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A New Transcriptional Effect Level Index (TELI) for Toxicogenomics-based Toxicity Assessment

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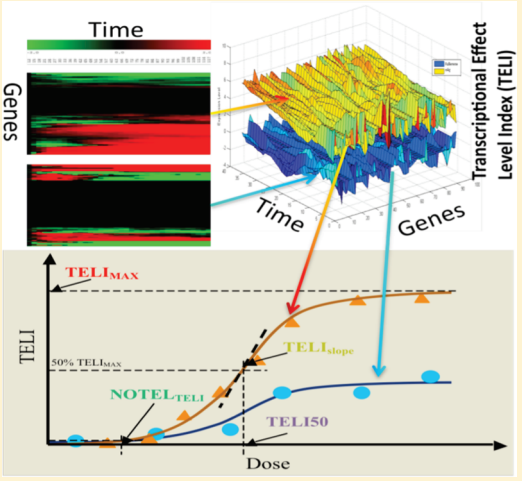
Supporting Information

ABSTRACT: This study proposes and demonstrates the potential application of a new Transcriptional Effect Level Index (TELI) to convert the information-rich toxicogenomic data into integrated and quantitative endpoints. A library of transcriptional fusions of green fluorescent protein (GFP) that includes different promoters for 91 stress-related genes in *E. coli* K12, MG1655 is employed to evaluate the gene expression alteration induced by exposure to four nanomaterials (NMs), nano silver (nAg), nano titanium dioxide anatase ($nTiO_2$_a), nano titanium dioxide rutile ($nTiO_2$_r), and fullerene soot. TELI is determined for each toxicogenomic assay, and it incorporates the number and identity of genes that had altered expression, the magnitude of alteration, and the temporal pattern of gene expression change in response to toxicant exposure. TELI values exhibit a characteristic "sigmoid" shaped toxicity dose−response curve, based on which $TELI_{MAX}$ (the maximal value of TELI), TELI50 (concentration that yields half of $TELI_{MAX}$), $NOTEL_{TELI}$ (TELI-based no observed transcriptional effect level), and $Slope_{TELI}$ (the slope of TELI-dose response curve) are obtained. TELI-based endpoints are compared to currently used endpoints such as EC50 and no observed transcriptional effect level (NOTEL). The agreement of $NOTEL_{TELI}$ and NOTEL values validates the concept and application of TELI. Multiple endpoints derived from TELI can describe the dose response behavior and characteristics more completely and holistically than single points such as NOTEL alone. TELI values determined for genes in each stress response category (e.g., oxidative stress, DNA repair) indicate mode of action (MOA)-related comparative transcriptional level toxicity among compounds, and it reveals detailed information of toxic response pathways such as different DNA damage and repair mechanisms among the NMs. This study presents a methodology for converting the rich toxicogenomic information into a readily usable and transferable format that can be potentially linked to regulation endpoints and incorporated into a decision-making framework.



INTRODUCTION

The concern of emerging contaminates, such as endocrine disrupting compounds (EDCs), pharmaceuticals and personal care products (PPCP), and nanomaterials (NMs), are anticipated to greatly increase the demands for their ecological effects and risk assessments, as indicated by various new toxicity assessment and regulation programs, such as the EPA Tox21 in U.S. and Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) program within the EU.[1−5] Presently, virtually all ecotoxicological tests rely on whole animal exposures with endpoints derived from adverse effects such as survival, growth, and reproduction (e.g., EPA Whole Effluent Toxicity (WET)).[6] These standard tests have been proved to be valuable and relatively efficient in risk assessments and regulatory decision-making. However, it is also recognized that these tests are resource- and time-intensive. Unless the approaches can be revised, the time required to handle the anticipated toxicity testing efforts for the large and ever-increasing number of emerging contaminants will be measured in decades.[7] In addition, there is also an evident growth in the complexity of testing with respect to the environmental sample matrix (e.g., effluents, sediments, synergistic effects of mixtures). Therefore, an urgent demand exists for less costly and more rapid, yet informative and reliable ecotoxicity screening and testing methods.

Recent advancements in the emerging field of toxicogenomics, which examines the global molecular-level activity in response to environmental stressors, provide a significant advance in toxicant evaluation and understanding toxicity mechanisms and pathways.[8−10] In addition to the better determination of Mode of Action (MOA), another most significant advantage of toxicogenomics is that it provides crucial and multiple information

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to help reducing the uncertainty in risk assessment of chemicals.[11] The cellular-level and sublethal impact observed may also be more indicative of chronic effect than phenotype endpoints. Furthermore, the toxicogenomic data can be potentially used to identify and classify compounds with similar MOAs and gain diagnostic insights into identifying of causal agents (e.g., comparing to "reference" toxicants with established MOAs). At last, incorporation of toxicogenomic-based screening assays into ecological risk assessment framework can help guide and optimize the resources and minimize the animal use.[11]

