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# Food for Thought ...
## Green Toxicology

*Alexandra Maertens [1], Nicholas Anastas [3], Pamela J. Spencer [4], Martin Stephens [1], Alan Goldberg [1] and Thomas Hartung [1,2]*

[1]Johns Hopkins University, Bloomberg School of Public Health, CAAT, Baltimore, MD, USA; [2]CAAT-Europe, University of Konstanz, Germany; [3]EPA Region 1, Boston, MA, USA; [4]The Dow Chemical Company, Midland, MI, USA

**Summary**

*Historically, early identification and characterization of adverse effects of industrial chemicals was difficult because conventional toxicological test methods did not meet R&D needs for rapid, relatively inexpensive methods amenable to small amounts of test material. The pharmaceutical industry now front-loads toxicity testing, using in silico, in vitro, and less demanding animal tests at earlier stages of product development to identify and anticipate undesirable toxicological effects and optimize product development. The Green Chemistry movement embraces similar ideas for development of less toxic products, safer processes, and less waste and exposure. Further, the concept of benign design suggests ways to consider possible toxicities before the actual synthesis and to apply some structure/activity rules (SAR) and in silico methods. This requires not only scientific development but also a change in corporate culture in which synthetic chemists work with toxicologists. An emerging discipline called Green Toxicology (Anastas, 2012) provides a framework for integrating the principles of toxicology into the enterprise of designing safer chemicals, thereby minimizing potential toxicity as early in production as possible. Green Toxicology's novel utility lies in driving innovation by moving safety considerations to the earliest stage in a chemical's lifecycle, i.e., to molecular design. In principle, this field is no different than other subdisciplines of toxicology that endeavor to focus on a specific area – for example, clinical, environmental or forensic toxicology. We use the same principles and tools to evaluate an existing substance or to design a new one. The unique emphasis is in using 21st 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. Starting with the formation of a steering group and a series of workshops, the Green Toxicology concept is currently spreading internationally and is being refined via an iterative process.*

*Keywords: Green Toxicology, benign design, design out, product development*

### Introduction

Over the past few decades, there has been an increase in consumer demand for less toxic, more environmentally-friendly products, as well as increasing regulatory and economic pressures for more sustainable products, less wasteful manufacturing, and a switch to renewable resources as source materials – in essence, a Green Chemistry approach (Anastas and Warner, 2005) that puts environmental and sustainable principles at the forefront of chemical design.

In order for Green Chemistry to flourish, however, there must be a parallel paradigm change in toxicology: less toxic chemicals cannot be designed unless scientists have the necessary tools to quickly and accurately assess chemical hazards. Toxicology has been little concerned with developing tools to help chemists better understand toxicity and to design better alternatives. The principle of "benign design" has been part of the 12 founding principles of Green Chemistry from its inception, and principles three and four directly address this (Box 1). Other principles aim to reduce waste and use of chemicals and thus limit exposure in the environment and the workplace.

The current industrial product development paradigm relies on time-consuming, expensive animal studies and is too slow to keep pace with technological change (Hartung, 2009, 2010). For example, a typical two-generation reproductive study costs more than $500,000, uses more than 3000 rats, and takes

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**Box 1**

**The 12 principles of Green Chemistry** (Anastas and Warner, 1998)

1. It is better to prevent waste than to treat or clean up waste after it is formed.
2. Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
3. Wherever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
4. Chemical products should be designed to preserve efficacy of function while reducing toxicity.
5. The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.
6. Energy requirements should be recognized for their environmental and economic impacts and should be minimized. Synthetic methods should be conducted at ambient temperature and pressure.
7. A raw material or feedstock should be renewable rather than depleting wherever technically and economically practicable.
8. Reduce derivatives – Unnecessary derivatization (blocking group, protection/deprotection, temporary modification) should be avoided whenever possible.
9. Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. Chemical products should be designed so that at the end of their function they do not persist in the environment and break down into innocuous degradation products.
11. Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
12. Substances and the form of a substance used in a chemical process should be chosen to minimize potential for chemical accidents, including releases, explosions and fires.

15 months to complete. For this reason, toxicity testing is typically reserved for the latter stages of chemical/product development (after the chemical or product is determined to be commercially viable). Consequently, toxic effects are identified closer to commercialization when few options for design changes exist and after significant investment of time, resources and money. Today, rapidly evolving, 21st century safety assessment methodologies have the potential to transform how companies develop and commercialize new products and chemicals.

