ET&C Focus

Focus articles are part of a regular series intended to sharpen understanding of current and emerging topics of interest to the scientific community¹.

EcoToxChip: A Next-Generation Toxicogenomics Tool for Chemical Prioritization and Environmental Management

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SIGNIFICANT CHALLENGES TO EFFECTIVE CHEMICALS MANAGEMENT

Chemical contamination of natural ecosystems is regarded as one of our planet's greatest threats (Landrigan et al. 2018). Contaminant-related phenomena such as malformed frogs, fish with tumors, and dwindling bird populations increasingly fuel societal <ZAQ;1>concerns. Legislation in North America and Europe mandates the assessment and reduction of risk for thousands of commercially important chemical substances used by society and released into the environment. For example, large-scale efforts such as the Chemicals Management Plan in Canada, the European Union's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program, and the US Environmental Protection Agency's (USEPA's) ToxCast program under the Toxic Substances Control Act (1976) were implemented to address legislative obligations to identify, prioritize, and take action on chemicals found to be harmful. However, these regulatory programs face significant challenges. The number of chemical substances for which toxicity data are required is tremendous and backlogged (e.g., 23 000 initially in Canada; 85 000 in the United States; upward of 101 000 in the European Union) and continues to grow by approximately 500 to 1000 new substances each year. In addition, regulations such as Canada's Wastewater Systems Effluent Regulations (section 36, Fisheries Act), the US National Pollutant Discharge Elimination System, and the European Union's Water Framework Directive (2000/60/EC) mandate the monitoring of municipal and industrial effluents with regard to their potential impacts on aquatic ecosystem health. These programs require the testing of complex environmental samples (e.g., water, effluents, and sediments) for compliance;

however, treatment and remediation efforts represent huge, unresolved challenges for chemicals management stakeholders, including those in regulatory agencies and industry.

The major concerns associated with existing testing strategies under the above legislations are that these strategies rely on extensive animal testing and extrapolation from standard laboratory model species to native species of relevance in local ecosystems (Hecker 2018). In addition to significant ethical concerns for animal welfare, these practices result in prohibitive time and monetary costs, as well as inaccurate estimations of risk to native species and ecosystems (Villeneuve and Garcia-Reyero 2011). For example, USEPA researchers estimate that traditional testing of a single chemical can take up to 4 yr and cost US \$1 to 20 million (Martin et al. 2012). In one realistic scenario, researchers calculated that 54 million vertebrate animals and US \$13.6 billion were needed to achieve registration goals for the European Union's REACH program (Rovida and Hartung 2009). Such costs represent major barriers to fulfilling legal obligations to manage chemicals effectively. As a result, regulatory agencies and industry are moving toward alternative, mechanism-based, and high-throughput approaches to support chemical safety evaluations to meet new legislative mandates, while reducing animal use, costs, and time required for testing.

TOXICITY TESTING IN THE 21ST CENTURY

"Toxicity Testing in the 21st Century" is the title of the landmark 2007 US National Research Council (NRC) report that advocates harnessing recent advances in biology and biotechnology to transform toxicity testing. Since the release of the NRC report, significant efforts have been made to develop and establish alternative testing approaches in both human and environmental toxicology arenas (Ankley et al. 2010; Villeneuve et al. 2014; LaLone et al. 2017). The ultimate goal of these efforts is to transform conventional testing approaches, which

are based on apical and largely qualitative measures (e.g., survival, growth, development), to a model founded on contemporary scientific understanding, new technologies (e.g., toxicogenomics, high-throughput screening), and alternatives to animal testing strategies (i.e., in silico, in vitro, and embryo-based approaches that incorporate Russell and Burch's [1959] "3 Rs" principle [reduce, refine, replace]). The big data that emerge from these new approaches, when integrated using systems biology frameworks, promise to predict apical outcomes and, thus, significantly improve the ability of scientists and regulators to prioritize chemicals more effectively based on their mode of action (Kavlock et al. 2018). In addition to clear scientific, ethical, and regulatory benefits, there are important economic drivers for this shift in testing strategies. For example, it has been estimated that such strategies have the potential to reduce testing costs by up to 70% (Bourdon-Lacombe et al. 2015) or even 25-fold (Martin et al. 2012) while reducing animal use and improving regulatory decision-making. There is now a rapidly growing interest among regulators in exploring the potential of such data for use in ecological and chemical risk assessment and management. For example, there has been a steady increase in the number of Health Canada assessments that use gene expression data: from 2% in 2009 to 25% in 2012 (Bourdon-Lacombe et al. 2015).