Applications of toxicogenomics for environmental toxicity assessment have been demonstrated. Poynton et al. showed that that gene expression analysis with *Daphnia magna* could predict environmental exposure to metals in effluents from two copper mines in California (e.g. Cu, Pb, and Zn).[12,13] Recently, our group showed that the toxicogenomics approach using recombinant whole-cell arrays could also be employed for toxicity evaluation and potential identification of pollutants.[14,15] Compared with traditionally microarray technology whose procedure includes multiple steps including RNA isolation, PCR amplification, labeling, and hybridization, our direct GFP signal detection method is simpler, faster and less costly, and therefore it is more feasible for high-throughput screening of a large number of chemicals. In addition, it provides multidimensional transcriptional level effect information, by adding a temporal dimension to the gene expression data and therefore can more accurately reflect the chemical-induced cell responses that are time-dependent.[14,15]

However, challenges remain on the application of toxicogenomics for ecological assessment and regulatory decision-making. One of the greatest initial challenges is how to convert the rich toxicogenomic information into readily usable and transferable format that can be potentially linked to regulation endpoints and incorporated into decision-making framework. NOTEL is the currently accepted endpoint that has been used with toxicogenomic data, and it indicates the threshold for the altered gene expression induced by a toxicant to be observed.[16] Although NOTEL indicates the relative toxicity level of a compound, it does not fully reflect the rich and specific toxicant-induced genomic information that can be obtained from toxicogenomic assays (e.g., MOA, response at higher concentration above NOTEL). Furthermore, there is no accepted quantitative toxicogenomic endpoint that incorporates the important factor---time-dependence of the genomic response.

In this study, we propose and validate a Transcriptional Effect Level index (TELI) that incorporates both the number and magnitude of genes with altered expression induced by chemicals as well as the temporal pattern of response. Dose−response curves of TELI as a function of concentrations are established, and the related toxicity endpoints derived from the TELI dose response curves are proposed. To validate the proposed quantification method, the derived TELI from toxicogenomic data are compared to those previously established endpoints including NOTEL, EC50 (half maximal effective concentration), and BOD (Biological Oxidative Damage).[17] The potential application and importance of the proposed quantitative ecotoxicogenomic endpoints are discussed. A number of nanomaterials are selected due to the current concern of their recognized as well as unknown environmental impact and health risks.[5,15]

MATERIAL AND METHODS

Nanomaterials Tested for Toxicity. Nano silver (nAg, ~60 nm, NanoDynamics Inc., Buffalo, NY, USA, 1424), nano titanium dioxide rutile ($nTiO_2$_r, ~10 nm thick, 40 nm laterally, Sigma-Aldrich, 10024JH), nano titanium dioxide anatase ($nTiO_2$_a, ~10 nm, NanoStructured & Amorphous Materials, Houston, Texas, USA, 5425HT), and fullerene soot (M.E.R.Co, Tuscon, AZ, USA) were prepared in M9 medium (minimum medium for bacteria culture) for a stock concentration of 1 mg/mL, which contains 1% of crude Bovine Serum Albumin (BSA) (ACROS, NJ, USA) as a dispersant. The stock solutions are sonicated in a High energy Cup-sonicator, at ~90 W power for at least 15 min to maintain a better dispersion before the toxicity assays. Detailed physical and chemical characterization of these same nanomaterials used are described by Bello et al.[17] and in STable 1.

Measuring the Temporal Gene Transcriptional Activity in E. coli. upon NMs Exposure. A library of transcriptional fusions of green fluorescent protein (GFP) that include different promoters for 91 stress-related genes in *E. coli* K12, MG1655 is employed in this study, and the detailed information for the library construction and library validation can be found in previous reports.[18,19] The selected stress genes and their main functions are described elsewhere and given in STable 2. In this library, each promoter fusion is expressed from a low-copy plasmid, pUA66 or pUA139 that contains a kanamycin resistance gene and a fast folding gfpmut2 gene, therefore allows for continuous and real time measurements of the promoter activities. The stability of plasmid and the identity of the promoter regions were verified by the previous report.[18]

To measure the transcriptional level effect induced by the toxicant, bacteria are grown in black 96-well plates with clear bottom (Costar, Bethesda, MD, USA) for 2 h at 37 °C until the cultures reached early exponential growth in M9 media (OD600 about 0.1). Duplicate tests for each treatment were performed. NM stock solution is added into the microplate well for the targeted concentrations. Then the plate is put in a Microplate Reader (SynergyTM Multi-Mode, Biotech, Winooski, VT, USA) for simultaneous absorbance (OD600) measurement (cell growth) and fluorescent readings (GFP level, EX 485 nm, EM 528 nm) at a time interval of 3 min for 2 h. We chose 2 h exposure time for this study because our intention was to develop a relatively fast toxicity assessment and screening methods for evaluating a large number of contaminants.