This rapid, high-throughput, high-content *Green Toxicology* paradigm can work in tandem with R&D by providing answers about mechanisms of toxicity quickly, inexpensively, and with the small quantities of material typically available for R&D. Green Toxicology combines the *in vitro* and *in silico* tools of predictive toxicology with the principles of chemical design to develop chemicals that have negligible toxicity. It also enables early elimination of candidates possessing undesirable traits by "failing early and failing cheaply" – or, to put it more positively, innovation through early and inexpensive evaluation of hazard.

## Consideration 1: The first principle of Green Toxicology – "Benign design"

The idea is simple: toxicologists partner with synthetic chemists to understand what chemical moiety may impart undesired hazard traits as early as feasible in product development. Toxicology is in the midst of a major transition from animal-based methods that are slow, expensive and suffer from low-throughput to more modern approaches utilizing cheminformatics, cell cultures, genomics and computational biology to achieve greater speed and throughput, lower cost and – ultimately – more accurate predictions for humans and environmental safety (Leist et al., 2008; Hartung, 2009).

Programs based on structure activity relationships (SAR), for example, can be useful in guiding early selection of low hazard candidates for continued product development. A nice illustration is the "ultimate rat carcinogen" drawn by Tennant and Ashby (1991) showing the chemical features associated with mutagenicity in one theoretical molecule (Fig. 1). However, when challenged to prospectively predict the outcome for 30 chemicals to be tested in the US National Toxicology Program, the authors achieved only 50-60% prediction of the carcinogenic substances and wrongly predicted 40-50% of non-carcinogens as positive in the animal test (Benigni, 2004). This illustrates the limitations of SARs for such complex endpoints (Basketter et al., 2012) and therefore, as much as read-across and QSARs have helped to make testing more targeted and efficient, their utility lies in low cost, rapid "Tier 1" assessments of new candidate chemistries and sustainable alternatives.

It is worth pondering whether the existing QSARs will have adequate applicability for the more novel chemicals that emerge from Green Chemistry research – for example, QSARs developed for industrial synthetic chemicals may not be applicable for bio-based materials. Some positive examples exist, especially in the field of aquatic toxicity (Voutchkova et al., 2010a,b, 2011), but this is arguably an easy case, where lipophilicity is key to uptake and thus a hazard. This does not mean, however, that helpful estimates for more complex hazards, such as immunotoxicity (Hartung and Corsini, 2013), developmental neurotoxicity (Smirnova et al., 2014) or endocrine disruption (Juberg et al., 2014), could not be done.

Additionally, while QSARs have certainly proven their merit in the pharmaceutical industry, this success is unlikely, for a variety of reasons, to be repeated for industrial chemicals. Industrial chemicals may consist of polymers with a wide range of

