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Ecotoxicogenomics: Bridging the Gap between Genes and Populations

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Ecotoxicogenomics might help solving open questions that cannot be answered by standard ecotoxicity tests currently used in environmental risk assessment. Changes in gene expression are claimed to serve potentially as early warning indicators for environmental effects and as sensitive and specific ecotoxicological end points. Ecotoxicogenomics focus on the lowest rather than the highest levels of biological organization. Our aim was to explore the links between gene expression responses and population level responses, both mechanistically (conceptual framework) and correlatively (Species Sensitivity Distribution). The effects of cadmium on aquatic species were compared for gene level responses (Lowest Observed Effect Concentrations) and individual level responses (median Lethal Concentrations, LC_{50}, and No Observed Effect Concentrations, NOEC). Responses in gene expression were on average four times above the NOEC and eleven times below the LC_{50} values. Currently, use of gene expression changes as early warning indicators of environmental effects is not underpinned due to a lack of data. To confirm the sensitivity claimed by ecotoxicogenomics more testing at low concentrations is needed. From the conceptual framework, we conclude that for a mechanistic gene population link in risk management, research is required that includes at least one meaningful end point at each level of organization.

Introduction

The potential and actual damage of chemicals to the environment is estimated in risk assessment (*1–3*). To cover different levels of organization, effects on molecules, cells, organs, individuals, populations, and ecosystems may be taken into account (*4*). In ecotoxicity tests, however, the most commonly used end points are survival and reproduction of individuals (*5*). These standard tests have been proven useful and efficient in current risk assessments. However, they do not provide sufficient information in all situations, and for early warning they may respond too slowly (*6–8*). As a result, end points on subcellular and molecular levels have been proposed to provide additional support for risk assessment.

Since the late 1990s, when "omics" technology emerged and was applied in ecotoxicology, research on effects of various chemicals at the genetic level has given rise to the field of ecotoxicogenomics (*9*). Ecotoxicogenomics is the study of gene and protein expression integrating transcriptomics, proteomics, and metabolomics into ecotoxicology (*10*). Application of ecotoxicogenomic techniques in chemical screening, environmental monitoring, and risk assessment is currently being explored. These techniques have been applied, e.g., to study the interactions of different substances on organisms (*11*) and to determine how chemicals affect molecular pathways and biological processes within individuals (*12, 13*).

The potential applications are widely recognized and it has been suggested that these "omics" technologies can provide a broader understanding and prediction of the effects of chemicals on populations and ecosystems (*14*). Changes in gene expression are claimed to serve as early warning indicators for environmental effects and as useful biomarkers for chemical exposure (*15–18*), because they can be detected at low concentrations of chemicals and before morphological or reproductive effects become visible (*19, 20*), Overall, recent reviews concerning ecotoxicogenomics share a common opinion that this field is one of the most promising for environmental risk assessment in which responses in gene expression to chemicals can be considered as a new sensitive, specific, and informative ecotoxicological end point (*21–24*). However, skeptical attitudes toward application of "omics" research tools into ecotoxicology and the use of gene expression effects in risk assessment are also present (*25, 26*). While ecotoxicogenomics is developing, the focus of this field appears to be on the lowest rather than the highest levels of biological organization. Yet, a comprehensive overview of attempts to link these levels carried out so far is lacking. The aim of the present study was therefore to explore potential relationships, both correlative and mechanistic, between gene expression responses and population level responses to be used in risk assessment.

Materials and Methods

Literature concerning "omics" in ecotoxicology was explored in the Web of Science, Scopus and Scirus databases using different combinations of the keywords "ecotoxicogenomic", "ecotoxicology", "risk assessment", "gene expression", "microarray", "genomics", "transcriptomics", "metabolomics", and "heavy metals". Searches with keywords were supplemented with examination of the literature cited in the articles found. Priority was given to articles with (a claim of) an application in ecotoxicology.

For the construction of a framework linking genes to populations, we investigated studies on plant and animal species from various terrestrial and aquatic systems exposed to different stressors.

For the statistical analysis illustrating potential ways for gene to population extrapolation, we focused on effects of cadmium on aquatic species only, because of the relative large number of publications available. Data on toxic effects of cadmium at different levels of organization were collected from articles, reports, and from the database of the Dutch National Institute for Public Health and the Environment

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(RIVM): e-toxBase (*27, 28*). For the gene level response, we selected articles containing data on whole-genome expression profiling or specific gene expression in aquatic organisms under exposure to cadmium. If more than one concentration of cadmium was tested, the lowest concentration with a response deviating significantly from the control was used. This concentration served as the Lowest Observed Effect Concentration (LOEC) (Table S3 of Supporting Information). Obviously, one canot exclude that lower concentrations, if tested, would have induced an effect as well. For the individual level response, median Lethal Concentrations (LC_{50}) and No Observed Effect Concentrations (NOEC) for aquatic species were collected (Table S4 of Supporting Information).

