collaboration and infrastructure investments.

### 4.5 Current initiatives

Many research, regulatory, and commercial efforts are underway to facilitate adoption of green toxicology:

- Government programs like the U.S. National Toxicology Program are investing in alternative test method development and validation which will provide the underlying data to enable new risk assessment approaches (Thomas et al., 2017; Schmeisser et al., 2023).
- CAAT started a Green Toxicology program in 2014, which has continuously gathered and disseminated information on the use of alternative methods for early hazard avoidance (Maertens et al., 2014; Maertens and Hartung, 2018).
- Academic training programs are expanding to produce next-generation toxicologists adept in new methodologies like *in vitro* assays, computational modeling, and omics technologies as well as systems thinking and sustainability concepts. Examples are our free Coursera classes<sup>2</sup>, in which almost 20,000 students have enrolled.
- Public-private partnerships like the Green Chemistry and Commerce Council (GC3)<sup>3</sup> are engaging industry to develop tools

and approaches to advance application of green chemistry in product development including hazard assessment guidance.

- Non-governmental organizations provide certification programs like Cradle to Cradle<sup>4</sup> and GreenScreen<sup>5</sup> to guide manufacture of less toxic products as well as advocacy campaigns to accelerate policy reform.
- Businesses marketing greener products invest heavily in alternatives assessment using principles of green toxicology to demonstrate reduced risk profiles compared to traditional options (Malloy et al., 2017).

These efforts and others demonstrate growing momentum around adoption of green toxicology. However, considerable barriers remain, as discussed next.

## 5 Challenges and limitations

Green toxicology seeks to transform chemical risk assessment and management through use of alternative test methods and implementation of safer design concepts. However, adopting these newer frameworks faces substantial barriers. Institutional inertia poses a foremost challenge, as altering existing protocols and practices is difficult (Maertens et al., 2014). Regulators and industry have invested heavily in animal-based approaches and may resist deviations that necessitate major changes. Transitioning towards new methods requires surmounting this inertia across diverse stakeholders embedded in the *status quo*.

Uncertainty around data interpretation and legal constraints further hinders green toxicology uptake. While alternative test methods offer promise for reducing animal testing, connecting non-animal data to potential human health risks requires development of robust adverse outcome pathways (AOPs) and quantitative prediction models that have yet to be fully validated (Leist et al., 2017). Without confidence in these predictive tools, regulatory acceptance lags. Additionally, legal statutes often still mandate animal testing, constraining opportunities to fully implement alternative integrated testing strategies even when suitable non-animal approaches exist (Hartung, 2010b). Overcoming these barriers requires not just scientific advancement but policy changes, enabling data from new methods to substitute for animal studies.

Implementation barriers also pose substantial challenges (Zaunbrecher et al., 2017). The costs and expertise required to utilize newer methods can be prohibitive, especially for smaller programs with limited resources. Building out the physical and personnel infrastructure necessary requires substantial investment in equipment, facilities, and training. Many companies and institutions lack the financial capacity to overhaul existing systems centered on animal tests. Furthermore, lack of holistic lifecycle data and safer chemical alternative options severely hampers full application of green toxicology frameworks like the

<sup>2</sup> <https://www.coursera.org/learn/toxicology-21> and <https://www.coursera.org/learn/evidence-based-toxicology>

<sup>3</sup> <https://www.greenchemistryandcommerce.org>

<sup>4</sup> <https://c2ccertified.org>

<sup>5</sup> <https://www.greenscreenchemicals.org>



lifetime impacts concept and substitution efforts (Tickner et al., 2015). Gathering comprehensive data across chemical lifecycles poses scientific and logistical difficulties. And even when problems are recognized, identifying or designing equally effective but less hazardous alternatives represents a major challenge companies face in improving product safety.

Surmounting these myriad technological, economic, legal, and social barriers requires multifaceted partnerships across public and private entities to spur a culture shift away from entrenched systems. Transitioning toxicology towards more sustainable paradigms necessitates unprecedented cooperation of stakeholders to develop solutions addressing this complex array of challenges. A piecemeal approach may yield incremental gains, but coordinated efforts across disciplines and sectors can catalyze the field's transformation.

## 6 New approach methods to enable safer chemical substitutions

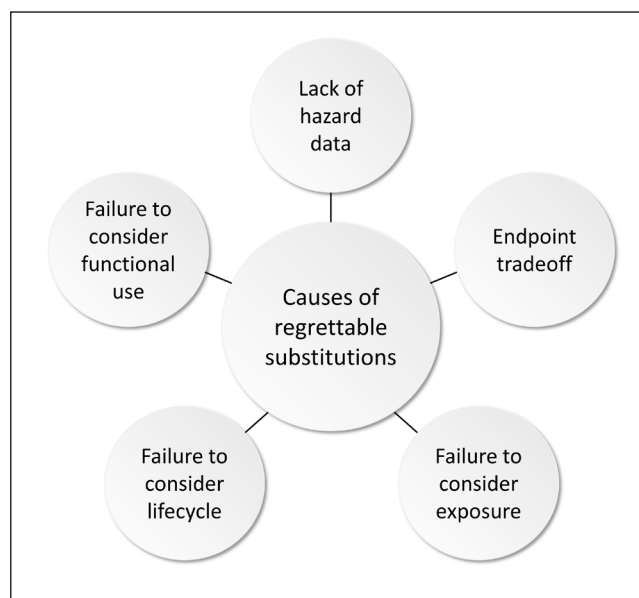
Alternative test methods that reduce or replace animal use are vital for implementing green chemistry principles of inherent safety by design. Emerging non-animal approaches, aka new approach methodologies (NAM), which enable assessment of potential toxicity liabilities early during molecular development when alterations are still feasible, are based on recent advances in high-throughput screening, computational toxicology, and microphysiological organ-on-chip systems applicable for evaluating industrial chemicals and guiding the design of less hazardous substitutes.

