A QUALITATIVE AND QUANTITATIVE EXAMINATION OF THE IMPACT OF CHEMICAL REGULATION LEGISLATION ON THE FIELD OF TOXICITY TESTING

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ABSTRACT

Proposals for revising the principal United States law governing industrial chemicals, the Toxic Substances Control Act (TSCA), are currently under consideration in the US Congress, and some version of legislation is likely to be passed in the near future. At the same time a desire to move away from current testing methods for ethical, scientific, and practical reasons has led to multi-million dollar investments in *in vitro* and computational toxicology methods and programs. Such investment has been endorsed by multiple scientific bodies, most comprehensively by the US National Academies of Science in its 2007 report, *Toxicity Testing in the 21st Century: A Vision and a Strategy*. Legislative language has the potential to endorse this transition and facilitate its fruition, or conversely enshrine *in vivo* testing methods and concepts for the foreseeable future. Additionally, legislative language and subsequent regulations have the potential to affect the numbers of animals killed in toxicity tests in the near term. There are a number of strategies and incentives that, used effectively, can reduce the overall number of animals who will be killed in tests required by new legislative mandates, while strengthening environmental and human health protections. We examine legislative and regulatory options for TSCA reform and their potential impacts on animal use and test method innovation, and the likelihood that such options will assist policymakers in successfully achieving desired legislative objectives, such as providing more information on potential chemical risks for a greater number of chemicals. Analyses like these are essential to judiciously select policies that reduce the use of animals in toxicity testing and protect human health and the environment.

Keywords: integrated testing, non-animal, Toxic Substances Control Act, chemical regulation

INTRODUCTION

In part spurred by changing chemical regulation legislation in the European Union with the adoption of the Registration, Evaluation, Authorisation and Restriction of Chemicals regulation (REACH) (EC 2006), the United States Congress has attempted to amend its own overarching industrial chemicals regulation, the 1970s-era Toxic Substances Control Act (TSCA) several times over the past few years. A preliminary attempt to change this legislation, the Kid Safe Chemicals Act, was introduced into both chambers in 2006 and 2008. In 2009 and 2010, both chambers held several hearings on the topic, and members in the Senate and the House of Representatives introduced separately named bills in April and July of 2010 respectively. The Safe Chemicals Act (SCA) (S. 3209)[1] and the Toxic Chemical Safety Act (TCSA) (H.R. 5820)[2] both require all existing and new substances and mixtures to be tested and assessed according to a minimum data set to be determined by the US Environmental Protection Agency (EPA), though the bills differ in important ways, most notably in the time allowed for testing and assessment of existing substances.

This paper examines the bills' potential impacts on the numbers of animals used for toxicity testing of industrial chemicals, both in terms of the testing the bills may require if enacted and the extent to which the bills support the development and use of non-animal test methods.

Efforts to amend TSCA are motivated by deficiencies in the current law. First, several thousand substances on the market when the legislation was enacted were not required to be systematically assessed for toxicity. Second, in order to take regulatory action, the EPA is required to prove that a proposed substance has or will cause harm. Much like REACH, the proposed legislation seeks to shift this burden to producers by requiring the collection of toxicity and use information on existing substances and demonstration of the safety of new substances before they can be marketed.

Because assessments need to be completed for perhaps tens of thousands of substances, the tests to be required and the test methods to be used are of critical importance. Traditional batteries of animal tests--acute, sub-chronic, and chronic tests assessing a substance's effect on various mammalian systems--are time-consuming, expensive, and ethically problematic. For these reasons it is essential that testing requirements in the legislation are flexible--a "one size fits all" approach, which is typical of previous chemical testing programs, will expend a large amount of resources without offering concomitant public health protection. A greater reliance on strategies, models, and tools that do not involve animals translates directly into an ability to generate more information on more substances more quickly (Bradbury et al 2004). These strategies include:

- Integrated Testing Strategies (ITS) and/or Tiered Testing
- Data read-across among categories of similar substances

[1] http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=111_cong_bills&docid=f:s3209is.txt.pdf
[2] http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=111_cong_bills&docid=f:h5820ih.txt.pdf

- Quantitative Structure Activity Relationship (QSAR) models
- *In vitro* assays
- Sharing of existing data or testing responsibilities
- Testing waivers
- Prioritization of substances or hazard endpoints.

