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Building on a solid foundation: SAR and QSAR as a fundamental strategy to reduce animal testing[$]

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The development of more efficient, ethical, and effective means of assessing the effects of chemicals on human health and the environment was a lifetime goal of Gilman Veith. His work has provided the foundation for the use of chemical structure for informing toxicological assessment by regulatory agencies the world over. Veith's scientific work influenced the early development of the SAR models in use today at the US Environmental Protection Agency. He was the driving force behind the Organisation for Economic Co-operation and Development QSAR Toolbox. Veith was one of a few early pioneers whose vision led to the linkage of chemical structure and biological activity as a means of predicting adverse apical outcomes (known as a mode of action, or an adverse outcome pathway approach), and he understood at an early stage the power that could be harnessed when combining computational and mechanistic biological approaches as a means of avoiding animal testing. Through the International QSAR Foundation he organized like-minded experts to develop non-animal methods and frameworks for the assessment of chemical hazard and risk for the benefit of public and environmental health. Avoiding animal testing was Gil's passion, and his work helped to initiate the paradigm shift in toxicology that is now rendering this feasible.

Keywords: Animal testing; (Q)SAR; read-across; safety assessment; visionary

1. Introduction

Tools based on (quantitative) structural activity relationships ((Q)SARs) have already begun to impact upon the use of animals in the testing of chemicals. Companies and government agencies around the world have invested in and relied upon these tools to estimate the potential hazards of chemicals in the pesticide, industrial, cosmetics and food additive sectors, among others. The general principles of grouping and categorisation that allow the "read-across" of information from one chemical to another have contributed more than any other factor to a reduction in the number of animals used in chemical assessment programs such as the US Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program. Chemical categorisation and read-across have also been implemented in the Organisation for Economic Co-operation and Development (OECD) HPV program, and

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[$]Dedicated to the memory of Dr. Gilman D. Veith (1944–2013).

are expected to reduce the numbers of animals used under the EU Registration, Authorisation, and Restriction of Chemicals (REACH) legislation [1].

The concept that a chemical's structure can inform or predict biological activity, particularly potential adverse effects, has made an increasing impact in recent years on chemical safety evaluation. Improvements in *in vitro* molecular data gathering technology (e.g., high-content imaging and "omics" approaches) and in computer science (analytical tools, complex databases, and interpretive algorithms) have allowed the identification of chemical activity fingerprints, or biomarkers, that can be used in a variety of ways, including the prediction of overall toxicity (e.g. the EPA Toxicological Prioritization Index or "ToxPi" [2]). In addition, the recent application of pathway-based approaches – a combination of chemical structure knowledge and biological event assessment – to inform the interpretation of toxicological information has provided the opportunity to move away from animal testing by increasing the predictivity of molecular assays that query upstream events in a pathway, avoiding the need for the assessment of adverse outcomes in animals.

In large part the genesis, development, and uptake of these revolutionary approaches has resulted from the influence of Dr Gilman Veith. His scientific vision and tireless promotion of predictive tools have created a new climate that offers a more efficient protection of human health and the environment, at the same time greatly reducing reliance on laboratory animals for testing.

Dr Veith was instrumental in developing the QSAR and predictive toxicology program at EPA, and at the OECD in Paris he guided the development of the OECD QSAR Toolbox [3] and the initial principles that would lead to the results of tools in this toolbox being trusted by regulatory agencies throughout the world.

Following his tenure at the OECD Dr Veith founded the International QSAR Foundation, which was dedicated to the development of QSAR methods to replace regulatory animal tests. While our organizations supported Dr Veith's efforts at the IQF both materially and scientifically, our own work to reduce the use of animals in regulatory testing has benefited immeasurably from the education, outreach, and activism Dr Veith accomplished during the short time the IQF was in operation.

2. Reducing animal use through international cooperation

There are three major ways in which QSAR-based tools can reduce animal testing; these are category formation (including read-across), end point prediction, and hypothesis generation for directing testing. In general, chemicals with similar chemical structures can be expected to interact similarly with biological systems, providing the rationale for the formation of categories of structurally similar chemicals. If hazard data exist for some of these chemicals, one can then "read across" the category and it may be assumed – depending on a number of factors – that other category members share a similar potential for hazard. Using this approach an estimate of potency should be possible provided hazard potency within the category tracks structural characteristics, for example carbon chain length [4]. While it is possible to create categories manually, computational tools make the exercise much simpler.

Predictive models based on structural characteristics provide an estimate of the toxicity value of interest (e.g., LC50), based on the toxicity information used to build the model.