The data were plotted log−logistically to create Species Sensitivity Distributions (SSDs), representing the cumulative distribution of test end points data (LOEC, NOEC, or LC_{50}) (*29*). SSDs are used to represent stress to the ecosystem caused by chemicals. These distributions can be derived at the species level from toxicity data obtained from acute or chronic tests. Also, other end points on different levels such as effects on gene expression can be used. The Potentially Affected Fraction (PAF) in these distribution curves shows the proportion of the species affected as a function of stressor concentration. The "potential fraction" indicates the fraction of species estimated to be exposed beyond an effective concentration (*29*). The average sensitivity of the species is represented by the 50% hazardous concentration (HC_{50}) (*30*). The median hazardous concentration values (HC_{50}) and 5% hazardous concentrations (HC_5) were calculated according to Aldenberg and Jaworska (*31*) and compared in *t* tests.

Results

Linking Levels in a Hierarchical Framework. Research in ecotoxicogenomics focuses on the genome-wide expression analysis under exposure to various contaminants resulting in chemical-specific patterns of gene expression and on the development of a mechanistic understanding of chemical toxicity on various organisms (Table S2 of Supporting Information). Potentials and advantages of ecotoxicogenomics are recognized, and its usefulness for application in ecotoxicology is accepted, but evidence and successful examples of these aspects are often missing. This potential can be accomplished by making links between gene expression profiles, cellular level responses, and observed biological responses known to impair adverse impacts at individual and population level (*32*). In general, biomarkers are considered most useful for environmental risk assessment if they can predict the effects on survival, growth, or reproduction (*33*). Thus, to test the ecotoxicological relevance of changes in gene expression and to use these as biomarkers or as new end points, it is necessary to ensure that the changes in gene expression are integrated with individual and population level end points.

The linkages from gene to population level effects can be presented in a hierarchical framework (Figure 1). Following cascade effects, processes at one level are considered to be caused by processes at a lower level and result in consequences at a higher level (*34, 35*). The initial responses to a chemical interacting with the site of action can be observed at low levels of organization, e.g., gene transcription and protein synthesis (*36*). In case of continuing or increasing stress, effects on the molecular level will result in a local cellular response (*23*). Cellular effects in turn may lead to tissue damage and to physiological, biochemical, or behavioral changes at the whole organism level. Damage at these levels can potentially affect population dynamics and community structure (*34*).

Ecotoxicogenomic studies typically focus on the lower end of the framework (Table S2 of Supporting Information). Changes in gene expression can result from toxicity as either

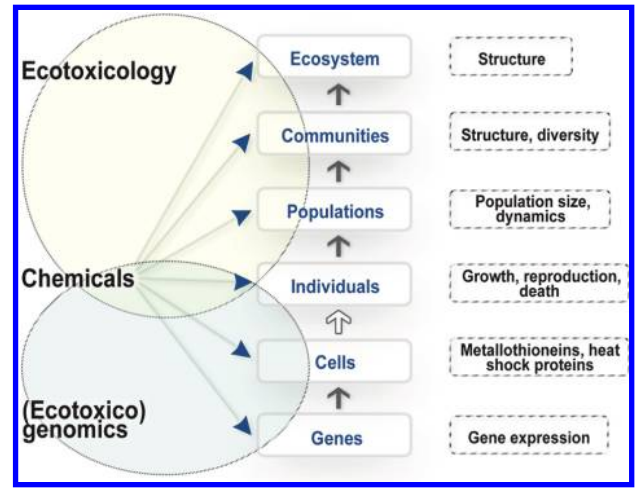

FIGURE 1. Hierarchical framework linking genes to populations throughout all levels of biological organization (vertical arrows). Ecotoxicogenomics investigate effects of chemicals at the gene level. Ecotoxicology covers the response at the individual and population level. The major gap identified in the present study is between gene and cell responses on the one hand and the individual and population responses on the other (white arrow). End points at several levels may help to link these parts and fill the gap (dotted boxes).

a direct or indirect response to chemical exposure (*7, 19, 24, 37*). Some genes are turned on or off. Alternatively, the level of expression of some genes is altered (*7*). Toxic end points at the cellular level such as inflammation, apoptosis, necrosis, and cellular differentiation are preceded by specific alterations in gene expression (*15*). Gene expression effects are generally specific for certain chemicals. By moving up in the organizational hierarchy, observed effects become less specific to the chemical tested (*38*).

The upper part of the framework relates to the area of classical ecotoxicology. For assessment and understanding the direct effects of toxicants on these levels, many approaches have been developed (*39*). The most commonly used test end points for organism level are the LC_{50} and the NOEC (*40, 41*). Often, however, environmental risk assessment aims to protect populations rather than individuals (*42–44*). Basic end points at the level of populations may include variables like the population density, productivity, and probability of extinction (*34*). Community level effects are often described by changes in species composition (types, diversity) (*2*). Effects on populations and communities are ecologically relevant but they often lack mechanistic explanations (*45*).