EMERGENT NOTIONS IN ECOLOGICAL RISK ASSESSMENT

Motivated by the 2007 US NRC report, an expert group of environmental scientists convened a pivotal Society of Environmental Toxicology and Chemistry Pellston workshop in 2009 to discuss strategies to develop and apply molecular approaches to advance ecological risk assessment (Villeneuve and Garcia-Reyero 2011). The approach that emerged from this workshop (and that has evolved at several subsequent meetings; e.g., Villeneuve et al. 2014; Brockmeier et al. 2017; Whittwehr et al. 2017)—and for which there is a growing international

consensus as to its potential for addressing the above <ZAQ;2>discussed needs—is one based on the adverse outcome pathway (AOP). An AOP is a conceptual and organizational framework that links an apical outcome of relevance to risk assessors (e.g., disruption of reproduction or development) to a specific molecular perturbation pattern within a biological system (Ankley et al. 2010). Based on the AOP framework, more sophisticated testing strategies have started to emerge including, for example, species and chemical read-across, quantitative models that link specific molecular initiating events with adverse outcomes, and tiered testing. International cooperation, for example, via the Organisation for Economic Co-operation and Development's (OECD's) Advisory Group on Molecular Screening and Toxicogenomics, has resulted in AOP-focused workshops and meetings, guidance documents for the development of AOPs, an AOP knowledge base (AOP-KB; aopkb.org), and a growing collection of documented AOPs (aopwiki.org).

Over the past decade there has also been rising interest in alternatives to animal testing strategies, termed “alternative testing strategies”, that embrace the “3 Rs” principle (Lillicrap et al. 2016). For example, early-life stage (ELS) tests with oviparous organisms are particularly promising because the developing organisms are not considered live animals until they have depleted their yolk sacs (e.g., fish embryos prior to swim-up, avian embryos prior to hatch), even though most of the biological processes of their adult counterparts are established. Spurred by a 2016 European Chemicals Agency workshop (European Chemicals Agency 2016), there has also been rising interest in the development and validation of new approach methods that encompass the taxonomy of new testing tools aiming to improve mechanistic understandings of the effects of toxicants on organisms. Both alternative testing strategies and new approach methods are active areas of research and development, amid a growing consensus that these new methods

need to be both scientifically sound and flexible; that is, they need to be “fit for purpose” (designed for a specific application) and “evergreen” (able to be updated and to evolve).

TRANSFORMING CHALLENGES INTO OPPORTUNITIES

The global ecological and chemical risk-assessment and management community (including regulators, industry, and academics) has coalesced around the imperative to transform toxicity testing and the vision of doing so by using mechanistic toxicity data to predict endpoints of regulatory relevance. This transformation is driven by mounting societal (including ethical and economic) pressures for change and is aided by the emergence of promising scientific frameworks and tools like AOPs, alternative testing strategies, and new approach methods, although there remain sizeable barriers (Figure 1). Foremost is that ecological risk assessment of chemicals tends to be a highly institutionalized field, with the methods, routines, and tools used by risk assessors typically taken for granted and, thus, difficult to change (Textbox 1). However, and as mentioned<Z&Q;3> above, regulatory changes in key jurisdictions present an unparalleled opportunity for the global community to transform toxicity testing.