These strategies allow authorities to focus on priorities for assessment and regulation, and shorten the time needed to generate information necessary to make an assessment, and ultimately decrease the number of animals used.

In an effort to address the shortfalls of current toxicity testing practice, the EPA commissioned an investigation by the National Academy of Sciences' National Research Council (NRC) to envision a "21st-Century" toxicology testing program. The group describes this vision in its 2007 report *Toxicity Testing in the 21st Century: A vision and strategy* (NRC, 2007). The report takes into account advances in cell and molecular biology and other disciplines, and the desire to increase the breadth and depth of investigation possible with current toxicology testing methods (i.e., non-human animals), and recommends a complete shift to a human cell-based, high-throughput approach that examines chemically-induced perturbations in normal cellular processes (pathways). These perturbations are linked to human health consequences via population-informed dose-response modeling. In addition to removing species-extrapolation considerations, this paradigm could conceivably assess thousands of substances a week, allowing a dramatic increase in any entity's ability to assess the potential hazards of substances, pollutants, contaminants, and mixtures of these.

This vision dovetails nicely with the desire for more information about the potential hazards of manufactured substances and products that revised legislation in the EU and US are intended to address. In fact a number of colleagues have stressed that the intent of REACH cannot be met without such a paradigm shift (Hartung 2009, van Leeuwen et al 2009). Therefore it is essential that new US legislation contain provisions for the funding and infrastructure needed to bring about this vision.

ASSESSMENT OF THE LEGISLATION: FLEXIBLE AND SUPPORTIVE?

Key Features

The bills contain a large number of provisions that will change the way EPA regulates industrial chemicals. For the sake of this analysis, this paper details only those that address the extent and type of toxicity testing that may be conducted.

Within one year, the bills:

- Require EPA to determine a minimum data set that should be collected for all substances [and mixtures]
- Require companies to declare substances under manufacture and submit exposure, use, and toxicity info

- Require EPA to set a “priority list” of 300 existing substances that will be tested and assessed first

With regard to the minimum data set, both bills allow for flexibility by requiring EPA to set the requirements, rather than doing so in the legislation itself. While the SCA allows for a tiered or varied data set depending on the substance or group of substances under consideration, TCSA requires such flexibility by stating that the minimum data set “shall include varied or tiered testing.” The bills also require the data set to be updated every five years, which will encourage the incorporation of advances in toxicology. Some substances are waived from the minimum data set requirement, including persistent, bioaccumulative, and toxic substances, listed “bad actors,” and substances designed to be safer alternatives to hazardous substances.

Both bills give EPA the authority to require additional testing beyond the minimum data set, but only in accordance with provisions in the section “Reduction of Animal-Based Testing,” intended to reduce *in vivo* testing. A cornerstone of both bills directs the EPA to eventually determine whether all substances meet a safety standard of “reasonable certainty of no harm,” including for vulnerable populations and taking into account cumulative and aggregate exposures. Manufacturers bear the burden of proving that substances and articles meet the safety standard.

The priority list of 300 substances is the main tool for collecting and generating information on existing substances and assessing whether their use meets the safety standard. Once a substance has been assessed, a new substance is placed on the list. EPA is to create the list with public input by considering a large number of factors, including production volume, exposure potential, existing hazard data, and physicochemical characteristics.

The deadlines for submitting the information in the minimum data set vary. Both bills require the submission of data for new substances when the manufacturer declares its intent to manufacture such substances. For existing substances on the priority list, data in accordance with the minimum data set must be submitted 18 months after a substance is placed on the list. For non-priority existing substances, the SCA provides a 14-year time period to submit existing data; TCSA contains very short deadlines for such substances depending on production volume. From the date of enactment of the legislation, manufacturers of high-, medium-, and low-production substances are given 3, 4, and 5 years respectively to submit the minimum data set.

