TOXICOGENOMICS *in* Regulatory Ecotoxicology

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Powerful new genomic techniques could significantly change how regulators evaluate contaminants; however, many practical and conceptual challenges remain before the promise of toxicogenomics can be achieved.

Recently, we have witnessed an explosion of different genomic approaches that, through a combination of advanced biological, instrumental, and bioinformatic techniques, can yield a previously unparalleled amount of data concerning the molecular and biochemical status of organisms. Fueled partially by large, well-publicized efforts such as the Human Genome Project, genomic research has become a rapidly growing topical area in multiple biological disciplines. Since 1999, when the term "toxicogenomics" was coined to describe the application of genomics to toxicology (1), a rapid increase in publications on the topic has occurred (Figure 1). The potential utility of toxicogenomics in toxicological research and regulatory activities has been the subject of scientific discussions and, as with any new technology, has evoked a wide range of opinion (2–6).

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The purpose of this feature article is to consider the roles of toxicogenomics in the field of regulatory ecotoxicology, explore current limitations in the science and practice of genomics, and propose possible avenues to approach and resolve some of the major challenges. A significant amount of input to our analysis came from a workshop sponsored by the Society of Environmental Toxicology and Chemistry (SETAC) in Pellston, Mich., in September 2005. A complete list of names and affiliations of the experts participating in that workshop is provided online in Table 1 of the Supporting Information for this paper.

Genomic methods and capabilities

The genome is the DNA sequence of an organism, and the number of species for which the entire genome has been elucidated is ever-increasing (e.g., humans, rats, mice, zebrafish [*7*], *C. elegans* [*8*]). Partial genome data exist for many others, including several species directly relevant to ecotoxicology (e.g., rainbow trout [*9*], Japanese medaka [*10*], fathead minnow [*11*], *Xenopus* sp. [*12*], *Daphnia pulex* [*13*]). Among other applications, genomic information can be used to design microarrays or "gene chips" for some or all of the genes in an organism. These chips can be used to determine which genes are up- or down-regulated (as transcribed messenger RNA [mRNA]) in a cell, tissue, organ, or organism under specific physiological conditions or in response to an environmental perturbation, such as exposure to a toxic chemical. The global detection and analysis of gene expression in this fashion are termed "transcriptomics".

Virtually all responses to external stressors, including toxicants, involve changes in normal patterns of gene expression (1,14). Some are a direct result of the chemical. For example, gene expression can be directed when a steroid hormone (or analog) binds to a transcription factor (receptor), forming a complex that modulates (enhances or depresses) transcription of specific genes. Other responses to toxic chemicals are compensatory, in that they reflect the response of the organism to molecular damage or cellular dysfunction.

Importantly, different mechanisms of toxicity can generate specific patterns of gene expression reflective of mechanism or mode of action (MOA; *14*). The number of genes needed to reflect an MOA will vary in a pathway-specific manner, but given the large number of genes that can be queried with microarrays (several thousand for some species), this is not likely to be a limiting factor in the application of transcriptomics to toxicity research (15,16). Although microarray research with mammalian models has been far more prevalent than with the species used for regulatory ecotoxicology, more and more examples exist of transcriptomic tools and research with ecologically relevant species (17–26).

Transcription of mRNA is only an intermediate step in converting genetic information into proteins, the biochemical bases of biological function. Not all mRNA sequences are transcribed, and many proteins are modified (e.g., by phosphorylation, posttranslational cleavage) before becoming physiologically active (27–29). Consequently, proteomics—the global evaluation of protein profiles—provides additional critical insights into biological pathways.

Alterations in protein profiles can be used, in conjunction with transcriptomics, to understand responses of an organism to toxicants (3,4). As with transcriptomics, a rapid evolution has occurred of proteomic methods capable of providing broad characterizations of the proteins expressed within cells, organs, or, in some instances, whole organisms, including species relevant to ecotoxicology (30,31). Methods vary, but they typically include protein isolation and separation steps with techniques such as 2D gel electrophoresis or high-pressure liquid chromatography, followed by mass spectrometry (MS) analyses to identify peptide profiles or amino acid sequences as a basis for determining specific proteins (32–34).

Metabolomics describes the global characterization of low-molecular-weight metabolites involved in all the biological reactions required for growth, maintenance, and normal function (35–37). The metabolome includes various polar organic compounds (e.g., amino acids, small peptides, glucosides); comparatively nonpolar molecules, such as lipids; and even inorganic chemicals. Metabolomics could be thought of as a sophisticated version of traditional tests for disease states, in which endogenous metabolite profiles can be used as a diagnostic tool (35). As such, metabolomics captures a more integrated assessment of the physiological state of an organism than transcriptomics or proteomics do (37). Most metabolomic research to date has focused on models of human health (rats, mice, and even people); however, recent work in the area has successfully used animals, including aquatic species, that are relevant to ecological risk assessments (38–40). Different high-resolution MS and nuclear magnetic resonance (NMR) techniques are the primary methods for generating metabolomic data (41).

Transcriptomics, proteomics, and metabolomics measure responses at different biological levels of organization and thus provide different insights into the biochemical and molecular status of an organism. However, all three approaches have an excellent potential for defining toxicity pathways, particularly if used together (2,3,37,42). These techniques also have many parallel challenges with regard to data collection, integration, and interpretation. For example, none would be considered routine for determining the biological endpoints typically measured and used for the environmental regulation of chemicals—survival, growth and development, and reproduction. Advanced expertise, complex reagents, and sometimes costly equipment are required to collect this genomic data.