Bridging the Gaps in Individual Studies. Examples of recent ecotoxicogenomic studies in the area of environmental toxicology, ecotoxicology and risk assessment are given in Table S1 of Supporting Information. In these studies, gene expression was used to distinguish the type of contamination or the mechanism of action of the chemicals rather than used to predict effects on exposed populations. Some of these studies report the effects on oxidative stress, detoxification, immune response, and energy metabolism, thereby qualitatively linking chemical-gene-cellular interactions. Some studies also incorporated effects at gene level with response at higher levels of biological organization.

Magrini et al. (*46*) investigated gene expression in *Arabidopsis thaliana* grown in soils contaminated by copper and lead in combination with individual level responses. Genes related to metallothioneins, heat shock proteins, protein synthesis, cellular transport, and wound stress response were up-regulated. Gene expression was used as an indication of soil contamination, which was also supported by reduced plant growth. This result suggests that exposure

to heavy metals can trigger specific gene activation as well as changes in general stress response genes expression.

In another study, rainbow trout (*Oncorhynchus mykiss*) showed unique response patterns when exposed to a series of model toxicants (*47*). The majority of responsive genes was specific to a single chemical, showing a link between gene expression profiles and the toxic mode of action (*47*). The specificity of gene expression in response to chemicals, the link between genes expression and mode of action provide another example that "omics" technologies can be potentially used for chemical screening. Roelofs et al. (*48*) identified differentially expressed genes in springtails (*Orchesella cincta*) exposed to cadmium in their food. These genes were associated with general stress response and involved in cadmium detoxification. They also found that different metals induced the same gene expression, which is similar in different species (*48*). Watanabe et al. (*49*) examined gene expression of *Daphnia magna* under the influence of $CuSO_4$ and H_2O_2. It was found that a high dose of $CuSO_4$ induced a similar gene expression profile as a high dose of H_2O_2. Some heavy metals, copper and cadmium, were also found to trigger similar gene expression patterns in fish (*50*). Sheader et al. (*51*) identified 27 up-regulated genes in European flounder (*Platichthys flesus*) under cadmium exposure and some candidate genes were selected as potential biomarkers for cadmium exposure.

These studies demonstrate how gene expression and "omics" technologies have been applied to study the effects of toxicants on biological pathways in organisms. In a few studies, changes in gene expression were measured and accompanied by simultaneous monitoring the responses at higher levels. In a study with *Daphnia magna* exposed to different concentrations of cadmium, changes in gene expression were combined with effects on other levels: cellular energy allocation, growth, energy reserve availability, and energy consumption (*52*). At higher concentrations and after prolonged exposure, the number of genes expressed differently increased and net energy budget and growth decreased. Changes in gene expression were associated with molecular pathways involved in immune response, stress response, digestion, oxygen transport, cuticle metabolism, and embryo development. Menzel et al. (*26*) combined gene expression profiling of *Caenorhabditis elegans* exposed to different levels of heavy metals and organic pollution in river sediments with the effects on other levels, including endocrine disruption and reproduction. They showed how changes in gene expression can be used as supplementary assay to screen pollution in "real world". Connon et al. (*53*) noted gene expression in *Daphnia magna* under cadmium exposure and linked this expression to somatic growth, development, and population growth rate. In another study, *Caenorhabditis elegans* gene expression was integrated with organism and population level end points exposed to silver nanoparticles (*54*). This experiment gives an example of how expression of specific gene can be related to decreasing reproduction potential.

By use of earthworms (*Lumbricus rubellus*) Spurgeon et al. (*35*) provided an example of how effects at different levels can be linked by the cascade concept. The earthworms were exposed to different concentrations of zinc, and their gene expression effects were the most sensitive end points. Destabilization of lysosomal membrane was the next level at which the effect of zinc was measured. Less sensitive were individual level effects (changes in reproduction at EC_{50}) followed by population size effects.

Bridging the Gaps across Individual Studies. In addition to linking effects at different levels within the same study, gene expression responses and population responses to the same substance can also be compared by merging data. In ecotoxicology, results from single species tests are often combined statistically by creating a SSD (*29*). In Figure 2, the potentially affected fraction of species is plotted as a function of the cadmium concentration for end points on different levels of organization. The response concentration LOEC for gene expression is roughly in between the NOEC and LC_{50} for individual level effects. Differences between gene expression responses and whole organism responses based on NOECs, and between gene expression responses and whole organism responses based on LC_{50} are statistically significant (Figure 2). The standard deviation of distributions reflects slopes of the SSDs and represents variation in sensitivity of the end points.