TCSA states that is the policy of the US to: “replace, reduce, and refine testing on animals by promoting and funding more efficient test methods and strategies.” Both bills contain a section intended to do just that. This section requires the EPA to “take action to minimize the use of animals in testing” and to “encourage and facilitate” the use of:

- Existing data
- Test methods that eliminate or reduce the use of animals
- Read-across within chemical categories

- Formation of industry consortia to jointly conduct testing
- Parallel submission of data from animal-based studies and emerging methods and models

The EPA is also required to:

- "Fund research and validation studies to reduce, refine, and replace the use of animal tests"
- "Develop a strategic plan to promote the development and implementation of alternative test methods and testing strategies to generate information used for safety standard determinations"
- Biennially report on its progress to Congress
- Within 1 year, and triennially thereafter, create a list of "demonstrated test methods that reduce the use of animals"

Alternative test methods and testing strategies referred to above include:

- Toxicity pathway-based risk assessment
- *In vitro* studies
- Systems biology
- Computational toxicology
- Bioinformatics
- High-throughput screening

Both of the bills allow for the waiving or adaptation of tests in certain circumstances including if:

- Weight-of-evidence demonstrates a substance does or does not cause a particular toxicological effect
- Testing is not practicable (e.g. volatility)
- Testing would result in severe pain or distress (e.g. corrosive materials)
- Data are already provided or being developed for that substance or an equivalent substance

Finally the bills retain the potential for manufacturers to obtain testing exemptions for small volumes of substances produced for purposes of test marketing, research or product development, or "temporary existence" within a chemical reaction or process. TCSA requires the manufacturer to prove that the substance meets the safety standard of reasonable certainty of no harm despite any waived testing, but it also allows the EPA to determine that certain substances, based on "intrinsic properties" can be exempted from testing.

Data Requirements Impact Animal Use

Historically, the extent to which a substance was tested and assessed has been driven primarily by regulatory sector and subsequent use; chemical identity and properties may

also be considered. Pesticides and pharmaceuticals, which are designed to have biological effects and are often consumed, are assessed much more extensively than industrial chemicals or cosmetics, which are not. For example, current US pesticide regulations, though tailored slightly according to use, require a long list of toxicity tests for registration (Table 1) (Cooper et al 2006, Doe et al 2006) and are generally considered inflexible (Carmichael et al 2006). Much of these data are then set aside as restrictions are set according to a key finding sometimes from a single study (Doe et al 2006, Linsday 2006).

Previous regulations governing industrial chemicals such as TSCA did not require the collection of a “minimum data set” either to allow continued manufacture of an existing substance or to manufacture a new substance. Instead, the EPA used computational tools like Structure-Activity Relationships (SAR) and Quantitative SAR (QSAR) to categorize and comparatively assess groups of similar substances to highlight potential concerns (Zeeman et al 1995).

Interested parties have participated in voluntary efforts to make hazard data for industrial chemicals public and generate additional data. The Organisation for Economic Co-operation and Development’s (OECD) Screening Information Data Set (SIDS) is a worldwide cooperative effort to generate a “minimum data set” on substances produced at high volumes worldwide (HPV) (Gelbke et al 2004). The EPA also administered its own voluntary HPV program, using the SIDS data set as a base set of data that should be collected (Table 2).

Any legislation that would require testing for thousands of existing substances will result in a large increase in the number of animals used for toxicity testing. For strict minimum data sets, estimating the numbers of animals used in testing schemes can be a relatively simple exercise. Many consider a SIDS data set a minimum estimate of the number of animals used to test each substance, while the battery for conventional pesticides might be considered an upper bound (Table 3). While for many substances regulated under the new US legislation the number of animals used is likely to be somewhere in between these sets, because of the flexibility built into the currently-proposed bills not every substance will be tested according to the same set of testing requirements, making an estimation of the numbers of animals who could be used quite difficult.