Futhermore, well-trained experts and advanced capabilities are needed for data analysis. Because of the amount of information generated, the analysis of toxicogenomic data requires sophisticated bio-informatic (or chemometric) approaches that consider possible changes in thousands of data points per sample (3,37,43,44). For example, the human transcriptome is composed of ~30,000 genes, the proteome may have 100,000 (or more) proteins, and the metabolome contains ~2000–20,000 components (36). Ecotoxicologists historically have seldom dealt with data sets of this magnitude, so current infrastructure—both IT facilities and training—will need to be expanded to enable meaningful analysis of genomic data.

A final challenge for scientists using toxicogenomic techniques for ecotoxicology research or regulation involves knowledge of what exactly is changing when a treatment causes alterations in gene, protein, or metabolite expression. The genomes of most species traditionally used for regulatory ecotoxicology have been characterized only to the point that a mere handful of genes and translated proteins are well understood in terms of identity or function. By contrast, toxicologists involved in human-health research have extensively characterized the genomes of many experimental models (as well as humans). Even the zebrafish, which has a relatively well characterized genome, lacks robust annotation for many gene products (7). Hence, although tools exist to detect changes in transcriptomic or proteomic data in various nonmammalian species used for regulatory ecotoxicology, the baseline information needed to fully interpret these data is generally lacking.

Metabolomic data have similar limitations, albeit from a slightly different perspective. Although specific metabolites are much less likely to exhibit a high degree of species-specificity, the software and the libraries for identifying specific metabolic products from NMR or MS spectra are not yet extensive enough to exhaustively probe analytical data that may represent hundreds to thousands of unique molecules.

Ongoing research will, in the long term, obviate issues related to the global identification of gene products, proteins, and metabolites in test species that are relevant to ecological risk assessments. But for the near-term, approaches exist that will still allow toxicogenomic data

to be used in certain applications. For example, identification of key genes, proteins, or metabolites associated with specific pathways of concern can be done in a focused fashion for key test species, thereby enabling insights into toxic MOAs. Profiling techniques also could be used to support aspects of regulatory decision making in ecotoxicology. These techniques rely on patterns of responses in a particular suite of analytes, rather than identification of all components that have changed, to provide insights on toxic MOAs. Profiles (fingerprints) obtained from "unknown" chemicals then can be compared with those generated from exposures to toxicants with established MOAs, and hence potential toxicity pathways can be identified (e.g., *14*, *15*, *17*, *45–48*).

Current and emerging regulatory challenges

The need for improved safety data from human and ecological risk assessments is growing. New testing efforts such as the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program within the EU and the High Production Volume (HPV) challenge program in the U.S. promise to significantly increase the number of chemicals for which toxicity data may be needed (49–51). Emerging pollutants of concern, such as human and veterinary pharmaceuticals, are also anticipated to increase demands for regulatory testing for ecological effects (52). Against this backdrop of increased testing, the registration of new chemicals and the re-registration of existing materials (e.g., pesticides) continue. A steady growth is also taking place in toxicity testing of complex environmental samples, such as ambient waters, effluents, and sediments, for compliance monitoring and the evaluation of treatment and remediation efforts.

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The complexity of testing, both prospective and diagnostic, is also increasing. For example, regulatory programs worldwide are incorporating tests with endpoints that capture the effects of chemicals with the potential to disrupt specific endocrine pathways in animals (53). Thus, ecological testing and screening programs need to become more thorough, less costly, and more rapid.

Presently, virtually all methods used for regulatory decision making in ecotoxicology rely on whole-animal exposures and adverse effects on survival, growth, and reproduction. These types of tests are resource- and time-intensive, especially as studies shift from short-term lethality assays to partial- and full-life-cycle tests. Unless approaches can be developed to streamline this process, the time required to handle all of this anticipated new testing will be measured in decades. As outlined next, toxicogenomic tools offer a way to effectively address a number of the challenges currently confronting risk assessors and regulators.

Potential benefits

Arguably, the most significant impact of genomic data on either human or ecological risk assessments for chemicals would be better definition of toxicity pathways or MOAs. Benefits could include enhanced resource utilization and reduced uncertainty in regulatory decision making.

For example, regulatory bodies worldwide are currently focusing on the potential risk of endocrine-disrupting chemicals (EDCs) that operate through discrete pathways within the hypothalamic–pituitary–gonadal (HPG) or hypothalamic–pituitary–thyroid (HPT) axes (53–55).

Thousands of current-use and new chemicals could act as EDCs. It is impractical, if not impossible, to test all of them with the types of long-term assays necessary to detect some of

the more subtle, but nevertheless potentially adverse, effects on HPG or HPT function. Therefore, a pressing need exists to prioritize lists of chemicals with respect to their EDC potential via what are, in many cases, relatively well defined pathways. If short-term tests with either in vitro or in vivo systems can be used to develop profiles of genomic responses to EDCs linked to known MOAs, the results of these tests could be used to prioritize chemicals with unknown MOAs for more elaborate and expensive long-term testing (56).