For a number of years the US EPA has relied on tools such as ECOSAR [5] and Oncologic® [6] to estimate the potential for new chemicals to harm organisms in the environment [7], allowing regulatory decisions to be reached in the absence of specific test data. The concepts underlying these tools were built on the pioneering work of Dr Veith and

his colleagues at the EPA National Health and Environmental Effects Research Laboratory (NHEERL) in Duluth, MN (e.g. Russom et al. [8]). These and similar tools were made publicly available, firstly on the EPA website and later as part of the OECD QSAR Toolbox, allowing all stakeholders to benefit from this important work.

In the late 1990s the EPA embarked on the HPV Chemical Challenge Program (HPV Challenge), aimed at collecting hazard data for chemicals produced in or imported into the US in annual volumes of 1,000,000 lb or more. Animal protection groups, including our own organizations, provided advice to the EPA and to companies and consortia on strategies for reducing animal testing. The authors' experience [9] and that of others is that SAR concepts have more than any other strategy dramatically reduced the number of animals killed within this program. In particular, van Leeuven, Bishop and co-workers [7,9] found that the majority of human health and ecotoxicology data gaps could be filled using read-across and QSAR tools. The successful use of read-across in the HPV program paved the way for current EPA chemical assessment activities and provided a foundation for legislative efforts to improve chemical assessment and regulation in the US.

Between 1992 and 2008 almost 50% of the 850 chemicals assessed within the OECD HPV program were ascribed to a chemical category, avoiding the need for *de novo* testing for end points of interest, as presented by van Leeuwen et al. [7]. Estimates carried out by the present authors on the list of chemicals currently sponsored in the OECD HPV database (http://webnet.oecd.org/HPV/UI/Default.aspx) have shown that this ratio has remained constant since 2008.

Since the initial publication of the OECD QSAR Toolbox in that year, stakeholders (including our organizations) have used these tools to provide more helpful and scientifically relevant comments to parties within a diverse set of programs, including the EPA and OECD HPV programs, REACH [10], the US National Toxicology Program and the EPA Office of Pesticide Program testing requirements. The OECD QSAR Toolbox has also been used to address specific information needs within industry and in other regulatory programs. These tools, created from the research and work of Gil and his colleagues, have saved the lives of countless laboratory animals.

REACH, the European legislation which collects comprehensive toxicity data on all substances in the European market, allows for the use of (Q)SAR tools, in fact it requires that alternatives strategies of information gathering are explored before resorting to animal testing. Before the legislation came into force it was expected that alternative strategies would dramatically reduce the use of animals required, with read-across making the largest contribution [7, 11]. However, since testing requirements focus on registration of substances by production volume rather than by structure, concerns were raised, e.g., by Schaafsma et al. [12], that the potential for formation categories and read-across for preventing testing could not be fully realized. While (Q)SAR models and read-across have to some extent been used, established (Q)SAR models are used less often than might be expected [13], and there has been little real effort on the part of the European Chemicals Agency (ECHA) to use (Q)SAR models in targeting testing requirements. Coupled with the limited acceptance of read-across within ECHA [14], these factors have already led to the sacrifice of hundreds of thousands of animals to fulfill REACH requirements (see Rovida [15]).

3. The International QSAR Foundation (IQF): Advocating a new toxicology paradigm

The IQF was formed in 2004 as an *ad hoc* collection of scientists interested in extending the boundaries of QSAR use in regulatory applications. The original description and mission

statement of the IQF demonstrated Gil's long-term commitment to moving away from animal testing:

> "The International QSAR Foundation to Reduce Animal Testing was created to accelerate the development of QSAR methods for use in safety assessment and product discovery programs. In addition to funding new QSAR research, the Foundation hosts independent peer consultations on existing QSAR models that show promise for estimating specific regulatory endpoints. These peer consultations provide an independent critique of QSAR models and a roadmap for improving them in the near future."

Activities began in 2006 with a series of workshops to refine and expand the models already in use. Models included CATABOL, a QSAR approach to modeling the biodegradability of chemicals [16], and the Tissue Metabolism Simulator-Skin Sensitisation (TIMES-SS) [17–19] for the identification of chemical allergens, developed by the Laboratory of Mathematical Chemistry in Bourgas, Bulgaria.

The computation of chemical reactivity to predict skin sensitization became a major project within the IQF, three dedicated workshops being created between 2006 and 2010, and the formation of a Consortium for Skin Sensitization supported by ExxonMobil, Unilever, Procter and Gamble, the Research Institute for Fragrance Materials, L'Oreal, Dow Chemicals, DuPont, Givaudan, and the Danish Institute for Toxicology and Risk Assessment. The workshops were successful in relating intermediate empirical measures of chemical reactivity to downstream biological effects in order to allow the deduction of reactivity directly from structure [20]. The workshops also refined the models of nucleophilic activity and associated databases used to predict adverse effects, including skin sensitization, and inhalation, hepatocyte, and fish toxicity.