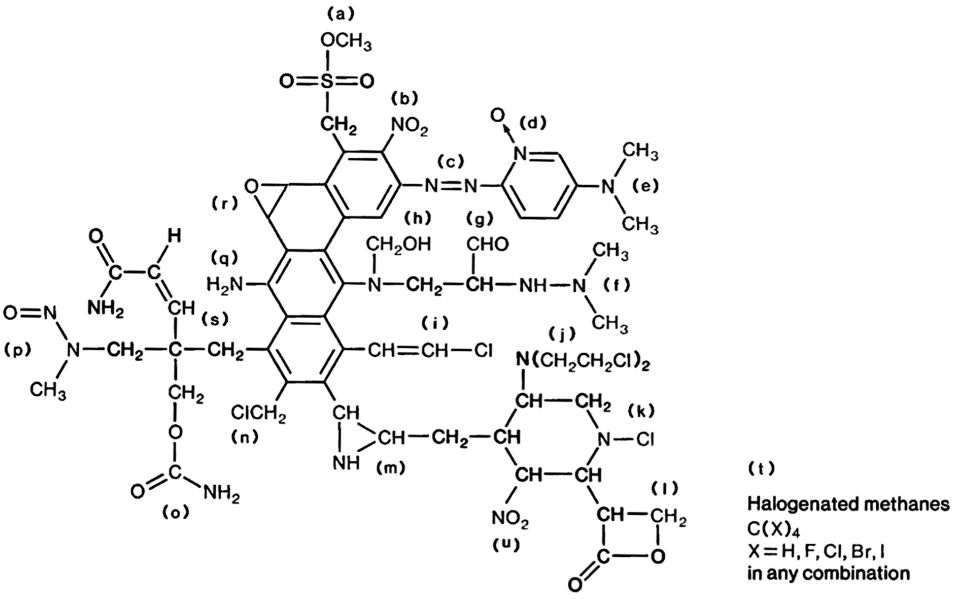


**Fig. 1: The "ultimate rat carcinogen" showing the chemical features associated with mutagenicity in one theoretical molecule**
Reproduced with permission from Tennant and Ashby (1991).

molecular weights, various impurities, left over reagents, etc., while the majority of drugs fall into a more narrow chemical space often referred to as the Lipinski rules (Lipinski, 2004):

– No more than 5 hydrogen bond donors (the total number of nitrogen-hydrogen and oxygen-hydrogen bonds)
– Not more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
– A molecular mass less than 500 daltons
– An octanol-water partition coefficient log P not greater than 5

This is a nice example (though not for safety but efficacy) of how structure considerations can help substance design.

More fundamentally, QSARs developed for the pharmaceutical industry have a domain defined by suspected biological activity and may simply lack the accuracy necessary when the overwhelming number of chemicals, in fact, lack toxicity, as is the case for many industrial chemicals (Box 2); the respective estimates reflect internal ECVAM (European Centre for the Validation of Alternative Methods, now EURL ECVAM) analyses of the European New Chemicals Database, which includes new industrial chemicals registered since 1981 under the Dangerous Substance Directive, around 2005 (Hoffmann et al., 2005; Hoffmann and Hartung, 2005). Therefore, while QSARS likely will have a role to play in the development of benign alternatives, it is equally important that toxicology develop other techniques and approaches that link molecular structure with toxic outcomes in a way that can be useful to synthetic chemists.

**Box 2**
**Most chemicals are not toxic:**

| | |
|---|---|
| 90% | not acutely toxic (EU New Chemicals Database) |
| 97% | not skin corrosive (EU New Chemicals Database) |
| 93% | not skin irritant (EU New Chemicals Database) |
| 97% | not teratogenic (expert estimate, about 60% not positive in single species two-generation studies) |
| 80-95% | not carcinogenic (expert estimates, 47% not positive in rodent bioassay) |
| 80% | not eye irritating (EU New Chemicals Database) |
| 65% | not skin sensitizing (EU New Chemicals Database) |

## Consideration 2: The second principle of Green Toxicology – "Test early, produce safe."

The pharmaceutical industry has developed the concept of *fail early*, *fail cheap* as a consequence of the cost explosion and high failure rates in late clinical development (Hartung and Zurlo, 2012; Hartung, 2013). It was noted that in the 1990s, for example, a large number of drugs failed because of pharmacokinetic problems, i.e., the active agent did not reach sufficient concentrations in the targeted organ in patients. Addressing this early and with human-relevant methods markedly reduced this type of failure (Singh, 2006; Tsaioun and Jacewicz, 2009).

This approach can also be adapted for front-loading toxicity testing of industrial chemicals. In the short term, predictive safety assessment offers a way to enrich the R&D pipeline for chemicals that are most likely to clear challenging regulatory hurdles. Because predictive methods focus on the root causes of toxicity at the cellular and molecular levels, they also gener-

ate new knowledge to inform the design of safer and more sustainable products. Traditional toxicity costs a total of several million dollars for a product to reach the marketplace. These studies also take a lot of time, in some cases requiring years to complete. The rat cancer bioassay, for example, entails two years of treatment plus time for planning, histopathology and reporting. And often, at the end of this process, the results are equivocal and may be of questionable relevance for humans (Basketter et al., 2012). If the results are positive, such bioassays typically provide no mechanistic information that would help a synthetic chemist to design a less toxic alternative. Under the pressure of *time-to-market* and the running clock of the patents and competitive economic pressures, these are clearly not the best tools for early decision making.