In addition, genomics could provide insights about whether a compound possesses an unanticipated MOA. For example, in regulatory situations like pesticide registration, a priori knowledge exists concerning a probable toxic MOA. However, chemicals often cause toxicity via multiple pathways. At present, pesticide regulators require numerous different types of tests with multiple species and endpoints to ensure that unanticipated toxicity pathways are not "missed". These are, of course, expensive and time-consuming and can result in large amounts of data that ultimately never contribute to decision making. However, short-term assays focused on genomic responses could help identify unanticipated toxicity pathways by comparison of data from test chemicals to information derived from "reference" toxicants with established MOAs (1). Insights gained through this type of analysis could be used to customize test designs and endpoints such that suites of assays are optimized for a given chemical. Thus, the use of genomics to better target MOAs would help focus the investment of resources into tests that would impact risk assessments, rather than generate costly data of little utility.

A better definition of a MOA could also provide information that is critical to reducing uncertainty in risk assessments. For example, a significant source of concern for risk assessments involves predicting the toxicity of chemical mixtures. Genomic profiles could be used to identify compounds with similar versus dissimilar MOAs and thereby selectively "bin" chemicals for which additive effects might be a reasonable expectation.

Extrapolation of effects of chemicals across species is another area where a better understanding of MOA via genomics could provide useful insights (57). For example, some toxicity mechanisms are quite well conserved across species, whereas others are not. Toxicogenomic techniques could lead to defining these similarities and dissimilarities across species, thereby helping confirm where extrapolation of chemical hazards from one species to another is technically valid.

Finally, knowledge of a MOA provides important insights for diagnostic assessments. In most environmental settings, organisms are exposed to various stressors, both chemical and nonchemical. Hence, in situations where undesirable effects are known to occur, identifying the stressors that cause the impacts can be challenging.

A comparatively simple example of this from a regulatory perspective involves effluent toxicity. In the U.S., and increasingly elsewhere, effluent quality is monitored, at least partially, through toxicity tests in the laboratory or with caged animals in the field (58). However, when an effluent is deemed toxic, identifying causal agents (and thereby developing effective remedial strategies) can be exceedingly difficult because of the complex mixture of chemicals typically present (as well as uncertainties related to contaminant bioavailability in a complicated matrix). This led to the development of biologically based sample fractionation procedures, often termed toxicity identification evaluations (TIEs), to help pinpoint specific chemicals responsible for sample toxicity (59). These approaches have been successfully adapted to various species, endpoints, and test matrices; however, the methods can be time-consuming and, occasionally, ineffective. Using toxicogenomic data to identify biologically relevant toxicity pathways in organisms exposed to complex mixtures of contaminants would greatly assist the TIE process.

Other benefits could be derived through the use of genomics in regulatory testing. For example, at present, toxicity data are available or generated for only a comparatively small subset of all existing and new chemicals because of the resources required (60). The net result is a significant degree of uncertainty about the safety of many chemicals present in or released to the environment. The REACH program seeks to address this uncertainty by requiring safety assessments for thousands of chemicals in the coming years (50,51). This EU program, as well as similar regulatory efforts that inevitably will arise elsewhere, will likely require the collection of empirical toxicological data to provide insights about potential chemical risk. However, this information will need to be generated in an era of modest, if any, increases in resources, as well as in a sociocultural environment where an increasing amount of scrutiny is given to animal testing. Toxicogenomic techniques could well result in the generation of relevant screening data that reflect multiple potential toxicity pathways from a limited number of samples. This approach optimizes resources and limits animal use. The broad application of genomics to risk assessments for chemicals that currently undergo no testing is not a development that could reasonably be expected in the short term, but this approach is conceptually sound.

Potential applications

The benefits of toxicogenomic information and tools in regulatory ecotoxicology are only starting to be elucidated. Several specific examples follow of how and when genomics could impact ecological risk assessments. We present this analysis from the standpoint of both prospective and diagnostic assessments. Of course, regional and jurisdictional variations exist in implementation of regulations and how support testing is conducted; however, the generic tiered testing scenarios we present provide a reasonable representation of the types of approaches used worldwide for regulatory decision making. It is important to note that, at present, *none* of the toxicogenomic tools available to the community of experts involved in ecological risk assessments is suitable for regulatory decision making. However, the potential exists for some techniques to be useful in the next 3–5 years, provided research and implementation needs discussed in this paper can be addressed.

Figure 2 depicts a generic tiered testing framework typically used for prospective assessments of the potential risk of a chemical (or chemicals) that may be released into the environment. This particular framework consists of five hierarchal tiers in which data and models of increasing complexity and expense are used. Ideally, most chemicals would be eliminated from further testing early in the process, thereby saving resources for those few with enough potential hazard or exposure to warrant long-term laboratory testing or field analyses (tiers IV, V).

However, eliminating any chemical from all potential concern with a tiered testing framework is usually very difficult, because without full life-cycle or multigenerational tests and advanced exposure analyses the possibility of unacceptable risk always remains. Hence, an important function of the analyses conducted at lower tiers in the framework is prioritization of chemicals for testing at higher tiers—that is, optimization of available resources to focus on chemicals with the greatest potential risk.

Figure 2 also shows potential uses of genomic data at each of the different steps for the hazard-identification portion of the framework. As previously stated, identification or confirmation of a toxic MOA would benefit testing at several junctures. For example, arrows in Figure 2 indicate specific points in the framework where knowledge of a MOA would streamline testing by assisting in prioritization. Specifically, if profiling analyses were conducted in conjunction with currently used in vitro or short-term (acute) in vivo assays (tier II), this information could enable the rapid identification of chemicals for higher-tier testing. This profiling would identify chemicals with the potential to exert toxicity through specific pathways of concern, thereby

"flagging" those compounds for the more-extensive testing. Different classes of EDCs (e.g., estrogens) would be amenable to this type of approach (56), as would contaminants that operate via specific pathways and mechanisms, such as human and veterinary pharmaceuticals (52).