Data Processing and Analysis. All data are corrected for various controls, including blank with medium control (with and without NMs) and promterless bacterial controls (with and without NMs). The alteration in gene expression, also called induction factor I (I = Pe/Pc), for a given gene at each time point due to chemical exposure, is represented by the ratio of the normalized gene expression GFP level ($Pe = (GFP/OD)_{experiment}$) in the experiments condition with NMs exposure to that ($Pc = (GFP/OD)_{control}$) in the control condition without any NMs exposure. Then the natural log of I value ($Ln(I)$) at every time point is compiled for further analysis. For down-regulated genes, absolute $Ln(I)$ values $[Ln(I)]_{abs}$ are applied to convert all altered transcriptional effect level to positive values. A conservative cutoff background noise threshold value of 0.4 ($[Ln(I)]_{abs} = 0.4$) is chosen based on previous reproducibility and control tests.[14]

Concept and the Determination of TELI. To quantify the transcriptional effect level induced by a given toxicant using the toxicogenomics data, we developed a Transcriptional Effect Level index (TELI) to convert the information-rich toxicogenomic data into an integrated and quantitative endpoint. The TELI considers and incorporates three factors: (1) the number and identity of genes that had toxicant-induced expression change, (2) the

magnitude of altered gene expression for each gene response to the toxicant exposure, and (3) most importantly, the time factor, namely, the temporal pattern of gene expression change. Figure 1 illustrates the conceptual construction and determination of TELI for each toxicity assessment. Figure 1(A) shows an exemplary temporal pattern of altered gene expression (transcriptional effect) level for a given gene (*fpr*), and we have shown that the temporal pattern varies for different genes.[15] For TELI determination, in order to quantify and compare the level of differentially expressed genes, we used absolute change folds values. For example, up-regulation 2 folds ($\mathrm{Ln}(I) = 0.69$) will be considered as the same magnitude of change for down-regulation of 50% ($\mathrm{Ln}(I) = -0.69$, $[\mathrm{Ln}(I)]_{\mathrm{abs}} = 0.69$). Control gene expression level ($I = 1$) is subtracted from each data point. Then, the accumulative transcriptional effect of a given gene over a 2 h exposure period is determined as the area (in blue color) defined by the curve over the *X*-axis as shown in Figure 1(A), which is calculated using the following equation

$$\mathrm{TELI}_{(genei)} = \frac{\int_{t=0}^{t} (e^{|\mathrm{ln}(\mathrm{I})|} - e^{|\mathrm{ln}(1)|})dt}{\text{Exposure Time}} \qquad \text{(Equation 1)}$$

Where, *t* is exposure time in h, and *I* was defined previously. To determine the TELI that reflects the overall transcriptional level response of the cell in exposure to the toxicant for a given time period, and over a range of genes (indicative of global response for most genes in the genome or, for any specific functional categories, e.g. stress genes in our library), the overall 3-D accumulative transcriptional effect levels for all the genes of interest is integrated as the volume of the "mountain peaks" as shown in Figure 1(B), which is calculated as the following equation

$$\mathrm{TELI}_{(total)} = \sum_{gene(i=1)}^{genei(i=n)} (\mathrm{TELI}_{genei}) \qquad \text{(Equation 2)}$$

Where, *i* is the number of genes in the assay library.

Determination of EC50 and NOTEL. The conventional 24 h EC50 values for the three NMs selected are determined using the same *E. coli* strain based on growth rate inhibition. *E. coli* K12 were incubated at 37 °C for about 2 h to obtain OD of about 0.1 before the NMs stock solution was added to obtain various NMs concentrations on microplates. Duplicates were performed for each concentration. After 24 h incubation, both colony-forming unit (CFU) counting and absorbance (600 nm) measurements were conducted to determine the extent of cell growth inhibition at various NM concentrations. EC50 was then calculated based on the dose−response curve of growth inhibition (%) using four-parameter logistic nonlinear regression model equation (GraphPad PRISM 5, La Jolla, CA 92037).

NOTEL has been proposed and applied for toxicogenomics-based toxicity assessment.[12,15] We apply the concept of NOTEL as the maximal concentration of a chemical at which less than 5% of the genes in the library are differentially expressed upon chemical exposure compared to control. The NOTEL is also determined by a dose−response curve of the percentage of genes that had altered expression in our "stress library" versus the dose concentration of chemicals, using the four-parameter logistic nonlinear regression model equation as described above.