Front-loading thus requires screening-level tests that are both less costly and much faster, and contribute to a smarter approach that begins with *in silico* screening to predict possible targets and progresses to targeted *in vitro* tests that can examine suspected Pathways of Toxicity (PoT) (Hartung and McBride, 2011; Kleensang et al., 2014). For those candidates that do move on to whole animal tests, a smarter testing approach might allow for reduced reliance on high-dose testing that causes gross pathological change as an indication of toxicity and focuses more precisely on the molecular initiating event at doses that can meaningfully be related to possible human exposures.

Another advantage of front-loading toxicity in the R&D process would be to reduce cases of *out of the frying pan, into the fire* – in other words, replacements that are promoted as alternatives to known "bad actors" often turn out to be not necessarily less *toxic*, but simply have less *data* (as was the case with flame retardants (Lakind and Birnbaum, 2010)). This creates a somewhat perverse incentive to avoid gathering toxicity data, which is compounded by the fact that consumer preferences can be markedly influenced by the results of toxicity tests taken out of context. More rigorous toxicity testing as an essential part of the R&D process would likely produce a more rational selection of benign replacements.

### Consideration 3: The third principle of Green Toxicology – "Avoid exposure and thus testing needs."

Traditionally, toxicologists are trained to think in terms of molecules and not in terms of the production processes behind them. Within the many steps involved in the production of industrial chemicals, however, small alterations can often achieve significant reductions in terms of exposure and therefore minimize risks – toxicity, for example, may reside in a catalytic agent that can be eliminated with alternative routes of synthesis. For many polymers, the final product has a sufficiently large molecular weight so as to preclude bioavailability, and any hazard is likely due to residual monomers. Consequently, small changes in the efficiency of the reaction or the purification step can drastically reduce the hazard while conserving resources. Similarly, a change to *one-pot synthesis* (meaning that all reactions take place in the same vessel) can decrease the number of exposed workers. In this respect, the goals of Green Toxicology dovetail with Green Chemistry's goal of improved efficiency and emphasis on the importance of close collaboration between the chemist and the toxicologist. Together their measures directly affect occupational health and then (via reduced exposure) influence risk assessment and management. Such scenarios are ripe for exposure-driven testing strategies, which can result in reduced testing demands. Reduced exposure also makes it more likely that thresholds of toxicological concern (TTC) (Kroes et al., 2005; Munro et al., 2008) are not exceeded – an example of