Genomic techniques also may offer the ability to make a decision often not possible in tiered testing—elimination of a chemical from consideration. Highlighted in tier II of Figure 2 is NOTEL—the no-observable-transcription-effect level. (Note that the following discussion could apply equally to changes in the proteome or metabolome as to those in the transcriptome, although different acronyms would be used). In the NOTEL concept for decision making, genomic profiling data would be generated in conjunction with existing tests (e.g., in tier II) to ascertain whether a chemical could elicit a toxic response via particular MOAs. If no evidence exists of interaction of the test chemical with the pathways of concern in terms of gene expression, a decision could be made that no further testing is needed.

It is important to reiterate that this use of NOTEL is not targeted to changes in the "global" transcriptome, because virtually any chemical exposure would be expected to change some aspect of gene expression. Instead, it would focus on groups or families of genes associated with specific toxicity pathways of regulatory concern.

However, for genomic data to be used in this manner, some important assumptions are made. It is critical that measured responses to chemicals at the molecular level be as much as or more sensitive than adverse responses observed in the test organism. If the correct genes, proteins, or metabolites are monitored, this assumption is biologically reasonable, provided that the in vitro or short-term in vivo test system used for the profiling data offers a coherent representation of what would be expected in longer-term exposures—from both biological response and chemical exposure perspectives. A simple example of a disconnect relative to the latter might involve aquatic exposures with chemicals that have log octanol–water partition coefficients >3.5. Specifically, these types of chemicals may not accumulate to a sufficient degree in short-term tests to elicit the molecular response that would be found with longer-term tests.

Approaches for diagnostic risk assessments are also typically tiered, and less-expensive data collection and analyses guide later, more-costly biological and chemical analyses (Figure 3). At tier I, available data are gathered and used to generate hypotheses to guide future tests. Tier II features initial data collection from the site(s) of concern, to ascertain occurrence and possible biological effects of contaminants. If tier II provides evidence of contaminant impacts, then tier III would feature controlled acute or chronic in situ or laboratory testing, often with multiple species. Tier IV, if required, involves extensive fieldwork to determine the extent of both contamination and biological impacts.

Toxicogenomic tools have the potential to assist investigations at several levels in the site-assessment process (Figure 3). Tier II genomic data could help establish that chemical exposure is occurring from a couple of different perspectives. For example, gene expression or metabolite profiles in organisms collected from the field could provide evidence of exposure to specific classes of chemical stressors, even in the absence of analytical measurements of contaminants.

Another challenge involves determining whether what is measured instrumentally is actually bio-available; genomic data could assist here, too. If the molecular profile of a particular chemical or class of chemicals is known or can be established for species of interest, failure to observe this pattern in exposed organisms (i.e., NOTEL) would provide strong evidence that chemicals are not bioavailable, regardless of whether they are detectable. So, with ever-increasing sensitivity of instrumental analyses for environmental contaminants, genomics can help provide insights about the biological significance of analytical measurements.

Toxicogenomic data also could be useful for aspects of tiers III and IV (Figure 3). For example, determination of specific gene, protein, or metabolite expression patterns associated with controlled testing of complex mixtures from or at the study site(s) could confirm chemical MOAs of concern that are hypothesized in tiers I and II, as well as highlight unanticipated MOAs (associated, perhaps, with unmeasured chemicals). As described earlier, genomic data could prove to be a very useful complement to TIE studies focused on associating biological responses with specific contaminants in complex mixtures.

Incorporating genomic studies into diagnostic assessments could provide noteworthy benefits for investigating endangered or threatened species. Relatively small amounts of biological materials, such as saliva, blood, surface mucus, urine, and those from needle biopsies—all of which can be collected via nonlethal approaches—are amenable to toxicogenomic analyses. Metabolomics may be particularly beneficial. Relatively small samples of biofluids are used for analyses, and metabolome data can be compiled and interpreted without extensive knowledge of the organism's genome. This typically would be the case for rare species.

Research needs

A tremendous amount of toxicogenomic research is currently taking place in the area of human health and, increasingly, with ecologically relevant organisms. Some of this effort will undoubtedly directly impact the use of genomics for regulatory purposes. However, various short- and long-term needs must be met if the full potential of toxicogenomics is to be realized.

For the near future, a pressing requirement is for standardization of data collection and analysis (including presentation) techniques. Researchers currently use a wide variety of approaches to generate data. For example, even for a species whose genome has been characterized, such as the zebrafish, commercially available microarrays differ in their coverage of the transcriptome as well as in the physical platform and associated measurement techniques used to collect the data. A similar situation exists for collections of proteomic and metabolomic data. Additionally, several different approaches—some of which are not particularly transparent—have been or are being used to analyze toxicogenomic data, and criteria for determining when changes actually have occurred are not well defined.

Although notable efforts have been made to formalize aspects of data generation and presentation, at least for microarrays (61), the current diversity of techniques and data analysis methods does not yet adequately support the use of genomics in regulatory testing. No tool or set of tools can or will be considered as viable for regulatory use unless the procedures have been subjected to formal standardization and validation through vetted approaches (62,63). Unfortunately, research for method standardization is expensive and often too routine and tedious to be attractive to many scientists. Yet, if standardization is not supported by both the relevant funding agencies and the technical experts involved in toxicogenomics, these tools will not be used for regulatory activities.