If followed, provisions in the legislation that promote the “3Rs” (replacing, reducing, and refining the use of animals) can have a significant impact on the use of animals. Manuppello et al (2009) estimated that use of existing data reduced the number of animals killed in the HPV program by 13%, and 77% of HPV substances fit into categories, allowing one study to provide data for at least one additional substance. Other analyses find similar results (van Leeuwen et al 2009). While it is possible that medium- or low-production volume substances are less likely to fit into categories, our own analysis of the EPA’s short-lived Chemical Assessment and Management Program reveals that 81% of the 303 MPV substances reviewed fit into categories. Within the

REACH legislation, Van der Jagt et al (2004) conclude that the use of 3Rs principles might reduce the use of animals by 33 – 49%.

Non-animal tools and strategies can also be used in the near term to increase the amount of useful information obtained on the potential toxicity of a substance while reducing animal use. These tools can be used to rank or narrow the substances or groups of substances to be tested to focus limited resources on substances more likely to be harmful first. Similarly, suites of *in vitro* tests can be used to develop a signature for single substances to highlight the most relevant toxicity deserving of additional scrutiny (e.g. neurotoxicity, reproductive fitness). The EPA's computational toxicology program recently demonstrated both of these principles with a set of substances being scrutinized for endocrine-disruptor potential in its ToxCast program (Reif et al 2010).

The bills have the potential to allow--and indeed encourage--the strategic use of non-animal tools and strategies to reduce the use of animals and speed the collection of information on substances. However, TCSA contains one serious impediment to the use of integrated strategies: extremely short deadlines for the submission of a minimum data set, especially for HPV substances. In order to meet these deadlines, companies may feel compelled to instead hastily plan to conduct the animal tests to fulfill the reporting requirements. However, the animal tests themselves are time- and resource-consuming, and relying on the results of these planned animal tests will ultimately take longer than if an informed, integrated strategy had been implemented from the start.

We recommend a number of improvements to ensure the bills more fully support the principles of the "3Rs" and ensure the development and use of non-animal test methods and strategies.

RECOMMENDATIONS

Already, lessons can be learned from the EU experience with REACH legislation. As we have stated, flexibility is key. Since REACH's data requirements are set into the legislation, it may be difficult to incorporate technology or policy developments. This is somewhat ameliorated by the requirement specified in REACH legislation to consider the use of animal tests a "last resort." Although difficult to enforce, this language sets a clear policy position that forces thought and caution before conducting any animal test.

To strengthen the stated US policy to "replace, refine, and reduce" animal tests, the bills should require the use of non-animal methods whenever possible. If a non-animal method is available, it should be used. Such a requirement spurs financial and organizational investment in new methods, as we have seen in the EU, where public and private sector investment in non-animal methods dwarfs investments in the rest of the developed world. Other incentives to use testing strategies and non-animal methods should also be explored, such as expedited substance assessment.

REACH not only requires the preference of non-animal methods over animal tests, but also requires registrants to abide by other strategies to reduce animal use, such as

formation of testing consortia and use of existing data. In fact, manufacturers hoping to register a new substance are required to check with the European Chemicals Agency (ECHA) to use any existing hazard data ECHA may possess before conducting new testing (ECHA 2010). While the SCA and TCSA allow manufacturers to waive testing if testing is being conducted or has been conducted elsewhere, without a requirement to check in with EPA, it is unclear how manufacturers will know such data exist. As is possible for minimum data sets for existing chemicals, data submission for new chemicals should be a flexible and iterative process instead of a one-time "data dump."

Finally the bills should recommend the use of exposure-based waiving, as REACH does. Both the level of release into the environment and the extent of actual bioavailability of the substance or its metabolites can render hazard testing for some or all endpoints moot. For example, substances that are not absorbed through human skin should not be tested for systemic toxicity by the dermal route (Stoick et al. 2007).