The IQF also sponsored a series of conferences titled the McKim Conferences (named after the eminent QSAR pioneer, James McKim) and held in 2006, 2007, and 2008, along with two special workshops on carcinogenesis in 2010 and 2012. The long-term goal of the McKim Conferences was to develop a new generation of tools and concepts to reshape the foundation of risk assessment from a "test-all-chemicals-for-all-hazards" approach to a more efficient hypothesis-driven testing approach, as described in [21] and [22]. The models would allow extrapolation of the existing experimental data on the chemicals tested to related untested chemicals, related species, or other hazard end points. In this approach QSAR, the interspecies extrapolation models and biological system models served as the prime elements of a new paradigm in risk assessment.

An important element of the McKim conferences was the identification and creation of methods for removing existing barriers to the development and use of the various models. For example, the 2006 McKim conference identified four major barriers to maximizing the use of existing test data:

(1) The data were scattered throughout the literature and private databases in an inconsistent non-digital format;
(2) The QSAR models for predicting the intrinsic behavior of chemicals did not adequately model reactive toxicity or hydrogen bonding at the receptor;
(3) Interspecies correlation models did not uniformly model the metabolic differences of species, nor predict their vulnerabilities; and
(4) The biology of the systems and the virtual animal models were in their infancy and did not adequately link the chemical perturbation of a system to its most important biological effects.

The conference then made a series of recommendations:

- Identify the adverse outcomes which formed the "big worries" to regulators, the adverse outcomes which provided the best current understanding of the mechanisms involved, and the QSAR and other models which were currently most useful, and build on them;
- Develop short, medium and long-term goals; and
- Engage experts offering a broad range of expertise.

The 2007 McKim conference explored the role of chemical categories in the hypothesis-driven paradigm, specifically the new QSAR models for estrogen receptor (ER) binding, plus the integration of QSAR and systems biology, in identifying the key molecular initiating events needed to predict the hazards of chemicals. The conference led to a number of papers covering a range of topics, including a QSAR model for the inhalation toxicity of narcotics [23], the use of solubility as a hazard identification reference point, a conceptual framework for toxicity pathways [20], and a category model for ER binding affinity, as described by Schmieder et al. [24] and developed with the US EPA, Duluth. The work in Schmieded et al. [24] aimed to screen large chemical inventories with experimentally supported QSAR models for nuclear receptor binding.

The 2008 McKim conference provided illustrations of the reasons the QSAR models for end points of complex effects required knowledge of toxicity pathways in order to simulate the results of animal tests, and provided the basic structure of a visualization system for toxicity pathways that described the biological effect linkages across different levels of biological organization. The IQF provided a visionary solution in its newly created *Effectopedia* [25] as a web-based tool to assist in the identification of biological response mechanisms.

The specialized McKim workshops on Data Redundancy in Cancer Assessment, held in 2010 and 2012, focused on streamlining the array of toxicity tests used in the assessment of carcinogenicity, including development of a hypothesis-driven testing framework for organizing short-term evidence of low incidence *in vivo* risks and more effective cancer assessment. As in other McKim workshops the scientific barriers, in addition to the critical paths required to overcome them, were explored. The initial goal of the workshops was to reinterpret the rodent cancer data and identify supporting evidence for carcinogenicity using more rigorous QSAR-based chemical categories by collating chemicals according to their molecular interaction with DNA and other macromolecules. Once categorized, the *in vitro* and *in vivo* evidence could be assessed using adverse outcome pathways to predict the assessment end points used by regulatory authorities. In other words, the goal was to identify chemical classes for which computational profilers and *in vitro* assays could accurately predict rodent cancer outcomes, avoiding the need for rodent testing for these chemicals [26].

The first workshop focused on genotoxic carcinogens and on the potential for a cell transformation assay to identify epigenetic carcinogens. The objectives of the second workshop, Reducing Data Redundancy in Cancer Assessment, were in the first place to review QSAR screening methods for grouping chemicals based on their potential to induce carcinogenesis through genotoxic and epigenetic pathways, and secondly to evaluate the combined use of structural domains and the *in vitro* data to avoid the need for rodent cancer bioassay. The activities planned in this workshop remain unrealized, although there are ongoing efforts to continue the development of certain carcinogenicity adverse outcome pathways and an integrated testing strategy [27].