Toxicogenomic techniques that show the most immediate promise for application to regulatory ecotoxicology are transcriptomics (e.g., microarrays) and metabolomics (NMR or MS). Proteomic techniques based on MS, although very important for providing insights on toxic mechanisms, currently are not as well suited for rapid global profiling. Therefore, the emphasis on standardization initially should be on microarray and metabolomic techniques for species commonly tested as part of ongoing regulatory activities. As part of this research, easily accessible libraries of profiling data need to be developed for a set of reference chemicals with well-defined, relevant MOAs. To support decisions such as those depicted in Figure 2, this baseline information should be generated using the same short-term designs and species currently employed for prospective or diagnostic assessments. In conjunction with these activities, "proof-of-principle" case studies should be conducted that demonstrate the utility

of genomic data to the risk assessment process. Only through demonstrated successes, such as focused case studies, can the regulatory community be expected to accept toxicogenomics as worthy of additional resources.

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Long-term research needs also exist to support toxicogenomics for regulatory applications. For example, for these techniques to be used fully, information on the genomes of ecologically relevant species is required. This includes organisms commonly used for laboratory testing (e.g., small fish, such as the fathead minnow and medaka; invertebrate models, such as cladocerans, amphipods, and chironomids) as well as species used in the field for monitoring, either through collection from extant populations or through caging studies. Increased coverage of genomic data for various species not only will support broader use of toxicogenomic techniques but also will enhance the understanding of across-species extrapolation of the effects of toxic chemicals (57).

Another long-term research need involves relating the molecular and biochemical responses measured by toxicogenomic methods to specific toxicity pathways and to (adverse) alterations in survival, growth, and reproduction (64). For decades, scientists have attempted to use biomarkers in regulatory ecotoxicology (65). Significant parallels exist between toxicogenomic data and the endpoints considered as potentially useful biomarkers (e.g., changes in CYP enzymes, metallothionein, heat shock proteins, vitellogenin). For example, both genomic and biomarker data attempt to reflect or-ganismic responses that are indicative of a MOA. This has always been a challenge for single-gene, protein, or metabolite biomarker data, because some question usually exists about whether the observed responses are specific to a single chemical stressor or MOA. For example, CYP enzymes can be induced by certain types of organic chemicals, but they are also influenced by variables such as diet, temperature, and the physiological status of the test organism. Through the more-global analysis of responses afforded by toxicogenomic techniques, the lack of pathway specificity encountered with single bio-markers should be obviated.

Another historical impediment to the use of bio-marker data for regulatory decision making has been a general inability to link responses at lower levels of biological organization to adverse outcomes in the whole animal (i.e., "phenotypic anchoring"). Although an argument could be made that a better understanding of toxicity pathways through the collection and analysis of toxicogenomic data should enhance prediction of adverse outcomes, this has yet to be established in a rigorous fashion.

One challenging aspect of phenotype anchoring is the interpretation of direct versus indirect (or compensatory) responses of organisms to chemical stressors. It is important to design experiments that carefully control for the time course over which gene, protein, or metabolite expression is evaluated and to note manifestations of toxicity and repair. Compensatory responses may prove to be particularly difficult to understand, because they could include alterations indicative of both a specific toxic MOA and more generalized responses to stress (66). Studies focused on phenotypic anchoring are not cheap or easy, because integrated responses across multiple biological levels of organization must be considered. However, this type of information is critical to supporting the use of molecular data in regulatory programs.

Implementation challenges

In addition to the research needs, several practical challenges exist to the use of genomics in regulation. One of the greatest initial challenges is educating the experts involved in risk assessments about what toxicogenomics can accomplish. Although toxicogenomic methods

and the resultant data are not necessarily difficult to grasp conceptually, most risk assessors lack the technical background to readily convert this information into a decision-making framework. This situation is due to numerous factors, including a relatively complicated and constantly changing set of terms and jargon; a lack of approaches to "condense" the large amounts of data into a readily usable form analogous, for example, to whole-animal no- and lowest-observable-effect concentrations; and difficulty in interpreting the biological—and, therefore, the regulatory—significance of the information. Standardization of terminology is a relatively straightforward activity that would help facilitate communication. Some of the data-reduction challenges could be addressed through adoption of concepts such as NOTEL. Unfortunately, readily transferable guidance does not yet exist for the interpretation of most toxicogenomic data. So, for the foreseeable future, the only feasible approach to closing this knowledge gap will be through direct interactions between genomic technical experts and scientists involved in risk assessments. As mentioned previously, a logical way to achieve this would be through the development of focused case studies with a limited set of chemicals and assessment scenarios.

As strategies are developed for the use of toxicogenomics in regulatory ecotoxicology, two factors need to be considered. First, scientists conducting certain types of human-health risk assessments (e.g., drug discovery and safety; *67*) are well ahead of those involved in ecological risk assessments with respect to how they may use toxicogenomic data. Hence, although regulatory challenges and scenarios can differ between human and ecological health, sometimes quite dramatically, ecotoxicologists need to keep abreast of advances in the use of toxicogenomics for human toxicology regulation. Second, given the increasing emphasis on international harmonization of regulatory test methods, efforts to introduce genomic approaches into ecological risk assessments likely would be best achieved through international collaborations. One agency that might help facilitate this is the Organisation for Economic Cooperation and Development (OECD), which already has initiated an expert group to consider the use of genomics in regulation (68).