### 6.1 Avoiding regrettable substitutions

A core aim of green chemistry is identifying safer, more sustainable alternatives to hazardous chemicals. However, substituting one chemical for another has inherent challenges and risks (Tickner et al., 2015). Regrettable substitutions occur when a replacement chemical is later found to have unanticipated toxicity or environmental trade-offs. This can erode public trust and demonstrates the need for robust evaluation frameworks. Several high-profile examples have highlighted the potential for regrettable substitutions:

- Refrigerants developed to replace ozone-depleting chlorofluorocarbons (CFCs) were later found to have high global warming potential (Velders et al., 2007).
- Bisphenol A replacements like bisphenol S and F have shown comparable endocrine activity to the original chemical (Eladak et al., 2015).
- Perfluorooctanoic acid (PFOA) substitutes including GenX chemicals display similar persistence and bioaccumulation (Lorenzo et al., 2016).

These cases illustrate the challenges around identifying innocuous alternatives that avoid “*regrettable substitution of one hazard for another*” (Tickner et al., 2015). Strategies to avoid regrettable substitutions include:



**Fig. 1: Causes of regrettable substitutions**

While this list is certainly not exhaustive, regrettable substitutions can largely be attributed to a handful of issues: (1) lack of hazard data on the replacement chemical; (2) trading off one hazard endpoint for another; (3) failure to consider exposure; (4) failure to consider lifecycle concerns; and (5) failure to consider functional use. Reproduced from our Maertens et al. (2021) publication as allowed by the publisher.

- Applying the precautionary principle during assessment of alternatives to support proactive evaluation even with data gaps (Geiser, 2015).
- Considering classes of chemicals rather than just one-to-one chemical replacements to expand options and identify inherently less hazardous functional groups<sup>6</sup>.
- Evaluating lifecycle impacts and avoiding burden shifting between environmental media, worker exposure, or across supply chains (Jacobs et al., 2016).
- Incorporating safer design principles like the 12 Principles of Green Chemistry during molecular development (Anastas and Eghbali, 2010).
- Using comprehensive hazard screening tools like GreenScreen<sup>5</sup> that consider multiple human and ecotoxicity endpoints (Clean Production Action).
- Monitoring substitutes after market adoption through biomonitoring studies to enable early detection of unanticipated effects (Viegas et al., 2020).
- The use of comparative exposure assessment to avoid overlooking viable alternatives (Meng and Zhou, 2023)

Though challenging, avoiding regrettable substitutions (Fig. 1) is critical for credible, ethical green chemistry that protects human and environmental health. It requires utilization of best prac-

<sup>6</sup> Mosley, M., Yordas Group, [https://static1.squarespace.com/static/5e66303d2a35d86b999ddf82/t/5e7b87369f0b2638fe1200c3/1585153849297/Substitution\\_Strategies\\_and\\_Green\\_Chemistry.pdf](https://static1.squarespace.com/static/5e66303d2a35d86b999ddf82/t/5e7b87369f0b2638fe1200c3/1585153849297/Substitution_Strategies_and_Green_Chemistry.pdf)

tices in alternatives assessment paired with continuous improvement and vigilance even after implementation.

## 6.2 High-throughput screening assays producing data for green toxicology

Cell-based assays allow rapid screening of chemicals for biological activity relevant to potential toxicities. Quantitative high-throughput screening (qHTS) assays can test thousands of chemicals at multiple concentrations to derive concentration-response profiles in just days (Xia et al., 2008). Screens have been developed to assess endpoints like cytotoxicity, receptor interactions, enzyme induction/inhibition, and reactive metabolite formation (Attene-Ramos et al., 2013; Schmidt et al., 2009). The Tox21 collaboration has generated over 50 qHTS assays covering stress response, nuclear receptor, and developmental/reproductive pathways that provide insight on ADME and toxicity processes (Huang et al., 2016). The emerging field of high-content imaging quantifies complex phenotypic changes like cell morphology and intracellular protein translocation to discern mechanisms of toxicity (van Vliet et al., 2014). When combined with computational modeling, large screening datasets establish structure-activity relationships, guiding design of less toxic alternatives and prioritizing candidates for targeted testing.

## 6.3 The rise of artificial intelligence and computational toxicology for green toxicology

Computational toxicology refers to the use of computer and information technology tools to collect, analyze, and model chemical toxicity data (Hartung and Hoffmann, 2009; Hemmerich and Eckler, 2020; Ferreira Matos et al., 2022). It represents a central pillar of 21<sup>st</sup> century green toxicology given its potential to dramatically increase human relevance, efficiency, and predictive capacity while reducing animal testing needs (Hartung, 2017). Major applications of computational toxicology include:

- Quantitative structure-activity relationship (QSAR) models that predict toxicity from chemical structures and properties (Cherkasov et al., 2014). Recent advances (Neves et al., 2018; Niazi et al., 2023) include the combination of cheminformatics and QSAR modeling with deep learning methods like DeepSnap for QSAR analysis without extracting descriptors, and fragment-based two-dimensional QSAR (FB-QSAR), which divides molecular structures in a family of drug candidates into several fragments.
- Virtual tissue models of ADME processes across biological barriers (Quignot, 2013; Bessems et al., 2014; Tsaïoun et al., 2016).
- Machine learning approaches like deep neural networks to find complex patterns in large toxicity datasets (Mayr et al., 2016).
- Organ-on-chip microphysiological systems linked to pharmacokinetic models to emulate human toxicodynamics (Leung et al., 2022; Deng et al., 2023; Komura et al., 2023).
- Virtual screening offers a fast and efficient way to identify new drug candidates and design new chemical compounds. High-throughput virtual screening of drug-like libraries can be used to triage millions of candidates (Graff et al., 2021). In recent years, “tangible” virtual libraries have made billions of mol-

ecules readily available; prioritizing these molecules for synthesis and testing demands computational approaches, such as docking studies (Lyu et al., 2023).

- *In silico* human clinical trial simulations for improving risk assessment and decision-making (Arsène et al., 2024).