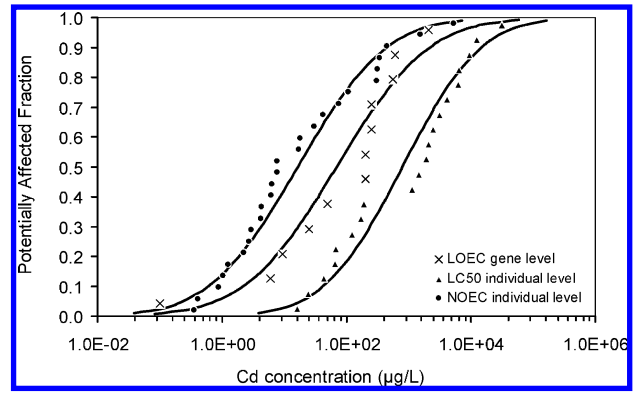

FIGURE 2. SSD with the PAF vs cadmium concentration for NOECs and median LC_{50}s for individual level responses as well as for LOEC for gene expression. The HC_{50}s of 16.5 μg/L (NOEC), 69.3 μg/L (LOEC), and 791.4 μg/L (LC_{50}) differ significantly (2-tailed *t* test, $\alpha = 0.05$), whereas the standard deviation equals 1.28 (NOEC), 1.17 (LOEC), 0.98 (LC_{50}). The corresponding HC_5s are 0.23 μg/L (NOEC), 0.72 μg/L (LOEC), and 18.3 μg/L (LC_{50}). Aquatic species included in these SSDs are listed in Table S3 of Supporting Information (gene level LOEC) and Table S4 of Supporting Information (individual level LC_{50} and NOEC).

Discussion

Perspectives for Mechanistic Links. Studies have shown that gene to cell level types of response are better traceable and can be easier linked to a specific cause than effects at higher levels. Each organism has specific mechanisms to cope with external stressors and to balance normal physiological conditions (*55*). Under stressful conditions, an organism will activate these stress-defense mechanisms acting on molecular up to organism levels. Mechanism-specific end points, however, are not necessarily predictive of an adverse outcome on ecologically relevant levels (*56*), and thus effects of chemicals should be tracked at each level. Examples of common stress responses known on different organizational levels are used as biomarkers of chemical exposure or effect. For instance, effects of chemicals can be marked by phase I and phase II metabolic enzymes, metallothioneins, antioxidant enzymes, and heat shock proteins (*34, 36*). Genes encoding these elements are commonly found to be differentially expressed upon chemical exposure too and can thus reveal respective cellular effects.

It should be possible to link cell stress reactions to organism level responses, by measuring metabolic products and processes such as proteins involved in digestion, oxygen transport, and total hydrocarbons concentration, because toxic defense and repair mechanisms are metabolically costly (*57*). As metabolic rates may be increased by some pollutants and decreased by others, the amount of energy left for survival, growth, and reproduction may be a better indicator (*58, 59*). Reallocation of energy to stress-specific responses might represent a general mechanism that occurs under stress exposure (*60*). Thus, mechanistic links could potentially be

TABLE 1. HC_{50} and HC_5 Values (μg/L Cd) Including Number of Species Used in Analysis (n) and Standard Deviation of the SSDs for LOECs (Gene Expression) and NOECs and LC_{50} (Both Individual Level Response) based on Selected Groups of Aquatic Species for which LOEC Values Were Available and for the Latter Two also on All Aquatic Species Present in the RIVM e-toxBase[a]

	NOEC				LOEC				LC_{50}			
	HC_{50}	HC_5	n	SD	HC_{50}	HC_5	n	SD	HC_{50}	HC_5	n	SD
selected species groups	16.5	0.23	26	1.28	69.3	0.72	12	1.17	791.4	18.3	20	0.98
all species	32.3	0.24	43	1.12					859.1	13.2	448	0.99

[a] Adding NOEC and LC_{50} values did not cause large shifts in the difference observed between HC_{50} and HC_5 values for different end points (compare also Figures 1 and S1).

explained by the energy budget models describing the effects of chemical stress on energy fluxes to maintain reproduction and survival of individuals and thus dynamics and existence of populations (*61*).

To be useful for implementation of such a mechanistic gene-population link in risk management, empirical research should include at least one meaningful end point at each level of organization. These end points may vary as shown in the Figure 1 but they will provide mechanistic information for quantifying effects of stressor if measured simultaneously. The major goal for these end points to be mechanistically linked to each other is to relate gene expression end points to parameters such as reproduction and growth and to ecologically relevant population and community responses.

Perspectives for Correlative Links. Data on gene expression LOECs collected in this study are less well standardized than individual-based NOECs and LC_{50}s. Comparing HC_{50}s calculated in this study showed that cadmium induced changes in gene expression at concentrations of about 4 times above the NOEC and 11 times below the LC_{50} for effects on individual level. At the HC_5 level, LOECs for gene expression and NOECs for individual level effects differed by a factor of 3. This indicates that cadmium effects on gene expression have not been observed at the NOEC level, the HC_5 of which is usually considered to be the threshold for protection of ecological structure (*62*). The observed difference between gene expression LOEC and individual level effects NOEC is not always reflected in data for the same species. Gene expression LOECs and individual level NOECs differed by a factor 5 in *Daphnia magna* and about 250 in *Oncorhynchus mykiss*. However, in *Chironomus tentans* gene expression LOEC was 1.5 times more sensitive than individual level NOEC.