Within the research community, there has been a relatively rapid uptake of novel approaches, in particular in the field of toxicogenomics. However, the toxicogenomic tools available are primarily geared toward assessing risks to human health and thus are of limited relevance to ecological risk assessments concerned with protecting native species of importance. Although genomic information is available for some nonmammalian models (e.g., *Xenopus*, zebrafish, rainbow trout, chicken, Japanese quail), this has yet to be translated into commercially available, standardized tests for these species. Further, there is a lack of tools focused specifically on native species in ecosystems targeted for protection. As a result, the assessment of risks to native species almost always relies on surrogate laboratory model species. This is

problematic because many types of fish, birds, amphibians, and other organisms—including endangered species—can be more sensitive or respond very differently to chemicals than standard laboratory models (Hecker 2018). Thus, the extrapolation of results from model species to native species of relevance often introduces significant uncertainties, complicates decision-making, and necessitates additional animal testing.

The increased interest in AOPs, alternative testing strategies, and new approach methods across the ecotoxicology scientific community has resulted in the development of numerous 'omics-based tools; however, many of these tools have not been validated or widely adopted. Given the contemporary challenges to effective chemicals management documented<ZAQ;4> above, the scientific community needs to do better to design and deliver decision-making tools. It is our view that, because the validation and adoption of new tools is in large part a social process in addition to a technical one, there is an opportunity to advance ecotoxicology through more interdisciplinary research and greater intersectoral dialogue. For example, tool developers could collaborate more closely with social scientists and end-users (e.g., government and industry partners) to codesign and codevelop tools that respond to the true needs of end-users, which only the latter can identify definitively. Such tools, if they were commercialized and made available in a format that was accessible, transparent, affordable, consistent, and reliable, could then feed directly into regulatory decision-making and render chemicals management more effective.

AN INTRODUCTION TO THE ECOTOXCHIP PROJECT

As discussed<ZAQ;5> earlier, there remains an urgent need for novel testing approaches that can help overcome some of the acknowledged limitations of traditional risk-assessment approaches. The EcoToxChip project featured in this Focus article represents an effort by researchers at McGill University, the University of Saskatchewan, and Environment and Climate

Change Canada (ECCC), along with a broad coalition of partners (in government and industry who drive the work), to develop, test, and validate one such novel testing approach. Specifically, the objective of the EcoToxChip project (www.ecotoxchip.ca) is to develop, test, validate, and commercialize quantitative polymerase chain reaction (PCR) arrays (EcoToxChips) and a data evaluation tool (EcoToxXplorer.ca) for the characterization, prioritization, and management of environmental chemicals and complex mixtures of regulatory concern. In addition to providing new tools, the project aims to provide guidance to regulators and other end-users (e.g., within industry or academia) with regard to the adoption of toxicogenomics testing tools in ways that support current risk-assessment activities and chemicals management programs. The present Focus article provides a high-level overview of the EcoToxChip project, and in the coming years we expect dozens of follow-up studies that include, for example, detailed results from our 52 distinct laboratory exposure studies, compilation of gene panels that articulate how they were designed as well as their performance in predicting apical outcomes of regulatory concern, and scientific efforts that scrutinize the validity and reliability of the project deliverables.

We note that the EcoToxChip project builds on our recent successes in developing and prototyping an Avian ToxChip to prioritize chemicals and assess contaminated sites (Figure 2) in terms of their potential risk to birds. A testing strategy using the Avian ToxChip has since been embraced by Canadian regulators and other chemicals management stakeholders, including industry and other government partners (Porter et al. 2014; Crump et al. 2015, 2016, 2017). It has also generated interest in scaling up research activities to develop a new tool—one that is appropriate for a range of model species used globally in ecological risk assessment as well as for native species of commercial, recreational, and cultural/aboriginal concern.