These capabilities will be increasingly vital for implementing green toxicology frameworks that minimize animal testing while still allowing rapid evaluation of chemical hazards and risks. However, computational models require extensive validation, combining multiple methods reduces uncertainties, and effective risk communication for new data types remains challenging.

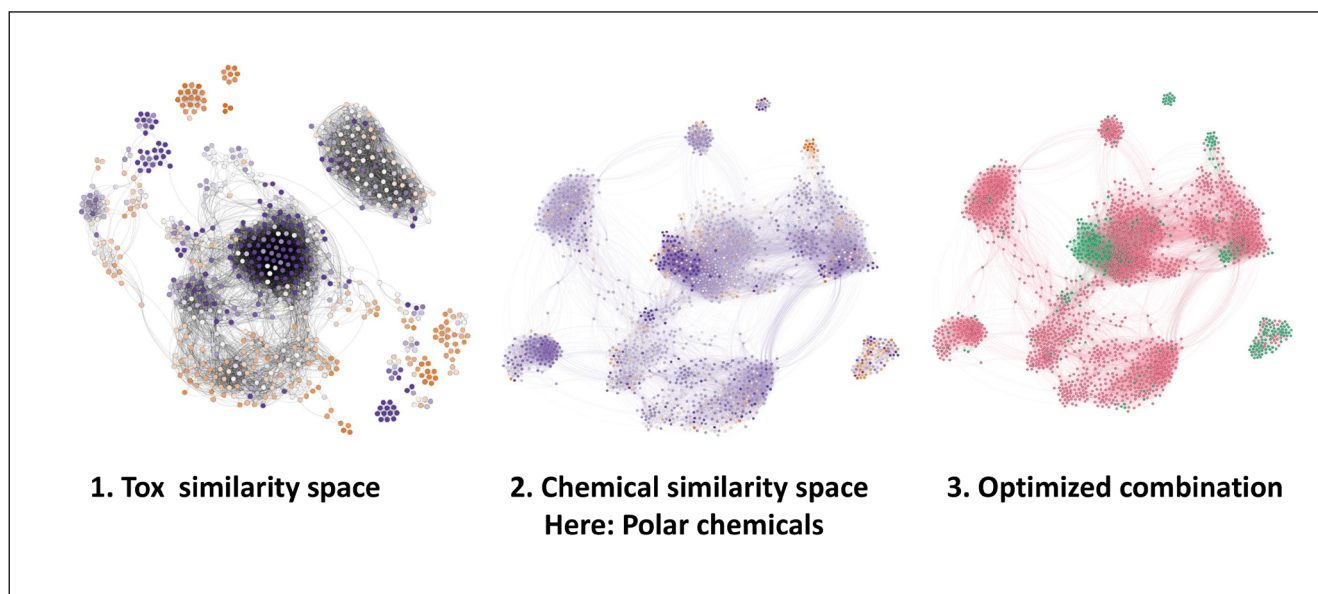
Rapid advances in artificial intelligence (AI) capabilities hold tremendous promise for accelerating green toxicology. Rather than relying solely on predefined expert rules, AI systems like deep learning neural networks can analyze enormous volumes of diverse data to find complex patterns and relationships (Hartung, 2023a,b; Kleinstreuer and Hartung, in press). This enables more accurate predictive modeling across a vast chemical space (Polishchuk et al., 2017).

Exciting AI applications in toxicology include:

- Natural language processing of scientific literature and reports to extract toxicity data for modeling (Corradi et al., 2022).
- Computer vision analysis of digital images from cell assays to quantify morphological changes and cytotoxicity. For instance, convolutional neural networks (CNNs) have been trained to assess cell cytotoxicity and predict the potentially toxic effects of new compounds, replacing some traditional testing methods (Tandon et al., 2022; Tran et al., 2023)
- Deep generative models that create novel molecular structures on demand with desired safety profiles (Putin et al., 2018).
- Unsupervised learning techniques to find outliers in chemical datasets indicating potential hazards (Glielmo, 2021).
- Integration of robotics with AI for automated high-throughput toxicity screening and drug discovery (Schneider, 2018).

A particular role AI can play is the identification of alternative chemistry to replace hazardous substances. Not only can approaches such as read-across-based structure-activity relationships (RASAR) predict physicochemical properties and hazards of a given structure (Hartung, 2016; Luechtefeld and Hartung, 2017; Luechtefeld et al., 2018a,b), but they can as well identify structures with a certain set of properties. This is illustrated in Figure 2, where the chemical similarity space of dichloromethane (polar solvent) was overlaid with the toxic properties of similar chemicals, and favorable chemistries (green) were identified. This unpublished proof-of-concept is only intended as an illustration of future opportunities. The ongoing ONTOX project (Vinken et al., 2021) is expanding the AI RASAR approach to hundreds of properties and promises tools useful also for proposing chemicals with desired properties.

The future of green toxicology will undoubtedly involve increasing incorporation of AI to transform data collection, analysis, risk assessment, and molecular design. The industrial chemical field can adopt many of the AI-driven advances of drug development (Paul et al., 2021). However, biases in training data and model interpretability remain challenges to the widespread adoption of AI systems. Responsible development and ethical consid-



**Fig. 2: Identifying favorable alternative chemistry by read-across-based structure-activity relationships (RASAR)**

The similarity in polarity and favorable toxic properties of dichloromethane were overlaid to suggest areas of chemical space with promising combinations (green) versus those that are either not similar enough or have predicted hazardous properties. Please note that the model (Luechtefeld et al., 2018b) included only nine acute, topical, and aquatic hazards. The unpublished results serve illustration purposes only.

erations are paramount. Overall, computational toxicology and AI are immensely powerful and fast-evolving capabilities that will enable greener approaches to chemical safety assessment. But human expertise, continuous model evaluation, and stakeholder collaboration remain essential to ensure these tools are leveraged appropriately and productively.