Some of the more notable challenges with using toxicogenomic data in human-health or ecological risk assessments lie not with the research needs and practical challenges but within current regulatory paradigms (67). For example, no precedent exists in regulatory ecotoxicology for the use of biomarker data in decision making (65). Hence, the first hurdle for acceptance of genomics data by risk assessors will be thorough documentation of the technical and practical utility, to an extent not previously achieved by those involved in biomarker research with ecologically relevant species. It is worthy of note that recent developments in the area of EDC regulation may provide an example of how a biomarker, vitellogenin, could be applied to regulatory testing and monitoring (69). Vitellogenin, commonly referred to as egg-yolk protein, is readily measured and highly specific for estrogenic materials (70). This sets it apart from the less-specific biomarkers (e.g., CYP proteins) used in the past. Well-characterized, specific molecular markers such as vitellogenin will quite possibly help pave the way for the routine use of toxicogenomic data in risk assessments.

Another paradigm shift involves moving from very prescriptive approaches for defining potential chemical risks to testing schemes that are more hypothesis-driven (60). For example, knowledge of probable toxicity pathways that is derived from genomic data would enable the streamlining of test programs, such as pesticide registration, that require significant amounts of test data, some of which may not be used. Toxicogenomic information generated from inexpensive in vitro or short-term in vivo techniques also could be used to identify and prioritize chemicals of toxicological concern for further testing. Conceptualizing how these types of innovative approaches could be useful is easy. However, actual implementation will be challenging, because hypothesis-driven testing is a significant departure from how regulatory

programs have functioned. Changing attitudes about how best to apportion testing resources is not an insurmountable obstacle, but it does require a frank acknowledgment that more efficient approaches to testing may exist than those we currently use.

The challenges associated with incorporating toxicogenomic data into regulatory decision making are not trivial. However, the potential for toxicogenomics to spur significant advances in the field of regulatory ecotoxicology is undeniable, in terms of both reducing uncertainty and optimizing testing resources. This latter point is especially noteworthy because we work in an environment that sees little or no increase in testing resources yet experiences increasing demands for more and better safety data for existing and new chemicals. In fact, the most significant impediment to realizing the potential of toxicogenomics in the regulatory arena will probably be a lack of resources. As Figure 4 shows, initial incorporation of genomic data into existing regulatory frameworks would require resources, primarily for generating profiling data (genomic addons) with samples derived from existing test schemes (i.e., few additional animals would be used). In the somewhat longer term, the resources needed, including animals, would increase as some of the more difficult issues associated with phenotypic anchoring and validation would be confronted. Ultimately, the successful transition of toxicogenomics into testing programs should markedly decrease resource and animal use.