On individual level, NOEC differs generally by a factor of about 2 from the LOEC for the same chemical compound (*63*). Thus, if we had compared LOECs on individual levels with the obtained LOECs for gene expression, the difference would still be a factor 2. This comparison indicated that response concentrations in gene assays were relatively high. The NOECs and LC_{50} were obtained for the same groups of species (Pisces, Crustacea, Insecta, and Mollusca) for which LOECs at gene level were available. Obviously, the limited number of input data might have decreased the accuracy of the NOEC and LC_{50} SSDs. However, the SSDs for individual level effects based on data for all aquatic species available in the RIVM e-toxBase were comparable with the SSDs for this limited group of aquatic species (Figure S1 of Supporting Information). The differences in sensitivity between gene responses and individual level were similar in both cases (Table 1), with gene expression LOECs about 2 times less sensitive than individual level NOECs.

In a similar SSD approach, cellular biomarkers were found to be a factor of 35−50 more sensitive to oil than individual-based end points (NOEC) (*64*). In addition, in the example of Spurgeon et al. (*35*) it was shown that gene expression response was the most sensitive end point and effects of zinc at low concentrations were detected. These studies suggest that high sensitivity in gene expression responses may indeed be achievable. However, the currently available data for cadmium exposure on aquatic organisms do not yet confirm that responses on gene expression level (LOEC) are more sensitive than on individual level (NOEC). Overall, the use of gene expression responses as an early warning of population level effects and as a sensitive end point measured at low, environmentally realistic concentrations as suggested in the past (*65, 66*) is thus not underpinned by the available data yet. We conclude that more testing at low concentrations is needed to confirm the sensitivity claimed. This would also require standardization of gene expressions assays to obtain meaningful end points at the gene level and confirmation for other chemicals and species. Such an end point may be, for example, a No Observed Transcriptional Effect Level (NOTEL) obtained after a standard exposure period to be compared with existing toxicity data for individual-population level (*67, 68*). This will allow the derivation of an "extrapolation factor" linking directly gene and population responses, which is not yet possible with currently available data. Without such standardization, we should keep in mind that variability in data also reflects differences between protocols and end points, in addition to differences between species. While empirical underpinning is required, our study shows that SSD can provide a tool for a gene-population response linking, albeit of a correlative nature.

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Supporting Information Available

Figure S1 shows the SSDs where all aquatic species (e-toxBase) were included. Table S1 gives examples of articles studying gene expression with links to higher levels of biological organization. Table S2 gives examples of articles studying gene expression without other levels of biological organization. Table S3 shows aquatic species used for gene expression LOECs. Table S4 shows aquatic species under cadmium exposure used for LC_{50}s and NOECs for SSDs constructions. This information is available free of charge via the Internet at http://pubs.acs.org/.

Literature Cited

(1) Power, M.; Adams, S. M. Perspectives of the scientific community on the status of ecological risk assessment. *Environ. Manage.* **1997**, *21* (6), 803–803.
(2) Calow, P. Ecological risk assessment: Risk for what? How do we decide. *Ecotoxicol. Environ. Saf.* **1998**, *40* (1−2), 15–18.
(3) Moore, M. N. Biocomplexity: the post-genome challenge in ecotoxicology. *Aquat. Toxicol.* **2002**, *59* (1−2), 1–15.
(4) Markert, B. A.; Breure, A. M.; Zechmeister, H. G. *Bioindicators and Biomonitors: Principles, Concepts, and Applications*; Elsevier: Amsterdam, 2003.
(5) Miracle, A. L.; Ankley, G. T. Ecotoxicogenomics: Linkages between exposure and effects in assessing risks of aquatic contaminants to fish. *Reprod. Toxicol.* **2005**, *19* (3), 321–326.
(6) Moriarty, F. *Ecotoxicology: the Study of Pollutants in Ecosystems*, 3rd ed.; Academic Press: San Diego, 1999.
(7) Walker, C. H.; Hopkin, S. P.; Sibly, R. M.; Peakall, D. B. *Principles of Ecotoxicology*, 3rd ed.; CRC: Boca Raton, FL, 2006.
(8) Robbens, J.; Van der Ven, K.; Maras, M.; Blust, R.; De Coen, W. Ecotoxicological risk assessment using DNA chips and cellular reporters. *Trends Biotechnol.* **2007**, *25* (10), 460–466.