#### 6.4 Microphysiological systems advance green toxicology

Microphysiological systems (MPS), also known as organs-on-chips, are miniaturized 3D tissue constructs that emulate human organ structure, functions, and physiology *in vitro* (Marx et al., 2012, 2016, 2020; Edington et al., 2018; Roth et al., 2021). MPS use microfabrication approaches to recreate tiny functional units of living organs for more predictive toxicity testing. Microfluidic channels, biomimetic scaffolds, and mechanical actuators emulate key features of tissues and organs difficult to represent in conventional cultures. Modular interconnected platforms can model ADME processes across barriers to derive pharmacokinetic parameters for extrapolating dose responses. MPS offer potential for chronic dosing regimen studies over months to assess long-term impacts. Importantly, these advances enable evaluation of tissue-specific effects to guide structural modifications avoiding target organ toxicity during early development. Key benefits of MPS for toxicity assessment include:

- Recapitulation of complex multicellular architectures, spatiochemical gradients, tissue-tissue interfaces, and mechanistic cues like fluid shear stress found in the body (Bhatia and Ingber, 2014).
- Ability to evaluate organ-specific adverse effects relevant for human risk predictions that animal models cannot replicate (Marx et al., 2016).
- Compatibility with high-throughput screening, allowing rapid testing of chemical libraries to derive structure-activity relationships (Low and Tagle, 2017).
- Capacity for chronic dosing over weeks to months to assess long-term impacts like carcinogenesis unfeasible in traditional static *in vitro* cultures<sup>7</sup> (Wikswa, 2014).
- Potential for interconnected systems that model ADME pathways across multiple organs to provide integrated pharmacokinetic/toxicokinetic data (Vermetti et al., 2017).

However, barriers to adoption remain around cost, complexity, and validation against human outcomes. Nevertheless, the field is rapidly advancing with increasing investment from government, academia, and industry accelerating technical innovation. We have recently started organizing a series of MPS World Summits<sup>8</sup> and an International MPS Society<sup>9</sup>. Overall, leveraging microphysiological systems can transform green toxicology by providing human-relevant *in vitro* models to comprehensively evaluate chemical safety without sacrificing animals.

<sup>7</sup> <https://www.ddw-online.com/hype-or-hope-microphysiological-systems-13760-202110/>

<sup>8</sup> <https://mpsworldsummit.com>

<sup>9</sup> <https://impss.org>



## 7 Opposing views of environmental and animal welfare NGOs on animal testing

Non-governmental organizations (NGO) play a major role in influencing policy-making (von Aulock et al., 2022) relevant to alternative approaches. Animal experimentation is a contentious issue between environmental NGOs and animal welfare NGOs due to their differing perspectives on its implications. There are several instances where environmental NGOs and animal welfare NGOs have taken differing views on animal use<sup>10,11,12,13,14,15</sup> (Campbell, 2018); especially, there are instances where environmental NGOs have advocated for more animal testing, primarily due to the belief that such testing can provide crucial information for environmental conservation and human safety<sup>16</sup>, while animal welfare NGOs often oppose such practices due to the potential harm and suffering inflicted on the animals (Kiani et al., 2022). These differences often stem from the distinct focus of each group: Environmental NGOs typically prioritize the health of ecosystems and biodiversity, while animal welfare NGOs emphasize the well-being of individual animals.

One prominent example is the World Wildlife Fund (WWF). The WWF has lobbied for more animal experimentation to test the impact of different chemicals on the environment. They have been particularly successful in pushing for a large-scale animal experimentation program called the Endocrine Disruptor Screening Program, developed by the US Environmental Protection Agency (EPA). This program aims to test the hormonal impact of various chemicals on animals<sup>17</sup>. Another environmental NGO that has shown support for animal testing is the Environmental Defense Fund (EDF). The EDF believes that limited animal testing is still necessary to prevent even greater harm to our ecosystems and the animals within them. They argue that more upfront testing could have prevented significant ecological, animal, and human harm caused by substances like DDT, PCBs, asbestos, and leaded gasoline<sup>18</sup>.

The desire of environmental groups for more (animal) data, and the desire of animal welfare groups to limit exactly this, has often conflicted in the past. This lack of alignment has negatively impacted both groups' progress and is a key reason why animal welfare is not clearly associated with progressive forces in society. The World Organization for Animal Health (OIE) analyzed what animal welfare means (Lopez, 2007), coming to the conclusion, "An attempt at defining animal welfare is made in this section and

*at determining whether it is a scientific discipline. The term does not express a scientific concept but, because the scientific method is used to identify, interpret, and implement societal concerns about animal quality of life issues, animal welfare has evolved to become a scientific field. Although a precise scientific definition of animal welfare is impossible, a broad working description of welfare is suggested, which includes the notions of the animal in complete mental and physical health, the animal in harmony with its environment, the animal being able to adapt, without suffering, to an artificial environment provided by human beings and, somehow, the animal's feelings should be taken into account."*

Notably, there are basic differences between the concerns of animal welfare and animal rights, which both seek the protection of non-human animals, but differ in their approaches<sup>19</sup> "Animal rights and animal welfare are two currents of thought that seek the protection of non-human animals at different levels. Animal welfare actions seek that animals, mainly those that are used as goods or services, can live in conditions where they can reach the fullness of their biological capacities, avoiding their suffering as much as possible. Social movements in favor of animal rights consider that they should be able to enjoy the same rights as human beings from a legal perspective and therefore should be considered as people with the same right to life as a human."