Core aspects of the design of the EcoToxChip project are driven by the needs of regulatory partners and scientists in the Canadian government, with additional input from a range of industry, government, and academic partners (see *Acknowledgment*). The initial EcoToxChip design will be based on a standard 384-well microplate format (although there will also be designs for use in additional formats, e.g., targeted RNA sequencing [RNAseq]). EcoToxChips will consist of approximately 370 evidence-based gene targets, with the remaining wells consisting of quality control measures (e.g., reference genes, technical controls, contamination controls). Although we acknowledge that there are other state-of-the-art transcriptomics platforms available (i.e., RNAseq), we focus on the microplate format intentionally because it is familiar, cost-effective, and usable in virtually any basic molecular lab, thus helping to minimize some barriers to adoption. We also purposefully focus on 384 genes. Our discussions with a range of stakeholder groups concluded that, in the current era of big data, this number of genes not only is manageable but also covers a large biological space: preliminary internal analyses indicate that a gene panel of this size performs well compared to much larger ones. Genes (and the ~20 targeted biological pathways of regulatory relevance within which these genes reside) included on the EcoToxChips are being identified and vetted through a systematic design-thinking process that is informed by 3 disciplinary perspectives: 1) ecotoxicology, by reviewing the existing scientific literature, including research on AOPs and generating new data from exposure studies, described in more detail<Z&A;Q;6> below; 2) bioinformatics, by mining data sets like National Center for Biotechnology Information's Gene Expression Omnibus and Toxicogenomics Project–Genomics Assisted Toxicity Evaluation System (TG-GATES); and 3) regulatory, by incorporating genes that are familiar, understood, and trusted by end users. Once validated, EcoToxChips will be manufactured through a commercial

partnership with Qiagen in an International Organization for Standardization–compliant facility and will be available through a global distribution network. Initially, EcoToxChips will be designed with an emphasis on the liver, given the prominence of this organ across the toxicological sciences; but it is envisioned that the tool can and will be adapted to other tissues of interest. EcoToxChips can also be customized by users (i.e., they can swap in or out genes of interest to them), thus affording flexibility.

The other key deliverable of the EcoToxChip project is EcoToxXplorer.ca, an intuitive data-evaluation tool. EcoToxXplorer is being designed with an eye to overcoming existing barriers to adoption of novel toxicogenomics tools that are associated with the challenges of managing and analyzing big 'omics data sets (e.g., lack of standardization, methods, tools). This cloud-based tool will serve as the main portal for analyzing and interpreting EcoToxChip results as well as other transcriptomics data sets like RNAseq files, thus expanding the potential uses of this tool. EcoToxXplorer.ca contains 4 basic categories of tools for 1) data quality assessment, 2) statistical analyses, 3) visualization, and 4) the generation of tailored reports. It is modeled on successful, existing cloud-based tools, for example, MetaboAnalyst (metaboanalyst.ca) and NetworkAnalyst (networkanalyst.ca), which process >2 million jobs per year (Xia et al. 2015; Chong et al. 2018), and is thus designed to provide intuitive bioinformatics support to diverse types of end-users and their varied needs. The website contains demonstrations, frequently asked questions, tutorials, and other resources to best assist all types of users. Other notable components of EcoToxXplorer.ca include 1) an internal laboratory information management system to serve as a repository for all project data; 2) an FTP site modeled after the TG-GATES project that allows the broader community to access project resources, including, for example, information from our 52 exposure studies and associated RNAseq data; 3) a Galaxy-based

pipeline for raw data processing (i.e., quality control and extracting count tables from RNAseq raw reads files); 4) machine-learning and other algorithms that link exposures to outcome measures to result in hallmark gene lists; and 5) integration with other relevant databases (e.g., Comparative Toxicogenomics Database, USEPA ToxCast assays) and programs (e.g., SEQAPASS, BLAST, AOP-KB).

To position the project advantageously with regard to ensuring the adoption of EcoToxChips and EcoToxXplorer.ca into the toolkit of ecological risk assessors and managers, the study is also producing and leveraging social science research about “institutional entrepreneurship” (see Textbox 1; Maguire et al. 2004). In this project (funded by Genome Canada), such scholarship is referred to as “GE³LS” research, which stands for Genomics and Its Ethical, Environmental, Economic, Legal, and Social Aspects. Specific GE³LS research activities, which are described in detail below (see [ZAQ;7> GE³LS research](#)), will result in a range of outputs, such as peer-reviewed social science publications and briefing notes as well as maps and historical timelines, and will form the basis for a technical guidance document designed to help stakeholders use EcoToxChip results.

ACTIVITIES UNDERPINNING THE ECOTOXCHIP PROJECT

The EcoToxChip project is conducted in 2 project phases, each containing tiered activities and contingency plans (Figure 3). Notably, the work is being driven by end-users’ needs and considerations and is informed by social science research.