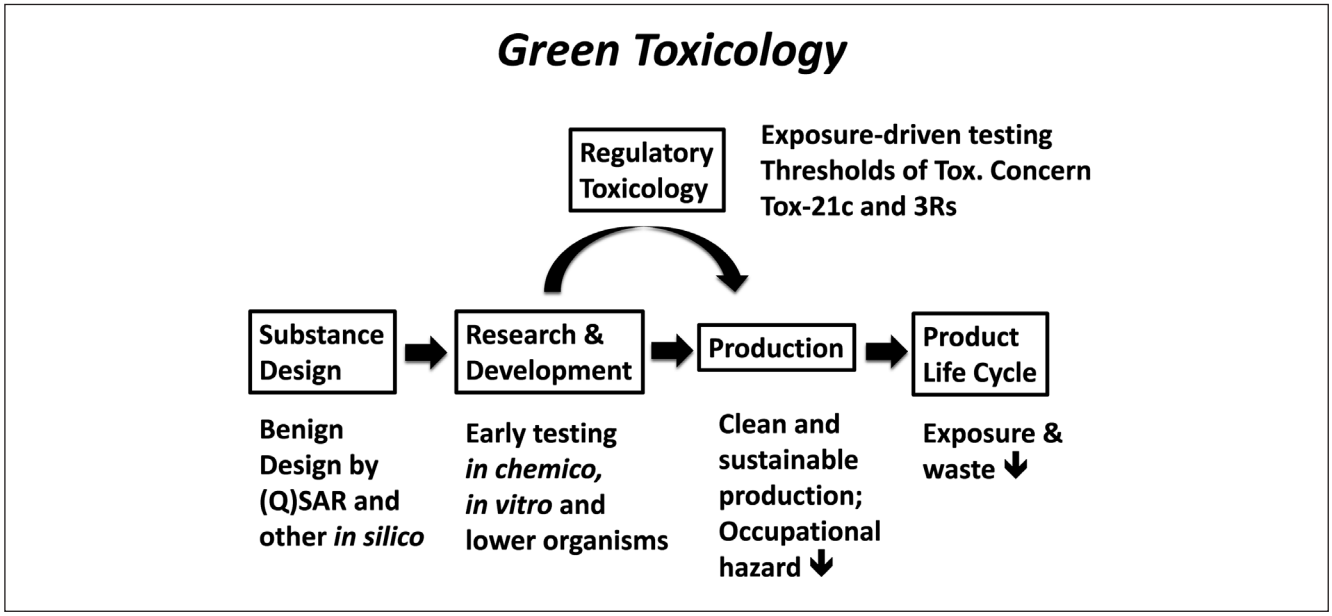


**Fig. 2: Principles and results of Green Toxicology**

a formalized, exposure-driven assessment. The idea is simple: We can assess how much of the known toxicants is necessary to exert a certain effect (which will give a distribution of doses) and then define a point-of-departure dose. With an appropriate safety factor it is unlikely that an unknown agent will exert this toxicity at lower doses. The concept has been pioneered for food (Kroes, 2004) and cosmetics (Blackburn et al., 2005; Kroes et al., 2007), but also adapted to pharmaceuticals, especially for genotoxic impurities. The World Health Organization, for one notable example, is currently reviewing the approach[2]. TTCs were first used for carcinogens, but the concept has also been adapted to reproductive toxicity testing (van Ravenzwaay et al., 2011). We argued in a similar way in the context of immunotoxicity (Hartung and Corsini, 2013), showing that clinical immunosuppressants require mg/kg quantities to exert their effects and that this could be used for establishing TTC for substances not optimized for this purpose.

In conclusion, Green Chemistry – by reducing exposure and thus testing demands – has more to offer toxicology, safety testing and risk management than just Benign Design and Early Testing (Fig. 2).

## Consideration 4: The fourth principle of Green Toxicology – "Make testing sustainable."

Animal testing is not just costly in terms of time and money, but is inefficient with regards to resources and energy and produces a great deal of biohazard waste. Consequently, we argue that the long-term use of animals is fundamentally unsustainable. It has been estimated that regulatory requirements in Europe require approximately 300 animals to test a new chemical compound and up to 10,000 animals for a pesticide (Bottini et al., 2007). The 10,000 animals per pesticide, it should be noted, do not include abandoned products. Before REACH in Europe, 90-140,000 animals were used for testing roughly 200 new chemicals per year, not including testing outside of Europe. In the US, discrepancies in testing demands are even stronger between different products, with seven out of eight new industrial chemicals having no toxicity data at pre-marketing notification under the Toxic Substance Control Act (Hartung, 2010) and similar requests for more than 30 animal tests for pesticides. With 40,000 animals tested for safety per new substance entering the market and 350,000 for R&D (factoring in the animals used for candidate drugs that do not succeed), the pharmaceutical sector still uses a large number of animals despite the impressive reductions in recent years. This is not only unsustainable but may impose an economic barrier that is prohibitive for niche chemicals with limited profitability. A smarter, *in vitro* testing strategy can reduce the use of resources for testing by better prioritization and more efficient screening-level tests. In the longer term, we hope agencies will find greater application of predictive methods to address some of the requirements of their programs.

## Consideration 5: "Early testing can use methods not yet mature enough for regulating."

Regulation tends to take a precautionary approach oriented towards minimizing mistakes rather than optimizing the cost/benefit analysis, making it profoundly difficult to change traditional approaches. Furthermore, a traditional validation study takes about one decade. Consequently, a validated test is "frozen in time," and it is simply impossible for regulatory mechanisms to keep up-to-date with the current rate of change in science (Hartung, 2007; Leist et al., 2012).

Front-loading toxicity at the research and development stage, however, allows a more flexible approach. Prioritization of substances for development can be based on methods which still have some margins of error. Early testing allows the use of methods that are not yet validated. *In silico* and *in vitro* tests that are individually too inaccurate for regulatory purposes will likely have a useful place in an integrated testing strategy (Hartung and Hoffmann, 2009; Hartung et al., 2013). Such strategies allow uncertainty in results and seek to combine data from multiple tests in a flexible manner that maximizes predictive power while also providing an estimate of the uncertainty in the data. This also helps to build capacity and capability to perform these assays for later regulatory use, if validated and accepted. In the meantime, front-loaded methods will be generating data, and thereby facilitating an assessment of the predictive value of these methods and thus contributing to the validation and acceptance process.