Examples of divergent views of environmental and animal welfare NGOs go beyond animal experimentation (Mitchell, 2022):

- Wildlife management: One example of this conflict can be seen in wildlife management practices (Owens, 1991). For instance, in some regions, certain animal populations (like deer or grey squirrels) may need to be controlled to maintain the balance of the ecosystem and protect other species. Environmental NGOs may support these measures for the sake of biodiversity, while animal welfare NGOs could oppose them due to concerns about the individual animals' welfare<sup>20</sup>.
- Use of animals in agriculture: The use of animals in agriculture can also be a contentious issue. Some environmental NGOs may support sustainable farming practices that include animal husbandry, if it is done in a way that minimizes environmental impact. On the other hand, animal welfare NGOs may oppose certain farming practices due to concerns about the treatment and living conditions of the animals<sup>21</sup>.
- Wildlife tourism: Wildlife tourism is another area where conflicts can arise. Some environmental NGOs may support wildlife tourism as a means of promoting conservation and provid-

<sup>10</sup> <https://www.newstatesman.com/ideas/2020/11/moral-conflict-between-environmentalism-and-animal-welfare>

<sup>11</sup> <https://aldf.org/article/environmentalism-as-a-form-of-animal-rights/>

<sup>12</sup> [https://en.wikipedia.org/wiki/Relationship\\_between\\_animal\\_ethics\\_and\\_environmental\\_ethics](https://en.wikipedia.org/wiki/Relationship_between_animal_ethics_and_environmental_ethics)

<sup>13</sup> <https://www.forbes.com/sites/briankateman/2023/02/01/the-environmental-movement-forgot-about-animals/?sh=41ce0d32175f>

<sup>14</sup> <https://fivethirtyeight.com/features/how-animal-rights-activists-and-environmentalists-became-unlikely-adversaries/>

<sup>15</sup> <https://sentientmedia.org/animal-advocates-environmentalists-clash-over-animal-testing/>

<sup>16</sup> <https://www.animal-ethics.org/animal-experimentation-environmentalist-purposes/>

<sup>17</sup> [https://19january2017snapshot.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-edsp-overview\\_.html](https://19january2017snapshot.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-edsp-overview_.html)

<sup>18</sup> <https://www.edf.org/animaltesting>

<sup>19</sup> <https://study.com/academy/lesson/animal-rights-ethics-arguments.html>

<sup>20</sup> <https://www.conservationjobs.co.uk/articles/animal-welfare-conservation-an-inevitable-conflict-of-interests/>

<sup>21</sup> <https://www.thecalltoconserve.com/blog/animal-rights-vs-welfare>



ing funding for environmental initiatives. However, animal welfare NGOs may oppose certain forms of wildlife tourism that can cause stress or harm to individual animals (Essen et al., 2020).

- Pet ownership: The practice of keeping wild animals as pets can also lead to disagreements. Some environmental NGOs may oppose this practice due to its potential impact on wild populations and ecosystems. Meanwhile, animal welfare NGOs might focus more on the welfare of the individual pet, advocating for proper care and treatment (Broom, 2019).

It is important to note that while these conflicts can occur, many environmental and animal welfare NGOs also find common ground and work together on issues such as promoting sustainable food systems, advocating for laws to protect animals and the environment, and raising awareness about the interconnectedness of animal welfare and environmental health<sup>22</sup>.

Faria and Paez (2019) took the extreme stance “*that animal ethics and environmental ethics are incompatible ethical positions. This is because they have incompatible criteria of moral considerability and they have, at least in some cases, incompatible normative implications regarding the interests of sentient individuals. Moreover, we claim that environmentalist views lead to an insurmountable dilemma between inconsistency and implausibility and fail to properly account for the importance of wild animal suffering. From this it follows not only that (a) we can endorse one of the two views but not both at the same time but also that (b) we have overriding reasons to reject environmentalism and endorse some animal ethics view.*”

From personal experience, our work on monitoring and predicting animal use for the European REACH regulation (Registration, Evaluation, Authorisation and Restriction of Chemicals), recently summarized in Rovida et al. (2023) and Knight et al. (2023), was attacked early on by the Environmental Defense Fund (EDF) represented by Richard Denison (Hartung and Rovida, 2009a). In 2009, we made a projection of what the application of the legislation to the likely numbers of chemicals and tonnage levels would mean (Rovida and Hartung, 2009; Hartung and Rovida, 2009b). It is interesting to revisit this projection 15 years later:

- The EDF/Denison questioned the accuracy of our estimates of the numbers of chemicals requiring testing and the numbers of animals to be used under REACH legislation, which we based on the expansion of the EU, growth of the chemical industry, additions to requirements, etc., all developments that already then invalidated the initial estimates. EDF questioned some of the assumptions and called the numbers inflated. The numbers of chemicals in higher tonnage bands estimated in our analysis were actually spot-on. These tonnage bands require most animal testing. What nobody anticipated was the low number of chemicals ultimately registered in the lower tonnage bands, indicating that either production of many was abandoned or they were never registered. It was also very surprising how few

chemicals earlier registered under the Dangerous Substances Directive required re-registration. The final numbers are still open until final decisions on the grouping of petrochemicals are taken, which would add these high-tonnage chemicals with high testing needs. In summary, there were indeed considerably fewer chemicals registered but only at tonnage levels that have little impact on the overall animal count.

- The views differed also on the extent to which existing data and alternative methods can substitute for new animal testing. Unfortunately, we were right here as numerous reports by the European Chemicals Agency (ECHA) have shown<sup>23</sup>. We will focus just on developmental and reproductive toxicity (DART), which is responsible for 80-90% of animal use (Rovida et al., 2011). We analyzed the guidance and availability of reproductive toxicity data and concluded that valid waiving opportunities are very limited. The 2020 report by ECHA showed that less than 1% of information needs for DART were satisfied with QSAR, about 2% with weight-of-evidence, and another 2% with “other” information (noteworthy, these data are rough estimates as they were only graphically displayed in Figure 4 of the ECHA report). About 20% of information requests were answered by providing a read-across, but as shown earlier, with acceptance rates of less than 2% (Ball et al., 2016). This means only about 5% of information (exactly the amount we assumed in 2009) was produced with non-standard methods. EDF in their critique implied more existing data and alternative approaches could be utilized to reduce testing. The 2023 ECHA report allows (combining Figures 3 and 4) to estimate how much pre-existing data, i.e., done before 2009, was used for registration: 3% for developmental toxicity and 24% for reproductive toxicity. We had assumed there would be data on 22% and 7% substances, respectively, in our 2009 study. We now further analyzed our recent study (Knight et al., 2023) and found 1378 dossiers using only data from before 2009 for reproductive toxicity (i.e., existing data), which translates to ~20% of all registered experimental data or data on ~6% of all substances in the tonnage bands requiring reproductive toxicity data – almost precisely the 7% we had assumed. For developmental toxicity, there was apparently less data available than we had assumed. Our analysis was correct for testing needs, however, almost 50% of developmental toxicity and 35% of reproductive toxicity data were simply not provided in the REACH registration dossiers, and a waiver was requested for another 7% and 11%, respectively, though the legal text does not give much grounds for this. Noteworthy, less than 1% of registrations included testing proposals for DART. This means that beyond the already documented animal testing (Knight et al., 2023) there is still much testing to be expected following ECHA’s compliance checks. So, in summary, the EDF assertion on the use of alternatives and available data for the most important DART testing did not hold.