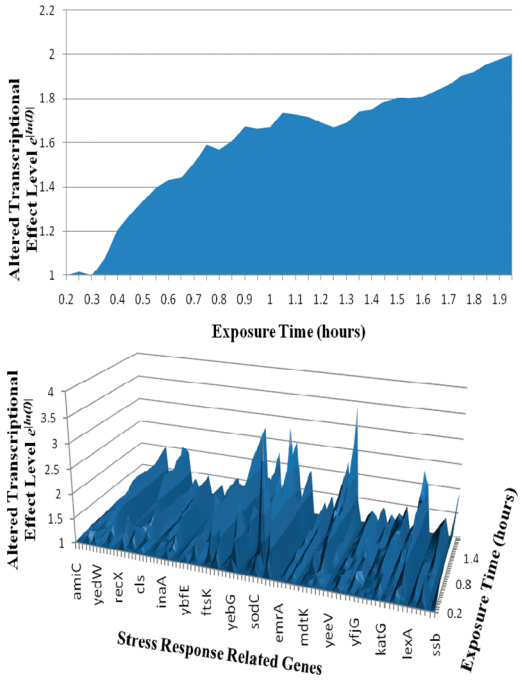

Figure 1. Determination of Transcriptional Effect Level Index (TELI) as a quantitative endpoint for toxicogenomics-based toxicity assay. (Top): Exemplary temporal altered transcriptional effect level for a given gene (*fpr*) over an exposure time of 2 hours to nAg (10 mg/L). Accumulative magnitude of transcriptional effect level for a given gene over a certain exposure time length is calculated as the integration of the altered gene expression level over time, indicated as the highlighted area. Y-axis: absolute altered transcriptional effect level, $\exp([\mathrm{Ln}(I)]_{\mathrm{abs}})$, a transformed value from induction factor I, which is calculated as the ratio of gene expression level in the experiment with toxicant exposure to that of the control without any toxicant exposure. (Bottom): 3-D altered transcriptional effect level profile compiled from the altered gene expression levels for all the genes in the test library. The TELI value is determined as the sum of accumulative transcriptional effect level (determined as shown in Figure 1A) for all the genes in the stress genes library (STable 2) used for the toxicogenomic assay. Note that only selected genes are shown in the X-axis.

RESULTS AND DISCUSSION

TELI as a Quantitative Toxicogenomic-Based Toxicity Assessment EndPoint. Three-dimensional real-time gene expression profiles, depicted as 3-D topography, are obtained for four nanomaterials (NMs), including nanosilver (nAg), nanotitanium dioxide anatase ($\mathrm{nTiO_2\_a}$), nanotitanium dioxide rutile ($\mathrm{nTiO_2\_r}$), and fullerene, and they are shown in SFigure 1. The 3-D altered gene expression profiles or fingerprints are distinctive for the four NMs evaluated, suggesting compound-specific cellular responses likely resulted from their different MOAs. TELI translates the transcriptional level effect of an organism in response to a toxicant into a quantitative endpoint value. TELI value is determined for each toxicogenomic-based toxicity assay upon NMs exposure, and its value allows for quantitative comparison of transcriptional-level effect induced by various NMs at different concentrations.

A currently used ecotoxicogenomics endpoint, namely NOTEL, is quantified based on gene expression data at one chosen time point.[11] It has been recognized that the cellular responses, measured as gene expression profiling, are dynamic over time.[20,21]

Although NOTEL has been applied and shown to be a useful indicator for quantifying the transcriptional effect level induced by a toxicant, it is evident that the NOTEL value dose not reflect other important dimensions of the toxicogenomic data such as the magnitude of differentially expressed gene levels for specific genes and the variation in response profile depending on the exposure time. The proposed TELI can incorporate all these factors and therefore is expected to quantify the transcriptional effect at higher resolution and with better accuracy.

It is important to note that our toxicogenomic-based toxicity assays record the transcriptional level response of the *E. coli* cells upon the exposure of various NMs at the concentrations much lower than that would lead to any noticeable inhibition effect (the maximum concentration examined caused less than 5% growth inhibition compared to control, data not shown). It reflects more subtle cellular response at doses far below those used in conventional toxicity assay that can lead to observable phenotype change such as growth inhibition or death. Therefore, it is conceivable that the TELI values likely entail more potential long-term impact of a toxicant on the cell, and the TELI can be obtained from short-term assay within 2 hours.

Dose−Response Curve Based on TELI and Determination of Toxicity endpoints. The TELI values exhibit concentration-dependent pattern and allows for the establishment of dose−response curve, as shown in Figure 2 and SFigure 2. The TELI-dose response curves are analyzed with a Four-parameter Logistic eq (4PL) model, and they exhibit a characteristic "sigmoid" shaped toxicity dose−response curve, indicating that TELI is a suitable response quantifying parameter. Note that the best fitting curves did not pass theoretical zero points for the NMs tested. The reason is likely that NMs alone may have background physical interference of the GFP reading, which cannot be quantified separately, and it gives a background reading slightly higher than theoretical zero (control with zero dose of NMs).