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A major challenge, therefore, will be justification of increased resource investment—both for research and for implementation—over the next few years, for a potential payoff years in the future. Although it is a daunting task, the successful incorporation of toxicogenomics into regulatory frameworks may someday be regarded as the most important intellectual and practical contribution from this generation of ecotoxicologists.
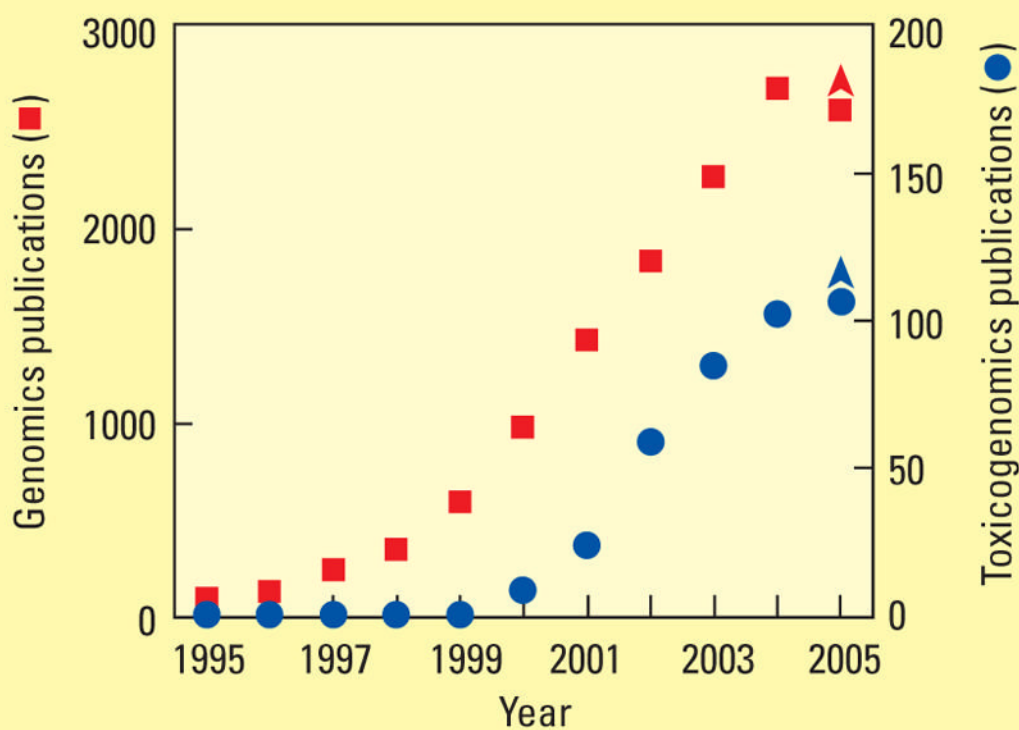
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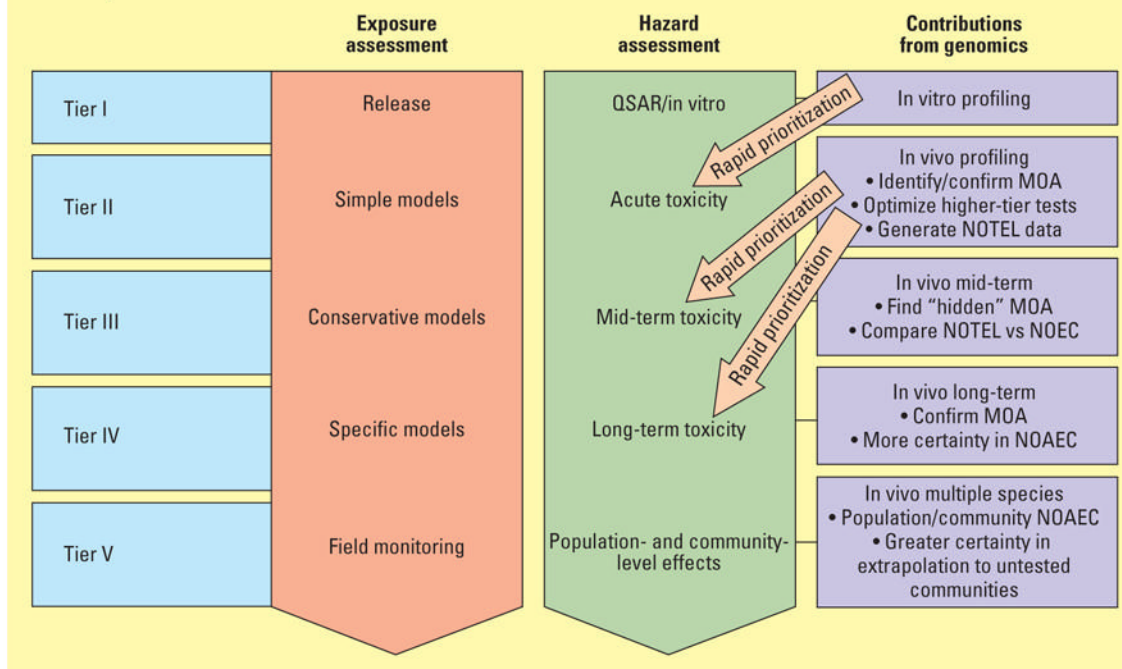
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FIGURE 1.
Increase in research activity in genomics and toxicogenomics over the past decade
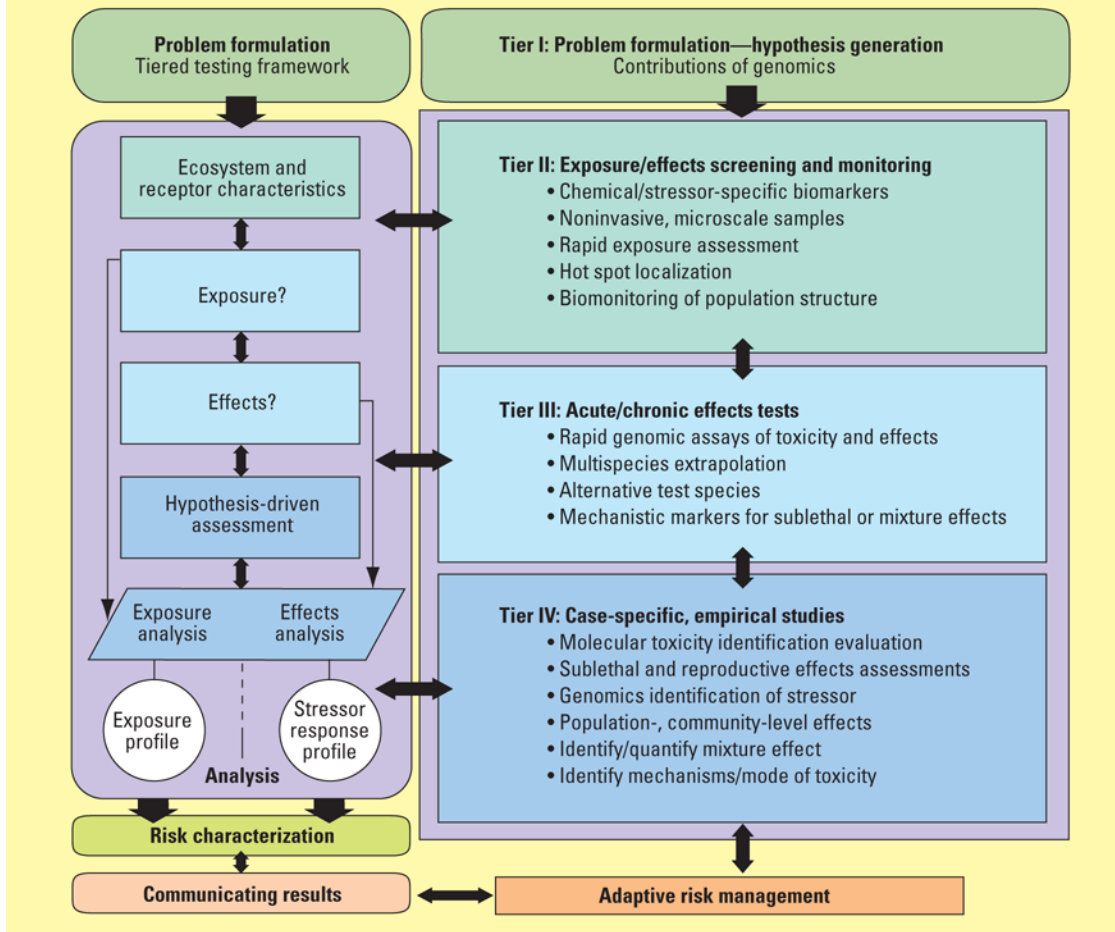
The different tiers of testing span problem formulation (tier I), acute effects (tier II), chronic effects (tier III), case-specific empirical studies (tier IV), and population effects (tier V). Exposure assessments across the progressive tiers start with simple information on whether the substance is released into the environment (tier I) and progress to complex information on distributions of exposure, with temporal and spatial resolution (tier V). For hazard identification assessments, complexity of study increases similarly with the progressive tiers—tier III focuses on sensitive life stages and critical processes, tier IV on life-cycle and multigenerational studies, and tier V targets microcosm, mesocosm, and field studies with multiple species. Rapid prioritization arrows indicate where toxicogenomics could be used to inform hazard assessment and better direct higher tiers of testing. Abbreviations: MOA, mode of action; NOTEL, no-observable-transcription-effect level; NOEC, no-observable-effect concentration; NOAEC, no-observable-adverse-effect concentration; QSAR, quantitative structure–activity relationship.