Project phase 1

Three species-specific EcoToxChips will be developed, tested, validated, and commercialized for laboratory model species representing the most important vertebrate groups in ecological risk assessment: for fish, the fathead minnow; for birds, the Japanese quail; and for

amphibians, *Xenopus laevis* (Table 1). For model species, we will study both adults and organisms in ELS, which will facilitate side-by-side comparisons of results from whole animals and alternatives. Model species will be exposed to 8 chemicals of environmental relevance in the laboratory using standardized tests (i.e., in line with current OECD, USEPA, and ECCC guidelines; Table 1). The 8 substances were selected for 4 main reasons: 1) they are chemicals of regulatory concern, 2) they represent different environmental contexts or sources (e.g., pharmaceutical, flame retardant, pesticide, combustion by-product, mining), 3) they have been shown to perturb a variety of biological systems (e.g., androgen, estrogen, AhR-active, selective serotonin reuptake inhibitor, anticholinesterase, carcinogen), and 4) each chemical has a relatively large knowledge base that we can draw from (activity 1). For each chemical we will test 3 concentrations that range from the environmentally relevant to the potentially harmful so as to help establish exposure–response relationships and to link transcriptomic signatures with expected adverse outcomes.

An integrative systems approach based on functional 'omics (combined global transcriptomic and proteomic profiling, targeted metabolome analysis) and physiological analyses (e.g., histology, enzyme assays) across levels of biological organization coupled with exposure information (residue measures) is applied to characterize the toxicity pathways that are perturbed (activity 2); from this and other resources species-specific EcoToxChips consisting of 384 environmentally responsive genes of regulatory concern will be informed, built, tested, and optimized (activity 3). EcoToxChip performance will be validated (and further optimized) through 2 main studies that will be informed by OECD Guidance Document 34's prevalidation module (activity 4). First, performance of EcoToxChips will be compared to the extensive 'omics (whole transcriptome, whole proteome, targeted metabolome) and physiological

(histology, biochemical markers, apical outcomes) analyses conducted as part of this study.

Second, partner laboratories will test the EcoToxChips alongside other, more familiar approaches when evaluating risks associated with variably contaminated sites in the Great Lakes region. Third, a ring test will be conducted in which 3 laboratories test the same 5 chemicals (Table 1). Additional studies with end users (both internal to the initial project team and new ones as we expand our coalition) are being considered to ensure a robust approach to validation as the project grows. The project team is open to involving new partners.

Project phase 2

The approach and knowledge from phase 1 will be translated to 3 native species of concern that are representative of Canadian ecosystems and actively monitored by current ECCC programs: for fish, the rainbow trout; for birds, the double-crested cormorant; and for amphibians, the leopard frog. This project phase serves as a “proof of concept” that EcoToxChips can be ported to other species. Exposure studies with a subset of 4 chemicals from project phase 1 (activity 5), ‘omics-informed EcoToxChip design (activity 6), and EcoToxChip development and use (activity 7) in native species (including the development of species-specific primers) will mirror steps described in phase 1 for the model species.

EcoToxXplorer

In parallel to, and informed by, activities 1 through 7, the research team will develop an intuitive online bioinformatics analysis tool (Figure 4) that is built initially to handle transcriptomic results (activity 8).

In terms of subactivities, the first was to establish a database (EcoToxDB) to serve internal project needs (laboratory information management system) and as a repository for all of the data generated in the current project as well as from elsewhere. From this database, the

second subactivity was to establish a knowledge base that consists of, for example, toxicity pattern recognition algorithms so that a list of prioritized genes (and associated pathways) for each test species can be identified for inclusion on the EcoToxChips. Finally, the Web interface for EcoToxXplorer has been developed with 4 basic categories of tools (data quality assessment, statistical analyses, visualization, and generation of tailored reports), with tool design being driven by user needs, experiences, and feedback. A range of qualitative and quantitative tools will be provided for key users (i.e., scientists from across sectors, government regulators, industry) and key use cases (e.g., screening and prioritization, mode of action assignment, dose–response modeling).