In traditional toxicity assays, the phenotype endpoints (e.g., growth inhibition, death) are clearly defined, and toxic response is normalized as a percentage of the maximal effect (e.g., 100% death). With toxicogenomic tests, we observe that there seemed to be a maximal TELI value that can be determined based on the TELI-dose response curve using 4PL model fitting, and it varies for different toxicants (Figure 2 (A)). It is recognized that cellular stress responses assist in maintaining homeostasis; however, they are also toxicity pathways in that they lead to adverse effects when stress is sufficiently high. It is therefore, reasonable to think that there may be a threshold condition when the transcriptional level effect, including both direct and compensatory effects here in our study, is at its maximal and beyond which cell damages start to occur and eventually progresses to observable phenotype damage endpoint such as growth inhibition or even death. This threshold corresponds to and can be indicated by the maximal value of TELI ($TELI_{MAX}$). The $TELI_{MAX}$ values for the four NMs are determined using model fitting of dose-response curves as shown in Figure 2(A). The $TELI_{MAX}$, referred as the maximal efficacy, reflects the limit of the dose−response relationship on the response axis to a certain chemical. It quantifies the maximal transcriptional level effect for a group of given gene that can be induced in a 2 h exposure.

Based on traditional toxicology, in which quantitative toxicity endpoints such as EC50, LC50 are determined as the concentration that leads to 50% of the maximal adverse effect, we apply the similar approach for determining TELI50. TELI values are normalized to $TELI_{MAX}$ determined as described above, and

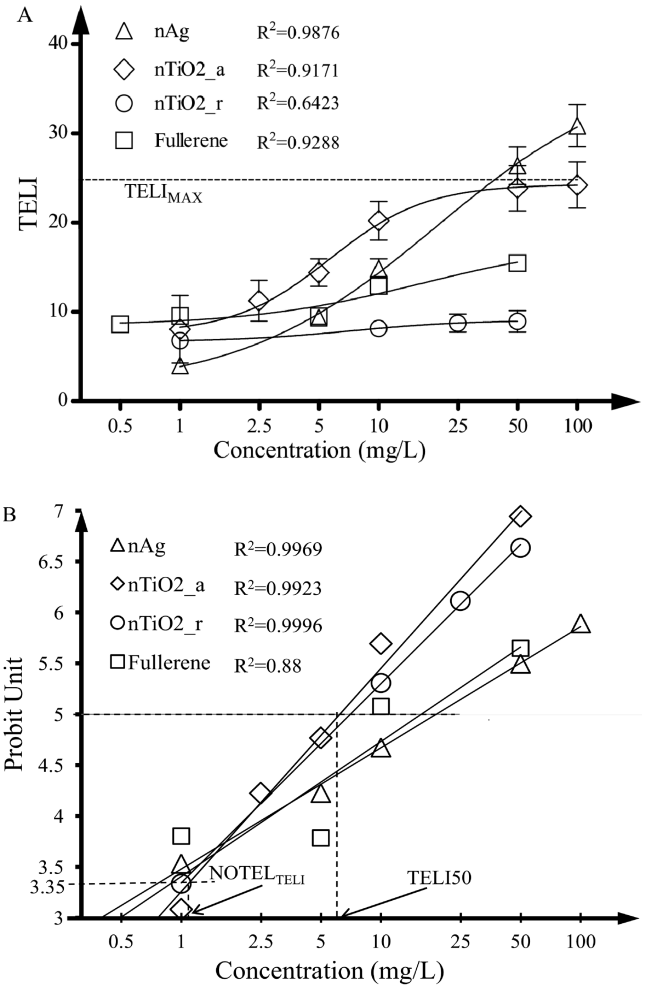

Figure 2. Dose response curves based on TELI values versus dose concentration for nAg, nTiO2_a, nTiO2_r, and fullerene, fitted by the Four-parameter Logistic Equation. Data points with an error bar represent the TELI value determined at each dose concentration. (A) TELI versus dose concentration for the NMs studied. $TELI_{MAX}$, the maximum value of TELI for each toxicant, is determined through model fitting. (B) Transformed dose response curve based on probability units. TELI values are normalized to the $TELI_{MAX}$. TELI50, $NOTEL_{TELI}$, and $Slope_{TELI}$ are determined via a linear fitting of *probit unit* versus dose concentration. As an example, the determinations of $TELI_{MAX}$, TELI50 and $NOTEL_{TELI}$ values for nTiO2_a are illustrated in the Figure.

the percent TELI response can be exhibited as *probit units* (*probability unit*) as the dose−response phenomena are usually normally distributed.[22] TELI50 is found via a linear fitting of *probit unit* verse dose, as shown in Figure 2(B), when *probit unit* equals to 5 (corresponds to 50% $TELI_{MAX}$ response). We also calculate TELI-based NOTEL, namely $NOTEL_{TELI}$, as the concentration at which *probit* unit equals to 3.355 (corresponds to 5% $TELI_{MAX}$ response). Slope of fitted line ($Slope_{TELI}$) is another endpoint since it reflects the rate at which the transcriptional effect increases with the increasing of concentration. The four endpoints defined, namely, $TELI_{MAX}$, TELI50, $NOTEL_{TELI}$, and $Slope_{TELI}$, together describe the characteristics of the transcriptional level response as a function of dose for a given NM.