<sup>22</sup> <https://proveg.com/press-release/united-nations-animal-welfare-environment-resolution/>

<sup>23</sup> [https://echa.europa.eu/documents/10162/0/alternatives\\_test\\_animals\\_2020\\_en.pdf](https://echa.europa.eu/documents/10162/0/alternatives_test_animals_2020_en.pdf), [https://echa.europa.eu/documents/10162/23919267/230530\\_117\\_3\\_alternatives\\_test\\_animals\\_2023\\_en.pdf](https://echa.europa.eu/documents/10162/23919267/230530_117_3_alternatives_test_animals_2023_en.pdf)

- EDF alleged that our study still used the unreliable preregistration data as “primary basis”, though after some analysis of the 2009 report, we came to exactly this conclusion and did not pursue the preregistration numbers but rather based estimates on corrected old figures.

In summary, the two sides disagreed over the methodology and assumptions underlying the estimates, the potential to avoid tests, especially for reproductive toxicity, and the role of the preregistration data in the analysis. However, we shared concerns over past lack of toxicity data and agreed on the need for new approaches to make chemical risk assessment more feasible. The strong reaction by this leading environmental group, however, illustrates the defense of animal use against showing the enormous extent of the endeavor, thus the title “*That which must not, cannot be...*” in our 2009 reply (Hartung and Rovida, 2009a).

In the US, a recent Pew Research Center survey revealed that 47% of Americans favor the use of animals in scientific research while 52% oppose the practice<sup>24</sup>. Interestingly, this does not at all follow party lines, with 50% of republicans and 45% of democrats supporting the practice. In contrast, environmental protection is much more partisan: A 2023 survey found that 78% of Democrats and 20% of Republicans believe environmental protection should be prioritized over economic growth<sup>25</sup>. This difference has political consequences. The U.S. Environmental Protection Agency (EPA) under Republican Administrator Andrew Wheeler had set a goal to reduce its requests for, and funding of, mammal studies by 30% by 2025 and to eliminate all mammal study requests and funding by 2035. Any mammal studies requested or funded by the EPA after 2035 would require administrator approval on a case-by-case basis<sup>26</sup>. With the new democratic government, however, the 2021 New Approach Methods Work Plan published by the EPA on December 12, 2021<sup>27</sup>, makes no reference to the memo signed by Administrator Wheeler in 2019, which directed the agency to aggressively reduce animal testing. This suggests that the EPA may have dropped the target date to end mammalian toxicity testing by 2035. This development appears to reflect the stronger influence of environmental groups, who have expressed opposition to the EPA plan to phase out animal testing. Their main concern is that the move could undermine the rigorous safeguarding of human health and the environment. For example, the Natural Resources Defense Council (NRDC), an environmental advocacy group, criticized the EPA’s decision, arguing that it eliminates tools that lay the groundwork for protecting the public from dangers like formaldehyde and PFAS<sup>28</sup>. This can be seen as another example of the contrary positions of environmental and animal welfare NGOs<sup>29</sup>.

In conclusion, the clash between environmental NGOs and animal welfare NGOs over animal experimentation stems from differing priorities: the former focusing on the potential benefits for environmental research and conservation, and the latter emphasizing the welfare of the individual animals used in these experiments. The obvious solution for this difference in views is the use of non-animal methods, which spare large numbers of animals from pain and distress, are often less expensive and time-consuming, and promise faster delivery of test results with greater applicability. Despite the growing evidence that animal testing is unreliable and the availability of non-animal alternatives, animal testing continues, largely due to old habits within the scientific community and a lack of knowledge and expertise in cutting-edge non-animal methodologies. However, change is happening, with global efforts to encourage scientists, companies, and policymakers to transition away from animal use in favor of 21<sup>st</sup>-century methods.

Environmental NGOs generally support the REACH regulation, recognizing its importance in protecting human health and the environment from risks posed by chemicals. However, they have also expressed concerns about the current implementation of REACH and have made several key demands for its improvement, which would require enormous increases in animal use<sup>30,31,32</sup> as analyzed earlier in this series (Rovida et al., 2023).

## 8 Green toxicology’s contribution to sustainable development

### 8.1 Alignment of green toxicology with the European Toxic-Free Environment program

The European Toxic-Free Environment program is an initiative coordinated by over 150 European NGOs. Its key objectives are to substantially reduce exposure to hazardous chemicals and prevent harm from chemicals to health and the environment by 2030. The program puts forth a series of policy demands focused on improving implementation of chemical regulations like REACH, the classification, labeling and packaging regulation (CLP), and policies on pesticides and endocrine disruptors. Core proposals include:

- Mandatory chemical substitution plans for highly hazardous substances,
- Applying an “essential use” concept to restrict chemicals that provide no net benefits to society,
- Enhanced monitoring and biomonitoring programs to track exposures,

<sup>24</sup> <https://www.axios.com/2018/08/21/americans-divided-on-use-of-animals-in-scientific-testing>

<sup>25</sup> <https://news.gallup.com/poll/474218/record-party-gap-environment-economic-growth-tradeoff.aspx>