4. Inspiration for the work ahead

Gil's passion for improving the scientific rigor and regulatory acceptance of QSAR tools made him one of the field's most ardent advocates, in both word and deed. A central Veith tenet was the necessity of applying models appropriately, and he expressed his concern by tireless education regarding the proper use of models, including a number of international pilgrimages to train potential users of the OECD QSAR toolbox.

In the recent past, guidance on the use and reporting of outcomes from QSAR tools has been published at the regulatory level, including the OECD Guidance Document on the Validation of (Quantitative) Structure–Activity Relationship [(Q)SAR] Models [28]. The QSAR Model Reporting Format (QMRF), a harmonized template for summarizing and reporting key information on (Q)SAR models, is published by the European Commission Joint Research Centre (JRC) and is based on OECD QSAR validation principles. A QMRF can be filled in using the JRC-developed QMRF Editor [29]. This guidance aims to increase the regulatory acceptance of QSARs. There are currently 70 QMRFs registered in the QSAR Model Reporting Format Inventory (http://qsardb.jrc.ec.europa.eu/qmrf/index.jsp).

As explored in the McKim conferences, the field of regulatory toxicology is currently undergoing a revolution, driven by the intention to predict better the human health and environmental consequences of exposure to xenobiotic agents, using an approach based on chemical structure-driven mechanisms. This framework will exploit *in vitro* and *in silico* models in order to predict *in vivo* results. Gilman Veith was part of a small group of people who predicted this promising future.

In 2003 and 2004 Veith and colleagues presented two papers outlining key barriers that would have to be overcome if QSAR was to make a greater contribution to chemical risk assessment [21,30]. Veith called for QSAR scientists to recover their field from skeptics in the regulatory setting, and from experimentalists who doubted whether QSAR would ever predict complex human health end points. It would do this by focusing on defining end points linked to chemical structure – not the "icities" familiar from apical animal studies, but mechanistic activities that resulted from the interaction of chemical structure and biological systems, leading ultimately to an apical adverse outcome via a "toxicity pathway" (see Figure 1 in Veith [21]). Ten years later this vision is well underway. QSAR models continue to be improved, providing the tools needed by risk assessors to increase the depth and breadth of toxicology assessments, while relying less and less on tests involving animals.

Major research efforts by regulatory agencies, both in the EU and US, have a common focus on linking the structures and properties of chemicals, via defined modes of action, to adverse outcomes at the organism or population level [31–36].

Academic efforts, aided by funding for "alternative methods", especially in the EU, have incorporated this practical pathway-based approach. As described in Ellison et al. [37], one can use structural alerts and physicochemical properties to determine whether a pharmaceutical compound can cause the very first stage of a toxicity pathway, known as a molecular initiating event. This information can be used to support the formation of categories of compounds or provide a first piece of evidence that warrants further Adverse Outcome Pathway (AOP) driven testing. Many industry scientists are contributing to these efforts. One team has conducted a set of blind case studies to test a QSAR framework for conducting quantitative read-across, in this case for developmental toxicity, and have found it possible to generate predictive read-across values for missing test data in some case studies [38,39]. It may be possible to augment this framework with mechanistic *in vitro* data to increase its success rate [21,40].

Scientific societies, such as the Society of Toxicology and the American Society for Cellular and Computational Toxicology, provide continuing education courses and other learning and collaboration opportunities, as do outlets such as the trade publication *Chemical Watch*. NGOs such as the International Council for Animal Protection in OECD Programmes (ICAPO) and the Human Toxicology Project Consortium work internationally to promote pathway-based approaches for regulatory use.

One key requirement is the creation of high quality databases that link structure and other characteristics of a compound to *mechanistic* data, not merely to apical outcomes [21]. While efforts have begun to construct such databases, some industry sectors, including pharmaceuticals and pesticides, have so far been reluctant to make this information public on a large scale. The long-term benefits of sharing such information and of contributing to the improvement of the regulatory risk assessment system outweigh short-term risks.

The ultimate realization of the objective of improving the assessment of regulated chemicals will require new tools to record and display the knowledge generated to inform the many AOPs upon which this new paradigm will be based. Gil envisioned Effectopedia as a means of collaboratively discussing, displaying, and discovering AOPs, especially quantitatively, and its creation is a critical building block for success. We are encouraged by recent commitments on the part of the European Commission and the OECD to fund and manage the development of Effectopedia as part of a set of tools termed the AOP–Knowledge Base (AOP–KB) and contributed to by OECD, the EC Joint Research Centre, and the US EPA. The eventual success of this set of tools will require the participation of experts from a variety of disciplines [41].

The inspirational groundwork laid by Gil and his colleagues has paved the way for the future of toxicology. This future includes not only a more efficient, effective and predictive science, but also an end to our current reliance on animal testing.

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