Table 1 summarizes the $TELI_{MAX}$, TELI50, $NOTEL_{TELI}$, and $Slope_{TELI}$ values determined based on the TELI-dose response relationships for nAg, $nTiO_2$_a, $nTiO_2$_r, and fullerene, as

Table 1. Summary of TELI-Based Toxicity Characterization Parameters and EndPoints[a]

	$TELI_{MAX}$	$Slope_{TELI}$	TELI50 (mg/L)	$NOTEL_{TELI}$ (mg/L)	NOTEL (mg/L)	EC50 (mg/L)	BOD (μmol/L)
nAg	41.72 (±8.57)	1.19 (±0.34)	18.92 (±1.63)	0.79 (±2.51)	1.08 (±1.23)	67.19 (±9.48)	116.4
nTiO2_a	26.98 (±1.48)	2.19 (±0.87)	7.02 (±1.30)	1.01 (±2.51)	1.01 (±1.32)	74.14 (±8.13)	64.9
nTiO2_r	10.08 (±3.56)	1.96 (±1.04)	6.23 (±5.19)	1.11 (±6.31)	1.17 (±2.45)	177.91 (±19.87)	12.6
fullerene	19.88 (±3.23)	1.33 (±0.33)	15.93 (±2.52)	0.92 (±2.52)	1.06 (±1.37)	87.22 (±12.31)	62.1

[a] Numbers in parentheses are the standard error.

shown in Figure 2. The $TELI_{MAX}$ value is the highest for nAg and lowest for $nTiO_2$-r, indicating a relatively higher transcriptional level effect induced by nAg than other three NMs to these stress genes. TELI50 and $NOTEL_{TELI}$ can be referred to as "potency", which refers to the dose required to produce a given response intensity. The $NOTEL_{TELI}$ values are in agreement with the conventional NOTEL values for the four NMs. Since both $NOTEL_{TELI}$ and NOTEL represent the same toxicant level at which no affect can be observed on the transcriptional level, the agreement of $NOTEL_{TELI}$ and NOTEL values is expected, and it validates the concept and application of proposed $NOTEL_{TELI}$ based on TELI. The difference in the dose response curve $Slope_{TELI}$ suggests that, for these four NMs, the transcriptional effects are induced at different increasing rates with an increase in dose.

For comparison of relative toxicity among toxicants based on transcriptional effect levels, employment of multiple endpoints is necessary to describe the dose response behavior and characteristics more completely and holistically. For example, although the NOTEL and $NOTEL_{TELI}$ values for TiO_2_r and TiO_2_a are similar with consideration of the variance of the data (Table 1), the dose−response curves for these two NMs are quite different as shown Figure 2, which can only be accurately described by using the multiple parameters, namely $NOTEL_{TELI}$, TELI50, $Slope_{TELI}$, and $TELI_{MAX}$, simultaneously. This is consistent with traditional toxicology that more than one endpoint of a dose−response curve should be considered to indicate the characteristic of the toxic response over the exposure of a toxicant.[23] Higher $TELI_{MAX}$ and EC50 values for TiO_2_a (Table 1) suggested higher toxicity level of this form of titanium nanomaterial than TiO_2_r. The denser arrangement of atoms and higher stability of TiO_2_r may explain why apparent lower toxicity of TiO_2_r compared to TiO_2_a have been observed (at transcriptional effect level), especially in nanosize.[24,25]

The Correlation between TELI and Other Established Toxicity endpoints. Another historical impediment to the use of toxicogenomic information for regulatory decision making has been the lack of consented approach and inability to link responses at lower levels of biological organization to adverse outcomes in the whole animal, so-called "phenotypic anchoring".[11] To probe the linkage between our proposed toxicity assessment index-TELI with apical endpoints, we conduct conventional toxicity assay to determine the EC50 and NOTEL for all the NMs and then compared the TELI endpoints with these conventional toxicity endpoints. Table 1 compares the TELI50 values with EC50, NOTEL, and BOD values for the NMs studied. BOD quantifies the oxidative damage potential, measured by a "ferric reducing ability of serum (FRAS)" assay in human blood serum as the difference of total antioxidant capacity between unexposed and NM exposed serum, and was proposed as indicator for quantifying oxidative damage-based nanotoxicity in eukaryotic cells.[17] BOD for each of the NMs is measured with 10 mg NM/mL serum at 37 °C after 90 min mixing and reported as trolox equivalent unit.[17] Table 2 shows the correlations coefficients (both pearson product-moment correlation coefficients and spearman's rank-order correlation coefficients) between the TELI-based toxicity endpoints with other accepted toxicity end points.[26] The $NOTEL_{TELI}$ values for the four NMs do not show significant difference ($p > 0.28$), indicating either there are similar levels of dose threshold to produce detectable transcriptional effect among the NMs studied or NOTEL may not be sensitive enough for differentiating the toxicity level among NMs used in this study, in comparison to other endpoint indicators such as EC50 or $TELI_{MAX}$ for instance. However, the resolution may potentially be improved by extending the exposure time or enlarging the gene library size.[16]

Table 2. Correlation Coefficients between TELI-Based Toxicity Endpoints with Other Toxicity EndPoints[a]

Correlation coefficient	$TELI_{MAX}$	$Slope_{TELI}$	TELI50	$NOTEL_{TELI}$
NOTEL (mg/L)	−0.51	−0.04	−0.20	0.40
	(−0.4)	(−0.4)	(−0.2)	(0.2)
EC50 (mg/L)	−0.82	0.39	−0.61	0.78
	(−1.0)	(0.4)	(−0.8)	(0.8)
BOD (μmol/L)	0.98	−0.63	0.80	−0.95
	(1.0)	(−0.4)	(0.8)	(−0.8)

[a] The values shown are pearson product-moment correlation coefficients, the values inside the parentheses are spearman's rank-order correlation coefficients.