|	Exposure assessment	Hazard assessment	Contributions from genomics
Tier I	Release	QSAR/in vitro	In vitro profiling
Tier II	Simple models	Acute toxicity	In vivo profiling • Identify/confirm MOA • Optimize higher-tier tests • Generate NOTEL data
Tier III	Conservative models	Mid-term toxicity	In vivo mid-term • Find "hidden" MOA • Compare NOTEL vs NOEC
Tier IV	Specific models	Long-term toxicity	In vivo long-term • Confirm MOA • More certainty in NOAEC
Tier V	Field monitoring	Population- and community-level effects	In vivo multiple species • Population/community NOAEC • Greater certainty in extrapolation to untested communities

Rapid prioritization (arrows)

FIGURE 2.
How toxicogenomic data could be incorporated into a generic tiered testing framework for prospective ecological risk assessments

The different tiers of testing span problem formulation (tier I), monitoring for extant effects and exposure (tier II), determining acute and chronic effects under controlled conditions (tier III), and conducting case-specific large-scale studies (tier IV). Potential genomic applications are indicated at each tier.

Problem formulation
Tiered testing framework

Ecosystem and receptor characteristics

Exposure?

Effects?

Hypothesis-driven assessment

Exposure analysis

Effects analysis

Exposure profile

Stressor response profile

Analysis

Risk characterization

Communicating results

Tier I: Problem formulation—hypothesis generation
Contributions of genomics

Tier II: Exposure/effects screening and monitoring
• Chemical/stressor-specific biomarkers
• Noninvasive, microscale samples
• Rapid exposure assessment
• Hot spot localization
• Biomonitoring of population structure

Tier III: Acute/chronic effects tests
• Rapid genomic assays of toxicity and effects
• Multispecies extrapolation
• Alternative test species
• Mechanistic markers for sublethal or mixture effects

Tier IV: Case-specific, empirical studies
• Molecular toxicity identification evaluation
• Sublethal and reproductive effects assessments
• Genomics identification of stressor
• Population-, community-level effects
• Identify/quantify mixture effect
• Identify mechanisms/mode of toxicity

Adaptive risk management

FIGURE 3.
Where toxicogenomic data could be incorporated into a generic tiered testing framework for diagnostic ecological risk assessments
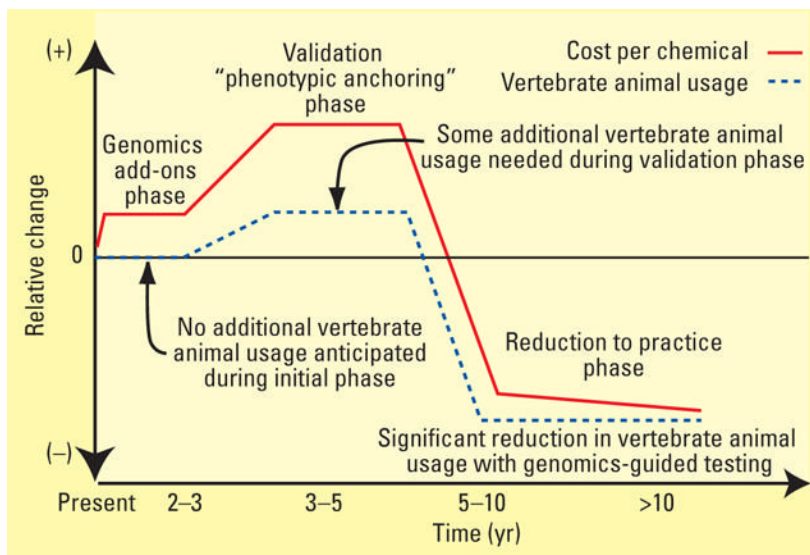
FIGURE 4.
Conceptual timeline and relative resource requirements for the development and integration of toxicogenomic data into regulatory programs