<sup>26</sup> [https://19january2021snapshot.epa.gov/research/epa-new-approach-methods-efforts-reduce-use-animals-chemical-testing\\_.html](https://19january2021snapshot.epa.gov/research/epa-new-approach-methods-efforts-reduce-use-animals-chemical-testing_.html)

<sup>27</sup> <https://www.epa.gov/chemical-research/epa-new-approach-methods-work-plan-reducing-use-vertebrate-animals-chemical>

<sup>28</sup> <https://www.npr.org/sections/health-shots/2019/09/10/759435118/epa-chief-pledges-to-severely-cut-back-on-animal-testing-of-chemicals>

<sup>29</sup> <https://geneticliteracyproject.org/2019/09/12/epa-plan-to-end-animal-testing-by-2035-pits-environmental-groups-against-animal-rights-activists/>

<sup>30</sup> <https://zerowasteurope.eu/library/delivering-a-toxic-free-environment-under-reach-eight-key-ngo-demands-to-improve-the-reach-regulation/>

<sup>31</sup> <https://eeb.org/library/ngo-key-demands-to-improve-reach-2022/>

<sup>32</sup> <https://www.wecf.org/delivering-a-toxic-free-environment-under-reach-8-key-ngo-demands/>



- Expanded public access to chemical safety data through transparency rules,
- Requiring companies to shoulder the burden of proving chemicals are safe before market approval,
- Incentivizing investment in green and sustainable chemistry,
- Additional focus on protecting vulnerable groups like pregnant women and children.

To accomplish its objectives, the Toxic-Free Environment program brings together a broad coalition of health, eco, consumer, labor, and other NGOs to leverage unified pressure on policymakers towards ambitious chemical legislation and strict enforcement.

The utopian slogan “toxic-free” left aside (Paracelsus is turning in his grave...), this movement has influenced politics. The European Commission’s Zero Pollution Action Plan is an example: This plan aims to reduce pollution in air, water, and soil to levels that are no longer considered harmful to health and natural ecosystems. It includes specific targets such as reducing the EU ecosystems where air pollution threatens biodiversity by 25%, reducing the share of people chronically disturbed by transport noise by 30%, and significantly reducing waste generation and residual municipal waste by 50%<sup>33</sup>.

The emerging green economy will depend on chemistry innovations to create less hazardous, lower carbon alternatives. Green toxicology provides the evidence base needed for informed substitution, fulfilling the vision of sustainability policies.

## 8.2 Alignment of green toxicology with the EU Green Deal and US Green New Deal

Both the European Union (EU) and United States (US) in 2019 launched sweeping policy initiatives aimed at spurring sustainability transitions across industries and supply chains. Known as the European Green Deal<sup>34</sup> and US Green New Deal, these proposals align closely with the principles of green chemistry and toxicology. As key elements are implemented, they will help drive adoption of greener approaches to chemical safety assessment and risk management.

The European Green Deal aims to cut greenhouse gas emissions by at least 55% by 2030 and make Europe climate-neutral by 2050. It promotes circular economy concepts across product life-cycles as well as safer choices in chemicals, materials, and consumer goods. The deal contains specific strategies around designing sustainable chemicals and minimizing hazardous substances. Europe’s strong regulatory frameworks on chemical controls provide policy levers to influence industry adoption of alternatives assessment and inherently safer design (Geiser, 2015).

In the US, the Green New Deal resolution focuses on achieving net-zero greenhouse gas emissions, investing in renewable energy and zero-emission vehicles and promoting climate and environmental justice (US Congress, *Recognizing the duty of the Fed-*

*eral Government to create a Green New Deal*. 116<sup>th</sup> Congress, 1<sup>st</sup> Session, H.Res. 109, 2019<sup>35</sup>). It specifically aims to spur economic transitions towards greener technology, infrastructure, and products that use sustainable materials and chemicals. Though lacking regulatory authority, the deal elevates public discourse and pressure on companies to align chemical management with ecological values.

These deals exemplify the growing focus of policymakers on sustainable systems transitions. As programs are funded and initiatives implemented, green toxicology will play an integral role by providing the tools and know-how to:

- Evaluate relative sustainability of chemical alternatives to avoid regrettable substitutions.
- Incentivize benign-by-design products formulated with inherently safer ingredients.
- Develop non-animal test methods tailored for rapid safety screening of green chemicals.
- Implement lifecycle thinking frameworks for holistic impact assessment across supply networks (Hunt and Franklin, 1996).

## 8.3 Alignment of green toxicology with the United Nations Sustainable Development Goals (SDGs)

Green toxicology is fully in line with the SDGs, i.e., the 17 global goals for sustainable development adopted by the UN in 2015 as part of the 2030 Agenda for Sustainable Development<sup>36</sup>. They provide a shared blueprint for peace and prosperity for people and the planet. The 17 SDGs are:

1. No poverty
2. Zero hunger
3. Good health and well-being
4. Quality education
5. Gender equality
6. Clean water and sanitation
7. Affordable and clean energy
8. Decent work and economic growth
9. Industry, innovation and infrastructure
10. Reducing inequality
11. Sustainable cities and communities
12. Responsible consumption and production
13. Climate action
14. Life below water
15. Life on land
16. Peace, justice and strong institutions
17. Partnerships for the goals

Each goal has specific targets to be achieved by 2030. Green toxicology relates most closely to SDG #3 (Good health and well-being) and SDG #12 (Responsible consumption and production), but it also supports many of the other SDGs in various ways through its alignment with broader sustainable development priorities:

<sup>33</sup> [https://environment.ec.europa.eu/strategy/zero-pollution-action-plan\\_en](https://environment.ec.europa.eu/strategy/zero-pollution-action-plan_en)

<sup>34</sup> [https://ec.europa.eu/info/sites/default/files/european-green-deal-communication\\_en.pdf](https://ec.europa.eu/info/sites/default/files/european-green-deal-communication_en.pdf)

<sup>35</sup> <https://www.congress.gov/bill/116th-congress/house-resolution/109/text>

<sup>36</sup> <https://sdgs.un.org/2030agenda> (accessed 26.12.2023)