The $TELI_{MAX}$ values among the NMs seem to be correlated relatively well with the EC50 ($r = -1$, for Spearman's rank-order correlation coefficients, Table 1). EC50 represents the phenotype changes such as cell growth being inhibited, which can only be observed at higher dose concentrations than those applied in our TELI assays.[27] As discussed previously, $TELI_{MAX}$ is assumed to indicate the threshold beyond which the phenotype damage may occur, which is, therefore, very close to the concentration that may cause phenotype damage. This correlation implies that "phenotype anchoring" is possible with our proposed $TELI_{MAX}$, therefore allowing for the linking of phenotype changes with cellular response.

The relative toxicity order of the four NMs based on values of $TELI_{MAX}$ also agreed well with that suggested by the BOD values, as demonstrated by the high correlation coefficient of $TELI_{MAX}$ with a BOD value ($r = 1.0$ with $p = 0.042$ for spearman's rank-order correlation coefficient; $r = 0.98$ with $p = 0.023$ for pearson product-moment correlation coefficients), respectively. This is consistent with the current understanding that oxidative damage, as resulted by the observed oxidative stress and Reactive Oxidative Species (ROS) production by these NMs, has been found to be the dominant toxic mechanism for these NMs.[28,29]

The toxicity order among the NMs revealed by the TELI50 seemed to be different from that indicated by the EC50 and BOD.

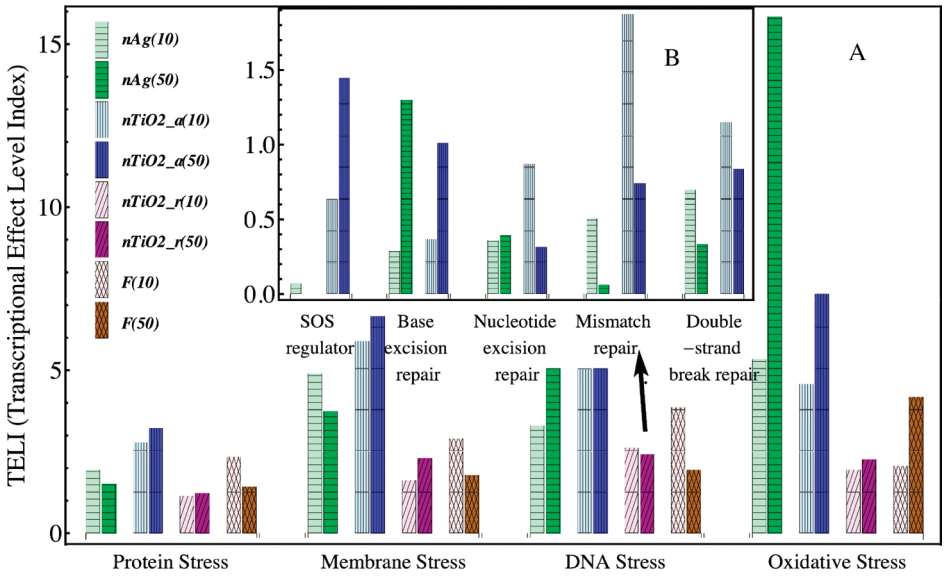

Figure 3. Comparison of TELI values for different stress functional categories among the four NMs tested. (A) TELI values determined for different functional stress gene categories, for toxicity assays of four NMs at two different representative concentrations. (B) TELI values determined for further division of DNA-stress related genes indicative of different DNA damage and repair pathways upon the exposure to nAg and nTiO2_a, respectively. (The number 10 and 50 indicate the exposure concentration of 10 mg/L and 50 mg/L, respectively.)

This is because the maximum transcriptional effect ($TELI_{MAX}$ value) varies with a different toxicant (Figure 2), whereas the maximal phenotype effect (such as death) in conventional toxicity tests is the same for all chemicals. Therefore, TELI50 here cannot be employed for direct comparison of relative "potency" or toxicity level among chemicals as EC50 implies. The $Slope_{TELI}$, determined as the shape of dose−response curve, is important in defining the shape of TELI-dose response curve for indicating the rate of relative percentage change in the response intensity (in relative to the maximum TELI) per unit change in dose. For example, comparing to $nTiO_2$, nAg and fullerene exhibited relatively less steep dose−response curves, indicating a slower relative increase in transcriptional effect while dose concentration increases. These results imply that the comparative toxic effect at the transcriptional level, exhibited at the concentration much below those that cause phenotype response and damage, may not be evaluated the same way as that at the phenotypic response level.