Flexibility should be extended to the safety standard (reasonable certainty of no harm). From the perspective of the "3Rs," there is a danger that this safety standard will be interpreted as direction to consider--and test for--every potential hazard endpoint, which is in contradiction to the rest of the bill. If the wording of the standard is to be kept and applied to all chemicals, the legislation should also define circumstances in which substances can meet the safety standard without a rigid list of testing requirements. For example, endpoint-specific integrated testing strategies often include "off ramps" where testing can be considered completed without *in vivo* testing for substances that meet certain characteristics.

CONCLUSIONS

As with REACH, passage of the SCA and TCSA will increase the use of animals in chemical toxicity testing significantly in the short term. However, passage of these bills will also increase the use of *in vitro* and other non-animal test methods and strategies. While the bills have the potential to be implemented in a flexible way and to help usher in "21st-Century Toxicology," key improvements must be made in order to maximize this potential, taking into account lessons from other programs and legislation. Finally, integrated testing strategy-approaches for information gathering may not be compatible with pressure to meet very short data submission deadlines.

REFERENCES

Bradbury, S.P., Feytel, T.C.J., van Leeuwen, K.J., 2004. Meeting the Scientific needs of Ecological Risk Assesment in Regulatory Context. Environ. Sci.Tech. 38, 463-470.

Carmichael, N.G., Barton, H.A., Boobis, A.R., Cooper, R.L., Dellarco, V.L., Doerrer, N.G., Fenner-Crisp, P.A., Doe, J.E., Lamb, J.C., Pastoor, T.P., 2006. Agricultural chemical safety assessment: a multi-sector approach to the modernization of human safety requirements. Crit. Rev. Toxicol. 36, 1-7.

Cooper, R.L., Lamb., J.C., Barlow, S.M., Bentley, K., Brady, A.M., Doerrer, N.G., Eisenbrandt, D.L., Fenner-Crisp, P.A., Hines, R.N., Irvine, L.F.H., Kimmel, C.A., Koeter, H., Li, A.A., Makris, S.L., Sheets, L.P., Speijers, G.J.A., Whitby, K.E., 2006. A tiered approach to life stages testing for agricultural chemical safety assessment. Crit. Rev. Toxicol. 36, 69-98.

Doe, J.E., Boobis, A.R., Blacker, A., Dellarco, V., Doerrer, N.G., Franklin, C., Goodman, J.I., Kronenberg, J.M., Lewis, R., McConnell, E.E., Mercier, T., Moretto, A., Nolan, C., Padilla, S., Phang, W., Solecki, R., Tilbury, L., van Ravenzwaay, B., Wolf, D.C., 2006. A tiered approach to systemic toxicity testing for agricultural chemical safety assessment. Crit. Rev. Toxicol. 36, 37-68.

EC (European Commission). Regulation No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/105/EC and 2000/21/EC (2006). Available at: http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32006R1907:EN:NOT

ECHA (European Chemicals Agency) 2010. Practical guide 10: How to avoid unnecessary testing on animals. Document ECHA-10-B-17-EN. Helsinki, Finland.

Gelbke, H.P., Fleig, H., Meder, M., 2004. SIDS reprotoxicity screening test update: testing strategies and use. Regul Toxicol Pharmacol. 39(2), 81-6.

Hartung, T., 2009. Toxicology for the twenty-first century. Nature 460, 208-212.

Linsday, A., 2006. Moving toward a hypothesis-driven toxicology-testing paradigm: Meeting common needs. Presentation before the 39th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides, and Biotechnology, OECD, 15-17 February 2006. Focus session on integrated approaches to testing and assessment. Available at: http://www.oecd.org/document/42/0,3343,en_2649_34365_36283562_1_1_1_1,00.html Accessed 10 September 2010.

Manuppello, J., Willett, C., Sandler, J., 2009. U.S. Environmental Protection Agency High Production Volume Chemicals Challenge Program: Accounting for the Animals. Poster presentation at 7th World Congress on Alternatives & Animal Use in the Life Sciences August 2007, Rome, Italy.