(9) Neumann, N. F.; Galvez, F. DNA microarrays and toxicogenomics: Applications for ecotoxicology. *Biotechnol. Adv.* **2002**, *20* (5−6), 391–419.
(10) Snape, J. R.; Maund, S. J.; Pickford, D. B.; Hutchinson, T. H. Ecotoxicogenomics: the challenge of integrating genomics into aquatic and terrestrial ecotoxicology. *Aquat. Toxicol.* **2004**, *67* (2), 143–154.
(11) Poynton, H. C.; Vulpe, C. D. Ecotoxicogenomics: emerging technologies for emerging contaminants. *J. Am. Water Res. Assoc.* **2009**, *45* (1), 83–96.
(12) Kammenga, J. E.; Herman, M. A.; Ouborg, N. J.; Johnson, L.; Breitling, R. Microarray challenges in ecology. *Trends Ecol. Evol.* **2007**, *22* (5), 273–279.
(13) Lettieri, T. Recent applications of DNA microarray technology to toxicology and ecotoxicology. *Environ. Health Perspect.* **2006**, *114* (1), 4–9.
(14) Steinberg, C. E. W.; Stürzenbaum, S. R.; Menzel, R. Genes and environment: Striking the fine balance between sophisticated biomonitoring and true functional environmental genomics. *Sci. Total Environ.* **2008**, *400* (1−3), 142–161.
(15) Pennie, W.; Pettit, S. D.; Lord, P. G. Toxicogenomics in risk assessment: An overview of an HESI collaborative research program. *Environ. Health Perspect.* **2004**, *112* (4), 417–419.
(16) Thomas, R. S.; Rank, D. R.; Penn, S. G.; Zastrow, G. M.; Hayes, K. R.; Pande, K.; Glover, E.; Silander, T.; Craven, M. W.; Reddy, J. K.; Jovanovich, S. B.; Bradfield, C. A. Identification of toxicologically predictive gene sets using cDNA microarrays. *Mol. Pharmacol.* **2001**, *60* (6), 1189–1194.
(17) Snell, T. W.; Brogdon, S. E.; Morgan, M. B. Gene expression profiling in ecotoxicology. *Ecotoxicology* **2003**, *12* (6), 475–483.
(18) Bartosiewicz, M.; Penn, S.; Buckpitt, A. Applications of gene arrays in environmental toxicology: Fingerprints of gene regulation associated with cadmium chloride, benzo(a)pyrene, and trichloroethylene. *Environ. Health Perspect.* **2001**, *109* (1), 71–74.
(19) Nuwaysir, E. F.; Bittner, M.; Trent, J.; Barrett, J. C.; Afshari, C. A. Microarrays and toxicology: The advent of toxicogenomics. *Mol. Carcinogen.* **1999**, *24* (3), 153–159.
(20) Klaper, R.; Thomas, M. A. At the crossroads of genomics and ecology: The promise of a canary on a chip. *Bioscience* **2004**, *54* (5), 403–412.
(21) Aardema, M. J.; MacGregor, J. T. Toxicology and genetic toxicology in the new era of "toxicogenomics": impact of "-omics" technologies. *Mutat. Res.-Fund. Mol. M.* **2002**, *499* (1), 13–25.
(22) Calzolai, L.; Ansorge, W.; Calabrese, E.; Denslow, N.; Part, P.; Lettieri, T. Transcriptomics and proteomics. Applications to ecotoxicology. *Comp. Biochem. Phys. D* **2007**, *2* (3), 245–249.
(23) Poynton, H. C.; Wintz, H.; Vulpe, C. D. Progress in ecotoxicogenomics for environmental monitoring, mode of action, and toxicant identification. In *Comparative Toxicogenomics*; Hogstrand, C., Kille, P., Eds.; Elsevier: Amsterdam, 2008.
(24) Ankley, G. T.; Daston, G. P.; Degitz, S. J.; Denslow, N. D.; Hoke, R. A.; Kennedy, S. W.; Miracle, A. L.; Perkins, E. J.; Snape, J.; Tillitt, D. E.; Tyler, C. R.; Versteeg, D. Toxicogenomics in regulatory ecotoxicology. *Environ. Sci. Technol.* **2006**, *40* (13), 4055–4065.
(25) Forbes, V. E.; Palmqvist, A.; Bach, L. The use and misuse of biomarkers in ecotoxicology. *Environ. Toxicol. Chem.* **2006**, *25* (1), 272–280.
(26) Menzel, R.; Swain, S. C.; Hoess, S.; Claus, E.; Menzel, S.; Steinberg, C. E. W.; Reifferscheid, G.; Sturzenbaum, S. R. Gene expression profiling to characterize sediment toxicity - a pilot study using *Caenorhabditis elegans* whole genome microarrays. *BMC Genomics* **2009**, *10*, 160.
(27) Crommentuijn, T.; Polder, M. D.; Van de Plassche, E. J. *Maximum Permissible Concentrations and Negligible Concentrations for Metals, Taking Background Concentrations into Account*; RIVM Report 60150100: Bilthoven, NL, 1997.
(28) RIVM e-toxBase. Database for Ecotoxicological Risk Assessment, National Institute for Public Health and the Environment. Available at http://www.e-toxbase.com.
(29) Posthuma, L.; Suter, G. W.; Traas, T. P. *Species Sensitivity Distributions in Ecotoxicology*; Lewis Publishers: Boca Raton, FL, 2002.
(30) Newman, M. C.; Ownby, D. R.; Mezin, L. C. A.; Powell, D. C.; Christensen, T. R. L.; Lerberg, S. B.; Anderson, B. A. Applying species-sensitivity distributions in ecological risk assessment: Assumptions of distribution type and sufficient numbers of species. *Environ. Toxicol. Chem.* **2000**, *19* (2), 508–515.
(31) Aldenberg, T.; Jaworska, J. S. Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. *Ecotoxicol. Environ. Saf.* **2000**, *46* (1), 1–18.
(32) Jha, A. N. Ecotoxicological applications and significance of the comet assay. *Mutagenesis* **2008**, *23* (3), 207–221.