GE³LS research

The social sciences component of the EcoToxChip project (activity 9) is designed to advantageously position the team to succeed as well as to provide guidance to the broader end-user community with regard to the adoption and use of toxicogenomics tools in ecological and chemical risk assessment (e.g., suggestions for how to overcome key barriers listed in Figure 1), while at the same time producing new social science knowledge about the phenomenon of “institutional entrepreneurship” (Textbox 1). To encourage and facilitate the adoption of EcoToxChips into accepted practices of ecological risk assessment and management, insights from a retrospective, qualitative case study of how ‘omics tools became accepted for human health risk assessment will be leveraged. Similarly, the project will also incorporate findings from a prospective Delphi study that, in drawing on survey research and quantitative methods, helps to forecast future end-user considerations. In addition, the efforts of project team members will be studied in “real time” using ethnographic methods, with regular feedback and coaching. Nested within this social science research are a series of activities aimed at making project team

members more effective innovators by bringing aboard design-thinking approaches, developing and weighing the benefits of different business models, and generating user-driven case studies.

SOCIOECONOMIC BENEFITS

The challenges to effective chemicals management posed by traditional approaches to toxicity testing are significant and have received much attention over the past decade. When coupled with increasing societal concerns over chemical pollution, they provide the impetus for a concerted drive toward transforming toxicity testing (Figure 1). Moving forward, the adoption of new approach methods, alternative testing strategies, and AOPs, including tools produced by efforts like the EcoToxChip project, can increase toxicological understanding (especially of chemicals with limited information) while decreasing the use of resources (money, time) and being more ethical (reduced animal tests). Although we do not yet have firm evidence from our project, based on our and end-users' experiences with the Avian ToxChip and other ongoing initiatives, we can develop informed estimates of the tangible socioeconomic benefits that the use of EcoToxChips may yield <ZAQ;8>(Figure 5).

For example, in the EcoToxChip project we will study at least 13 chemicals of concern. Testing these chemicals using whole-animal studies on 3 key ecotoxicology models (i.e., the fathead minnow, *X. laevis*, and the Japanese quail, which match the species in our phase 1 models) in accordance with international guideline tests is estimated to cost approximately US \$250 000 per chemical or \$3.25 million in total. As part of an alternative testing strategy, we estimate that screening these same chemicals in ELS of these same 3 test species using EcoToxChip measures would cost approximately \$30 000 per chemical or \$390 000. Assuming that 10% of the chemicals are flagged by the alternative testing strategy (ELS + EcoToxChip) for additional follow-up whole-animal tests, then the cost of this tiered approach would be \$715 000

or a dollar cost efficiency of \$2.5 million. Furthermore, there are estimated savings associated with time (i.e., assume 6 mo of person-time for the whole-animal tests vs 3 wk for the alternative testing strategy tests; 78 vs 17.6 mo results in a time savings of 60.4 mo) and animal lives (i.e., assume 830 animals for the whole-animal tests per chemical vs 10% prioritized; 10 790 vs 1079 animals results in a savings of 9711 animal lives) that can be considered. Scaling these calculations up to 140 chemicals per year (which is approximately the number tested annually by Canada's Chemicals Management Plan), the potential dollar cost efficiency (\$27.3 million/yr), time savings (721 mo), and animal lives saved (104 580) of adopting such an alternative testing strategy are significant. Such savings could help governments meet their regulatory obligations more effectively by allowing them to prioritize, more reliably, chemicals and complex environmental samples for additional toxicity testing and, in so doing, increase the efficiency of their resource utilization, accelerate and make more timely regulatory decisions and compliance determinations, and dramatically reduce the number of live animals utilized. We stress that the aforementioned calculations are estimates, and moving forward, the project will develop a series of case studies that better enumerate the socioeconomic benefits associated with the adoption of these new tools.