Therapeutic index (TI), usually used in pharmacology, exhibits the safety information of substances. Therapeutic index is a comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes drug toxicity. In traditional medical toxicology, TI is determined as the toxic effect of 50% of the population (TD50) divided by the minimum effective dose for 50% population (ED50).[22] We explore the possibility to apply the concept of TI to evaluate the ratio of dose that causes phonotype damage to that cause transcriptional effect. In this case, we define Pheno-to-Transcriptional damage Index (PTI) as the EC50 of growth inhibition divided by TELI50 to indicate the potential effect of NMs. The PTI determined for nAg, $nTiO_2$_a, $nTiO_2$_r, and fullerene are 3.92, 10.56, 28.41, and 5.46, respectively. In similar analogy to TI, lower PTI for nAg and fullerene suggest that the dose that leads to phenotype damage (growth inhibition) is not much higher than that cause observable transcriptional effect. PTI can potentially serve as another index for characterizing toxicity of environmental pollutants.

TELI for Effects Associated with Different Stress Functional Categories. The 91 stress genes in *E. coli* used in our library have been categorized into several functional groups based on their main function and involvement in different stress mechanisms, such as DNA stress (SOS response), protein stress, membrane damage (lipid stress), and oxidative stress (including ROS degradation and redox balancing) (shown in STable 2). Based on the definition and principle of TELI determination, it is reasonable to calculate TELI values for genes in each stress response category to quantify the corresponding transcriptional level response for each stress category. TELI indicates transcriptional effect level based on relative temporal altered gene expression levels for the selected genes in the assay, assuming equal weight for all genes. The TELI of each functional group were calculated and shown in Figure 3 (A).

The lowest TELI of $nTiO_2$_r of all the stress categories indicates the lowest toxicity among the NMs examined, as discussed in earlier sections. nAg has the highest TELI for genes involved in oxidative stress, indicating main toxicity mechanism as oxidative damage, which is consistent with previous reports.[30−32] Oxidative damage induced those genes related to ROS degradation and redox balancing. The key regulator *oxyR*, which serves as the transcriptional dual regulator to those involved in peroxide metabolism, peroxide protection, and redox balance,[33] was upregulated at an increasing level over time upon exposure to nAg (data not shown). Both nAg and $nTiO_2$_a exposures led to observable DNA stress and membrane damage. It is understood that oxidative stress and ROS production can lead to damage of DNA, RNA, proteins, and lipids.[34]

Not only TELI values can be determined for genes belonging to different functional categories, indicating MOA-related comparative transcriptional level toxicity among compounds as shown in Figure 3(A), TELI values can also be obtained for more in-depth pathways to reveal toxicity details. Figure 3(B) shows the TELI values specific for those genes that are indicative of specific DNA damage and repair pathways. Note that the genes that are shared by many DNA damage and repair pathways, such as DNA polymerase (polB, dinB) and helicase (dinG), are not included. As shown in Figure 3(B), although both nAg

and $nTiO_2$_a seem to cause DNA stress, they lead to different DNA damage type and repair pathways. In prokaryotic cells, DNA damage leads to activation of SOS DNA damge and repair regulation system, which is regulated by two SOS regulators, namely *recA* and *lexA*. The activation of the SOS system is a strong function of ssDNA length, for a minimum site size of 30−50 bases.[35] Some DNA damage type may not produce many ssDNA, such as base damage.[36] Comparing the two NMs that cause DNA stress, including nAg and $nTiO_2$_a, nAg did not seem to activate the SOS system; however, $nTiO_2$_a led to clear activation of SOS (as show in Figure 3(B)).

In summary, this study proposes and demonstrates the potential application of a new Transcriptional Effect Level index (TELI) to convert the information-rich toxicogenomic data into an integrated and quantitative endpoint. The TELI values exhibit concentration-dependent pattern and allow for the establishment of dose−response curve. Multiple transcriptional effect level based endpoints, describing the holistic toxic response characteristics, can therefore be determined. The validity of the toxicity assessment index, TELI, was demonstrated through comparison to other conventional or previously reported endpoints. Continuous advances in genomic technologies and in the toxicogenomics field will pose it as a promising and advantageous approach for mechanistic toxicity evaluation of pollutants. Methodologies for converting the rich toxicogenomic information into readily usable and transferable format that can be potentially linked to regulation endpoints and incorporated into decision-making framework is in great need and our proposes TELI may serve as such an effective index. Although we applied gene expression data in this study, the principle and concept of TELI can be potentially employed by proteomics, metabolomics, and others that examine global response of cell to toxicants.

■ ASSOCIATED CONTENT

Supporting Information. STables 1 and 2 and SFigures 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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