(33) Roh, J. Y.; Lee, J.; Choi, J. Assessment of stress-related gene expression in the heavy metal-exposed nematode *Caenorhabditis elegans*: A potential biomarker for metal-induced toxicity monitoring and environmental risk assessment. *Environ. Toxicol. Chem.* **2006**, *25* (11), 2946–2956.
(34) Newman, M. C.; Unger, M. A. *Fundamentals of Ecotoxicology*, 3rd ed.; Lewis Publishers: Boca Raton, FL, 2003.
(35) Spurgeon, D. J.; Ricketts, H.; Svendsen, C.; Morgan, A. J.; Kille, P. Hierarchical responses of soil invertebrates (earthworms) to toxic metal stress. *Environ. Sci. Technol.* **2005**, *39* (14), 5327–5334.
(36) Spurgeon, D. J.; Morgan, A. J.; Kille, P. Current research in soil invertebrate ecotoxicogenomics. In *Comparative Toxicogenomics*; Hogstrand, C., Kille, P., Eds.; Elsevier:: Amsterdam, 2008.
(37) Van Straalen, N.; Roelofs, D. Genomics technology for assessing soil pollution. *J. Biol.* **2008**, *7* (6), 19.
(38) Shugart, L.; Theodorakis, C. New trends in biological monitoring: application of biomarkers to genetic ecotoxicology. *Biotherapy* **1998**, *11* (2−3), 119–127.
(39) Forbes, V. E.; Forbes, T. L. *Ecotoxicology in Theory and Practice*; Chapman & Hall: London, 1994.
(40) Kimball, K. D.; Levin, S. A. Limitations of laboratory bioassays - the need for ecosystem-level testing. *Bioscience* **1985**, *35* (3), 165–171.
(41) Forbes, V. E.; Calow, P. Species sensitivity distributions revisited: A critical appraisal. *Hum. Ecol. Risk Assess.* **2002**, *8* (3), 473–492.
(42) Suter, G. W. II; Barnthouse, L. W. *Ecological Risk Assessment*; Lewis Publishers: Boca Raton, FL, 1993.
(43) Forbes, V. E.; Calow, P. Is the per capita rate of increase a good measure of population-level effects in ecotoxicology. *Environ. Toxicol. Chem.* **1999**, *18* (7), 1544–1556.
(44) Forbes, V. E.; Calow, P. Population growth rate as a basis for ecological risk assessment of toxic chemicals. *Phil. Trans. R. Soc. London B Biol. Sci.* **2002**, *357* (1425), 1299–1306.
(45) Clements, W. H. Integrating effects of contaminants across levels of biological organization: an overview. *J. Aquat. Ecosyst. Stress Recov.* **2000**, *7* (2), 113–116.
(46) Magrini, K. D.; Basu, A.; Spotila, J. R.; Avery, H. W.; Bergman, L. W.; Hammond, R.; Anandan, S. DNA microarrays detect effects of soil contamination on *Arabidopsis thaliana* gene expression. *Environ. Toxicol. Chem.* **2008**, *27* (12), 2476–2487.
(47) Hook, S. E.; Skillman, A. D.; Small, J. A.; Schultz, I. R. Gene expression patterns in rainbow trout, *Oncorhynchus mykiss*, exposed to a suite of model toxicants. *Aquat. Toxicol.* **2006**, *77* (4), 372–385.
(48) Roelofs, D.; Marien, J.; Van Straalen, N. M. Differential gene expression profiles associated with heavy metal tolerance in the soil insect *Orchesella cincta*. *Insect Biochem. Mol. Biol.* **2007**, *37* (4), 287–295.
(49) Watanabe, H.; Takahashi, E.; Nakamura, Y.; Oda, S.; Tatarazako, N.; Iguchi, T. Development of a *Daphnia magna* DNA microarray for evaluating the toxicity of environmental chemicals. *Environ. Toxicol. Chem.* **2007**, *26* (4), 669–676.
(50) Woo, S.; Yum, S.; Park, H. S.; Lee, T. K.; Ryu, J. C. Effects of heavy metals on antioxidants and stress-responsive gene expression in Javanese medaka (*Oryzias javanicus*). *Comp. Biochem. Phys. C* **2009**, *149* (3), 289–299.
(51) Sheader, D. L.; Williams, T. D.; Lyons, B. P.; Chipman, J. K. Oxidative stress response of European flounder (*Platichthys flesus*) to cadmium determined by a custom cDNA microarray. *Mar. Environ. Res.* **2006**, *62* (1), 33–44.
(52) Soetaert, A.; Vandenbrouck, T.; Van der Ven, K.; Maras, M.; Van Remortel, P.; Blust, R.; De Coen, W. M. Molecular responses during cadmium-induced stress in *Daphnia magna*: Integration of differential gene expression with higher-level effects. *Aquat. Toxicol.* **2007**, *83* (3), 212–222.
(53) Connon, R.; Hooper, H. L.; Sibly, R. M.; Lim, F. L.; Heckmann, L. H.; Moore, D. J.; Watanabe, H.; Soetaert, A.; Cook, K.; Maund, S. J.; Hutchinson, T. H.; Moggs, J.; De Coen, W.; Iguchi, T.; Callaghan, A. Linking molecular and population stress responses in *Daphnia magna* exposed to cadmium. *Environ. Sci. Technol.* **2008**, *42* (6), 2181–2188.
(54) Roh, J. Y.; Sim, S. J.; Yi, J.; Park, K.; Chung, K. H.; Ryu, D. Y.; Choi, J. Ecotoxicity of silver nanoparticles on the soil nematode *Caenorhabditis elegans* using functional ecotoxicogenomics. *Environ. Sci. Technol.* **2009**, *43* (10), 3933–3940.
(55) Van Straalen, N. M.; Roelofs, T. F. M. *An Introduction to Ecological Genomics*; Oxford University Press: Oxford, 2006.
(56) Ankley, G. T.; Bencic, D. C.; Breen, M. S.; Collette, T. W.; Conolly, R. B.; Denslow, N. D.; Edwards, S. W.; Ekman, D. R.; Garcia-