1. *Safer products*: By integrating toxicology principles early in the design and development of new chemicals and materials, green toxicology can help create safer products that have less environmental impact. This can contribute to sustainable development by reducing the potential harm to human health and the environment.
2. *Reduced waste and exposure*: Green toxicology aims to minimize potential toxicity as early in production as possible, subsequently reducing waste and exposure. By designing out undesired human health and environmental effects, green toxicology can help reduce the amount of waste generated.
3. *Economic benefits*: The principles of green toxicology can be economical for manufacturing companies. By identifying and addressing potential toxicity issues early in the development process, companies can avoid costly product recalls and legal liabilities associated with toxic products. This can contribute to sustainable development by promoting the long-term economic viability of companies.
4. *Integration of disciplines*: Green toxicology integrates toxicology principles with other disciplines, such as chemistry, materials science, and engineering. This interdisciplinary approach allows for a more holistic understanding of the potential hazards and risks associated with chemicals and materials, enabling the development of safer alternatives.
5. *Driving innovation*: Green toxicology drives innovation by placing safety considerations at the forefront of the design process. By integrating toxicology early on, researchers and developers can identify potential toxicity issues and make informed decisions to modify or eliminate harmful chemical structures. This promotes the development of novel, safer, and more sustainable products.

Keeling et al. (2019) have analyzed the relationship of the SDGs to animal welfare aspects. The current SDG framework lacks explicit targets around animal welfare including reducing animal testing or advancing alternatives, even though this aligns with multiple sustainability objectives. Attempts have been made to show the environmental impact of animal testing based on resources used in animal research, waste production in laboratories, sources of pollution, impacts on laboratory workers' health, and biodiversity impacts (Groff et al., 2014). Reducing reliance of animal models for chemicals and drug safety testing should thus become a priority target because it upholds ethical values of animal welfare and social justice. Millions of research animals are used annually (Taylor et al., 2008), often involving substantial pain and distress, despite viable alternatives. This contradicts sustainability ethics around valuing all life (Akhtar, 2015). Advancing environmentally-conscious life science innovation like organs-on-chips and organoids can provide human-relevant models for accelerated discovery of greener health and technology solutions as well as enabling smart resource efficiency. Animal testing consumes massive financial, time and trained personnel commitments better deployed toward advanced *in vitro* and computational models (Meigs et al., 2018). Developing integrated testing strategies and adverse outcome pathways linking non-animal data to human health outcomes

requires shared engagement across disciplines, fostering interdisciplinary and collaborative approaches (Leist et al., 2017). The UN should amend SDG targets to spur adoption of animal alternatives through:

- Explicit inclusion in SDG language such as adding a target under #3 SDG (Good health and wellbeing) to “End animal testing and promote alternatives”.
- Establishing working groups to assess viability of alternative methods and develop transition roadmaps.
- Creating public-private funding streams toward cause-driven social impact programs supporting alternative innovation networks.
- Measuring progress through defined metrics like numbers of animals used annually or research funding toward animal-free discovery methods tracked through mandatory reporting.

Embracing animal testing alternatives aligns with eight key SDGs toward responsible production and sustainable communities. It deserves acknowledgment within the development agenda.

## 9 Conclusions

Green toxicology offers a promising framework to meet growing calls for chemical risk assessment that is safer, cheaper, and more ethically conscious. While adoption remains incremental, its foundational principles are catalyzing valuable innovation in methodologies, tools, policies, and perspectives that collectively have potential to profoundly transform how chemical hazards are evaluated and managed. This paper provides an introduction and high-level synthesis of the field. Considerably more interdisciplinary research, demonstration projects, stakeholder engagement, and policy reform will be needed to advance widespread implementation of green toxicology approaches. But the conceptual foundation is steadily being built. Integrating green chemistry and toxicology can provide solutions that meet our societal needs for chemicals while protecting human and ecological well-being now and in the future.

The recent rise of various computational methods to predict potential toxicity liabilities based solely on chemical structure is fueled by the enormous progress of AI and its implementation. Quantitative structure-activity relationship (QSAR) models relate descriptors of physicochemical features to bioactivity/toxicity. Extensive public databases enable screening thousands of industrial chemicals, food additives, cosmetic ingredients, and drugs to predict many endpoints. More complex machine learning approaches like deep neural networks uncover subtle patterns among vastly more input features that improve model accuracy, applicability domain, and mechanistic interpretability. Binding affinity predictors estimate interactions with pharmacological targets but also unintended receptors that may cause side effects. Expert systems codify structure-toxicity relationships from decades of animal studies to avoid repeating tests on analogous chemicals. All these *in silico* methods enable rapid hazard profiling to prioritize safer candidates for synthesis or substitution over riskier alternatives.



In summary, high-throughput assays, computational prediction platforms, and organ-on-chip models enable rapid screening of toxicity liabilities using primarily human cell-based tests to inform molecular design. Adoption of these new approach methods serves dual aims of accelerating development of sustainable alternatives to hazardous chemicals while supporting reduced animal testing. Early integration of multiple complementary non-animal approaches via tailored integrated testing strategies in product development provides a path to confidently transition from traditional mainly regulatory toxicology. Comprehensive validation remains vital but steady progress toward human-relevant tools offers solutions to longstanding barriers hampering adoption of green chemistry innovations. It is time to implement, or as Christine Pelosi, daughter of Nancy Pelosi and a politician in her own right, underlines the proactive nature of environmental responsibility, put it, “*Being green and clean is not just an aspiration but an action*”.

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#### Conflict of interest

Thomas Luechtefeld is founder and owner of Tox-Track Inc. and Insilica LLC. He and Thomas Hartung are consultants for computational toxicology for Underwriters Laboratories (UL) and receive shares of their respective sales. Thomas Hartung is a member of Apple's Green Chemistry Advisory Board. He also holds stock options in and consults Tox-Track LLC Inc. and Insilica LLC.

#### Data availability

No datasets were generated in this study.

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