CONCLUDING REMARKS

We contend that the value proposition of new approach methods, AOPs, and alternative testing strategies, through initiatives like EcoToxChip, has the potential to be quite high. If designed with empathy for, and in collaboration with, stakeholders, project deliverables from such efforts are expected to provide a solution to the pressing practical problems confronting ecological risk-assessment and regulatory decision-making programs for chemicals management. The new approach methods and alternative testing strategies need to be in an accessible format

while also being affordable, reliable, fit-for-purpose and—most importantly—trusted by end users and society. If these features and characteristics can be achieved, then such tools, including EcoToxChip, will help governments to meet their regulatory obligations and to make more timely regulatory decisions and compliance determinations, while also dramatically reducing testing costs and the number of animals utilized.

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References

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrano JA, Tietge JE, Villeneuve DL. 2010. Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem* 29:730–741.

Bourdon-Lacombe JA, Moffat ID, Deveau M, Husain M, Auerbach S, Krewski D, Thomas RS, Bushel PR, Williams A, Yauk CL. 2015. Technical guide for applications of gene expression profiling in human health risk assessment of environmental chemicals. *Regul Toxicol Pharmacol* 72:292–309.

Brockmeier EK, Hodges G, Hutchinson TH, Butler E, Hecker M, Tollefsen KE, Garcia-Reyero N, Kille P, Becker D, Chipman K, Colbourne J, Collette TW, Cossins A, Cronin M, Graystock P, Gutsell S, Knapen S, Katsiadaki I, Lange A, Marshall S, Owen SF, Perkins EJ, Plaistow S, Schroeder A, Taylor D, Viant M, Ankley G, Falciani F. 2017. The role of omics in the application of adverse outcome pathways for chemical risk assessment. *Toxicol Sci* 158:252–262.

Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, Wishart DS, Xia J. 2018. MetaboAnalyst 4.0: Towards more transparent and integrative metabolomics analysis. *Nucleic Acids Res* 46:W486–W494.

Crump D, Farhat A, Chiu S, Williams KL, Jones SP, Langlois VS. 2016. Use of a novel double-crested cormorant ToxChip PCR array and the EROD assay to determine effects of environmental contaminants in primary hepatocytes. *Environ Sci Technol* 50:3265–3274.

Crump D, Williams KL, Chiu S, Letcher RJ, Periard L, Kennedy SW. 2015. Biochemical and transcriptomic effects of herring gull egg extracts from variably-contaminated colonies of the Laurentian Great Lakes in chicken hepatocytes. *Environ Sci Technol* 49:10190–10198.

Crump D, Williams KL, Chiu S, Zhang Y, Martin JW. 2017. Athabasca oil sands petcoke extract elicits biochemical and transcriptomic effects in avian hepatocytes. *Environ Sci Technol* 51:5783–5792.

European Chemicals Agency. 2016. New approach methodologies in regulatory science.

Proceedings of a scientific workshop. Helsinki, 19–20 April 2016. [cited 2018 August 31]

Available from:

https://echa.europa.eu/documents/10162/22816069/scientific_ws_proceedings_en.pdf

Hecker M. 2018. Non-model species in ecological risk assessment. In Murphy C, Reyero N, eds, *A Systems Biology Approach to Advancing Adverse Outcome Pathways for Risk Assessment*. Springer, New York, NY, USA, pp 107–132.

Kavlock RJ, Bahadori T, Barton-Maclaren TS, Gwinn MR, Rasenberg M, Thomas RS. 2018. Accelerating the pace of chemical risk assessment. *Chem Res Toxicol* 31:287–290.

LaLone CA, Ankley GT, Belanger SE, Embry MR, Hodges G, Knapen D, Munn S, Perkins EJ, Rudd MA, Villeneuve DL, Whelan M, Willett C, Zhang X, Hecker M. 2017. Advancing the adverse outcome pathway framework—An international horizon scanning approach. *Environ Toxicol Chem* 36:1411–1421.