Reyero, N.; Jensen, K. M.; Lazorchak, J. M.; Martinovic, D.; Miller, D. H.; Perkins, E. J.; Orlando, E. F.; Villeneuve, D. L.; Wang, R. L.; Watanabe, K. H. Endocrine disrupting chemicals in fish: Developing exposure indicators and predictive models of effects based on mechanism of action. *Aquat. Toxicol.* **2009**, *92* (3), 168–178.
(57) Calow, P. Physiological costs of combating chemical toxicants - ecological implications. *Comp. Biochem. Phys. C* **1991**, *100* (1−2), 3–6.
(58) Hendriks, A. J.; Maas-Diepeveen, J. L. M.; Heugens, E. H. W.; Van Straalen, N. M. Meta-analysis of intrinsic rates of increase and carrying capacity of populations affected by toxic and other stressors. *Environ. Toxicol. Chem.* **2005**, *24* (9), 2267–2277.
(59) Soetaert, A.; Moens, L. N.; Van der Ven, K.; Van Leemput, K.; Naudts, B.; Blust, R.; De Coen, W. M. Molecular impact of propiconazole on *Daphnia magna* using a reproduction-related cDNA array. *Comp. Biochem. Phys. C* **2006**, *142* (1−2), 66–76.
(60) Hoffmann, A. A.; Parsons, P. A. Selection for increased desiccation resistance in *Drosophila melanogaster* - additive genetic-control and correlated responses for other stresses. *Genetics* **1989**, *122* (4), 837–845.
(61) Kooijman, S. A. L. M.; Metz, J. A. J. On the dynamics of chemically stressed populations: The deduction of population consequences from effects on individuals. *Ecotoxicol. Environ. Saf.* **1984**, *8* (3), 254–274.
(62) Van Straalen, N. M.; Denneman, C. A. J. Ecotoxicological evaluation of soil quality criteria. *Ecotoxicol. Environ. Saf.* **1989**, *18* (3), 241–251.
(63) De Bruijn, J.; Crommentuijn, T.; Van Leeuwen, K.; Van der Plassche, E.; Sijm, D.; Van der Weiden, M. *Environmental Risk Limits in The Netherlands*; RIVM Report 601640001: Bilthoven, NL, 1999.
(64) Smit, M. G. D.; Bechmann, R. K.; Hendriks, A. J.; Skadsheim, A.; Larsen, B. K.; Baussant, T.; Bamber, S.; Sanni, S. Relating biomarkers to whole-organism effects using species sensitivity distributions: a pilot study for marine species exposed to oil. *Environ. Toxicol. Chem.* **2009**, *28* (5), 1104–1109.
(65) Reynders, H.; Van der Ven, K.; Moens, L. N.; Van Remortel, P.; De Coen, W. M.; Blust, R. Patterns of gene expression in carp liver after exposure to a mixture of waterborne and dietary cadmium using a custom-made microarray. *Aquat. Toxicol.* **2006**, *80* (2), 180–193.
(66) Galay-burgos, M.; Spurgeon, D. J.; Weeks, J. M.; Stürzenbaum, S. R.; Morgan, A. J.; Kille, P. Developing a new method for soil pollution monitoring using molecular genetic biomarkers. *Biomarkers* **2003**, *8* (3−4), 229–239.
(67) Lobenhofer, E. K.; Cui, X. G.; Bennett, L.; Cable, P. L.; Merrick, B. A.; Churchill, G. A.; Afshari, C. A. Exploration of low-dose estrogen effects: Identification of No Observed Transcriptional Effect Level (NOTEL). *Toxicol. Pathol.* **2004**, *32* (4), 482−492.
(68) Poynton, H. C.; Loguinov, A. V.; Varshavsky, J. R.; Chan, S.; Perkins, E. I.; Vulpe, C. D. Gene expression profiling in *Daphnia magna* part I: Concentration-dependent profiles provide support for the No Observed Transcriptional Effect Level. *Environ. Sci. Technol.* **2008**, *42* (16), 6250–6256.

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