This also opens up a role for new risk assessments based on toxicity "pathways" (cell/molecular level changes) and data-driven uncertainty factors (e.g., intra-human variability factors based on genetic analysis). It will take tremendous time to base regulatory testing on PoT, as the respective database would first need to be sufficiently and comprehensive and validated (Hartung and McBride, 2011). With each and every PoT identified, however, the respective assays can be included in integrated testing strategies. A pathway-based approach can also allow for more precise understanding of individual variation in response to toxicity as well as susceptible populations by illuminating more precisely the differences in PoT. Similarly, default safety and assessment factors might be replaced by knowledge on intra-species and inter-individual differences in PoT.

## Consideration 6: Green Toxicology as a driver of 21st century toxicology

Biology has been transformed over the last decade from a reductionist and largely qualitative science to a more quantitative approach requiring systems-level thinking, large-scale data analysis, and multi-scale modeling. Although certain areas of toxicology (such as PBPK modeling) have long embraced mathematical models and elements of systems-level thinking, the insights gained from systems biology have not generally been

[2] http://www.who.int/foodsafety/chem/ttc/en/

reflected in regulatory toxicology or hazard assessment (Hartung et al., 2012). Furthermore, the field of toxicology is only beginning to assemble the type of large-scale data sets that have been transformative in molecular biology. As the Green Toxicology paradigm of high-throughput, omics-based approaches for screening many compounds gathers data, this can act as a driver for transforming toxicology from a reductionist approach based on "feet-up/feet-down" assays (i.e., the $LD_{50}$) towards an approach that uses the insights of systems biology, computational modeling and exploratory data mining to locate the mechanism of toxicity in perturbed networks. Green Toxicology can serve as a bridge between 21st century toxicology methods and the development of safer, sustainable products.

This paradigm shift and transformation is necessarily a slow and lengthy process as the safety of workers and consumers is at stake. This delay makes regulatory science less attractive for academic research and even less so for the commercialization of test methods. If companies have to wait a decade for the regulatory acceptance of a test with unclear prospects for the validation phase, the return on investment is rather unlikely. Early non-regulatory testing creates an immediate market for new test methods, liberating the market forces necessary to standardize and disseminate tests internationally (Bottini et al., 2007; Bottini and Hartung, 2009, 2010).

## Consideration 7: The Green Toxicology Program

Following on the initial success of our Green Toxicology Information Day in November of 2013 and its forerunner at University of Connecticut in December 2012[3], a follow-up series of webinars is planned. In addition, a proposal for a session at the 2015 Society of Toxicology meeting has been accepted. Information sessions at the GlobalChem conference and the ACS Green Chemistry Conference are planned. A multi-day "Green Toxicology Workshop" is planned for the spring of 2015 in Washington State. Curricula for students of synthetic chemistry – who are typically given minimal training in toxicology – are a further goal. Development of dedicated scientific articles and a textbook on Green Toxicology, as well as a compendium of Design Rules for Reduced Hazard aimed at synthetic chemists (the "green toolbox"), will be significant products of the effort. Furthermore, the CAAT policy program will inform policy makers about the opportunities of a Green Toxicology approach. Key to this outreach will be bringing together two communities – toxicologists and chemists – that have long worked in parallel but have rarely worked collaboratively.

## Conclusions

Alternative methods in toxicology are increasingly enabling technologies, i.e., they can do more than optimize and replace current regulatory testing. The pharmaceutical field has for some time taken advantage of front-loading testing and mechanistic understanding for early determination of possible toxic liabilities. The chemical industry has begun to embrace similar concepts from the Green Chemistry movement. A Green Toxicology is emerging, which uses structure-activity relationships for the design of less harmful substances, tests early in the development process to prioritize less dangerous chemicals, and reduces exposures – thereby reducing risk and testing demands. These approaches promise to create opportunities for the development and use of alternative test methods and will support the transition to sustainable chemistry.

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[3] http://caat.jhsph.edu/programs/workshops/greenTox.html

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**Correspondence to**
Thomas Hartung, MD PhD
Center for Alternatives to Animal Testing
Johns Hopkins Bloomberg School of Public Health
615 North Wolfe Street
Baltimore, MD 21205, USA
e-mail: thartung@jhsph.edu