Landrigan PJ, Fuller R, Acosta NJR, Adeyi O, Arnold R, Basu NN, Baldé AB, Bertollini R, Bose-O'Reilly S, Boufford JI, Breysse PN, Chiles T, Mahidol C, Coll-Seck AM, Cropper ML, Fobil J, Fuster V, Greenstone M, Haines A, Hanrahan D, Hunter D, Khare M, Krupnick A, Lanphear B, Lohani B, Martin K, Mathiasen KV, McTeer MA, Murray CJL, Ndahimananjara JD, Perera F, Potočnik J, Preker AS, Ramesh J, Rockström J, Salinas C, Samson LD, Sandilya K, Sly PD, Smith KR, Steiner A, Stewart RB, Suk WA, van Schayck OCP, Yadama GN, Yumkella K, Zhong M. 2018. The Lancet Commission on pollution and health. *Lancet* 391:462–512.

Lillicrap A, Belanger S, Burden N, Pasquier DD, Embry MR, Halder M, Lampi MA, Lee L, Norberg-King T, Rattner BA, Schirmer K, Thomas P. 2016. Alternative approaches to vertebrate

ecotoxicity tests in the 21st century: A review of developments over the last 2 decades and current status. *Environ Toxicol Chem* 35:2637–2646.

Maguire S, Hardy C, Lawrence TB. 2004. Institutional entrepreneurship in emerging fields: HIV/AIDS treatment advocacy in Canada. *Acad Manage J* 47:657–679.

Martin MT, Knudsen TB, Judson RS, Kavlock RJ, Dix DJ. 2012. Economic benefits of using adaptive predictive models of reproductive toxicity in the context of a tiered testing program. *Syst Biol Reprod Med* 58:3–9.

National Research Council. 2007. Toxicity testing in the 21st century. National Academies, Washington, DC. <ZAJ;10>

Porter E, Crump D, Egloff C, Chiu S, Kennedy SW. 2014. Use of an avian hepatocyte assay and the Avian ToxChip PCR array for testing prioritization of 16 organic flame retardants. *Environ Toxicol Chem* 33:573–582.

Rovida C, Hartung T. 2009. Re-evaluation of animal numbers and costs for in vivo tests to accomplish REACH legislation requirements for chemicals—A report by the transatlantic think tank for toxicology (t(4)). *ALTEX* 26:187–208.

Russell WMS, Burch RL. 1959. *The Principles of Humane Experimental Technique*. Methuen, London, UK.

Villeneuve DL, Crump D, Garcia-Reyero N, Hecker M, Hutchinson T, LaLone CA, Landesmann B, Lettieri T, Munn S, Nepelska M, Ottinger MA, Vergauwen L, Whelan M. 2014. Adverse outcome pathway (AOP) development I: Strategies and principles. *Toxicol Sci* 142:312–320.

Villeneuve DL, Garcia-Reyero N. 2011. Vision & strategy: Predictive ecotoxicology in the 21st century. *Environ Toxicol Chem* 30:1–8.

Wittwehr C, Aladjov H, Ankley G, Byrne HJ, deKnecht J. 2017. How adverse outcome pathways can aid the development and use of computational prediction models for regulatory toxicology. *Toxicol Sci* 155:326–336.

Xia J, Gill E, Hancock REW. 2015. NetworkAnalyst for statistical, visual and network-based approaches for meta-analysis of expression data. *Nat Protoc* 10:823–844.

Figure 1. Key drivers and barriers underlying the transformation of toxicity testing, organized as an Ishikawa (fishbone) diagram. The drivers are highlighted in green and situated to the left of the angled line, whereas the barriers are highlighted in orange and situated to the right of the angled line.

Figure 2. A timeline of the period from research and development to application and adoption of the avian ToxCChip and the EcoToxCChip. ATS = alternative testing strategies; CMP = Chemicals Management Plan; ECCC = Environment and Climate Change Canada; NAM = new approach method; SETAC = Society of Environmental Toxicology and Chemistry; SOP = standard operating procedure.

Figure 3. Project overview indicating the workflow and integration of activities and deliverables. RNA-Seq = RNA sequencing.

Figure 4. Screenshot of the EcoToxXplorer.ca home page. Underneath we provide representative graphical outputs from the tool including (starting on the left) a principal components analysis plot, a heat map, a volcano plot, and a functional enrichment map.

Figure 5. How the EcoToxChips and EcoToxXplorer would be used for testing chemicals and environmental samples while realizing socioeconomic benefits.

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