NRC (Committee on Toxicity Testing and Assessment of Environmental Agents, National Research Council), 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. National Academies Press, Washington, DC. Available at: http://www.nap.edu/catalog.php?record_id=11970. Accessed 25 January 2009.

Reif, D., Martin, M., Tan, S., Houck, K., Judson, R., Richard, A., Knudsen, T., Dix, D., Kavlock, R., 2010. Endocrine Profiling and Prioritization of Environmental Chemicals Using ToxCast Data. Environmental Health Perspectives. Advanced online access: 10.1289/ehp.1002180 (available at http://dx.doi.org/) Online 8 September 2010.

Stoick, K., Nitschke, K., Sandusky, C., 2007. Systemic testing by the dermal route can be precluded by new non-animal percutaneous absorption strategies. ALTEX **14**, Special Issue, 561-564 Proc. 6th World Congress on Alternatives & Animal Use in the Life Sciences August 21-25, 2007, Tokyo, Japan.

Van der Jagt, K., Munn, S., Torslov, J., De Bruijn, J., 2004. Alternative approaches can reduce the use of test animals under REACH. Addendum to Assessment of additional testing needs under REACH. Effects of (Q)SArs, risk based testing and voluntary industry initiatives, Report EUR 21405, European Commission, Joint Research Centre, Ispra, Italy.

Van Leeuwen, K., Schultz, T.W., Henry, T., Diderich, B., Veith, G., 2009. Using chemical categories to fill data gaps in hazard assessment. SAR QSAR Environ. Res. 20(3-4), 207-220.

Zeeman, M., Auer, C.M., Clements, R.G., Nabholz, J.V., Boethling, R.S., 1995. U.S. EPA regulatory perspectives on the use of QSAR for new and existing chemical evaluations. SAR QSAR Environ. Res. 3(3), 179-201.

TABLE 1: Minimum pesticide testing requirements often include the following *in vivo* tests.

IN VIVO TEST	NUMBER OF ANIMALS
Eye irritation/corrosion	1-3
Acute systemic toxicity – oral	7
Acute systemic – inhalation	40
Acute systemic – dermal	20
Skin sensitization - Guinea pig	32
Gene mutation - in vivo	50
Metabolism and pharmacokinetics	8
Immunotoxicity	40
Repeat dose – dermal	40
Repeat dose – oral	40
Repeat dose – inhalation	40
Subchronic – dermal	80
Subchronic – oral	80
Subchronic - inhalation	80
Acute neurotoxicity	80

Subacute neurotoxicity – hen (conditional)	40
Subchronic (90 day) neurotoxicity – non-rodent (conditional)	80
Prenatal developmental toxicity (rat and rabbit)	80
2 – generation reproductive	2600
Carcinogenicity (mouse)	400
Chronic/Carcinogenicity combined (rat)	440
Developmental neurotoxicity (conditional)	1280
Acute fish toxicity	60
Early life stage - fish	420
Avian acute toxicity	60
Avian dietary study	90
Avian reproduction study	70

TABLE 2: Animal tests included in the OECD SIDS battery.

TEST	OECD TEST GUIDELINE NUMBER	ANIMALS PER TEST
Gene mutation - *in vitro*	471	-
Gene mutation - *in vivo* [for some]	475	50 mice or hamsters
Acute systemic toxicity (oral)	423, 425	7 rats
Repeat dose/reproductive/developmental	421, 422	675 rats
Short-term fish	203	60 fish

TABLE 3: Estimation of the numbers of animals minimum data sets consume.

NUMBER OF SUBSTANCES	HPV SIDS	CONVENTIONAL PESTICIDE[1]
Each	~742	~6000

300	222,600	1,800,000
30,000	22,260,000	180,000,000
80,000	59,360,000	480,000,000
[1]Does not include duplicative tests conducted for the same registration, for instance on the active ingredient and the final formulation.		