Review of the NTP Biomolecular Screening Branch

National Toxicology Program

Board of Scientific Counselors

Meeting Report November 30 – December 1, 2010

Introduction: The Board of Scientific Counselors (BSC) of the National Toxicology Program (NTP) conducted an intensive review of the Biomolecular Screening Branch (BSB) and Tox21 Initiative, with substantial effort, interest, and intensity. The Tox21 Initiative, a federal partnership transforming toxicology, was developed over the period 2005-2007 and encompassed the NTP Vision for the 21st Century and the National Academy of Sciences (NAS) report *Toxicology Testing in the 21st Century: A Vision and A Strategy*. The NTP Vision for the 21st Century focused on "supporting the evolution of toxicology from a predominantly observational science at the level of disease specific models to a predominantly predictive science focused upon a broad inclusion of target specific, mechanism-based biological observations." To implement this vision, the NTP developed a roadmap that included an initiative to develop a high throughput screening (HTS) program with three primary goals: (1) identification of the mechanism(s) of action (e.g., disease associated pathways) for further investigation, (2) prioritization of compounds for further in-depth toxicologic evaluation, and (3) development of predictive models for examination of *in vivo* biological response. The 2007 NAS report envisioned that routine toxicity testing would be accomplished in human cells or cell lines by monitoring chemical-mediated alterations in cellular responses in a battery of toxicity pathway assays using high throughput robotic-assisted methodologies. The strategy envisioned in the NAS report employed (1) development of a suite of *in vitro* tests employing human cells, cell lines, or cellular components; (2) targeted animal tests to complement the tests *in vitro*; (3) computational models of toxicity pathways to support application of *in vitro* test results in risk assessments; (4) infrastructure changes to support basic and applied research required for development of tests and pathway models; (5) validation of tests and strategies, and (6) evidence to justify the toxicity pathway approach as predictive of adverse health outcomes for use in decision-making.

Clearly, there was a convergence of philosophies in both strategic goals. The Tox 21 initiative thus represents a logical progression in the approach to accomplishing the identified strategies and the overall goals.

To implement this vision and strategy, a consortium of participants consisting of the NIEHS/NTP, the NIH Chemical Genomics Center (NCGC), the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) was constructed. The overall design and concept of this approach was published (FS Collins, GM Gray, JR Bucher, *Transforming Environmental Health Protection*, Science 319, 906, 2008 Toxicology Policy Forum). A number of key provisions were developed among the collaborative agencies, one of which was to build on existing and complementary expertise and overcome the resource limitations of a single agency. The development

of this consortium of agencies represented a significant step toward achieving the goals of the program. The overall goals of the Tox21 program are:

(1) to research, develop, validate and translate innovative testing methods for characterizing the toxicity pathways of compounds;
(2) to identify compounds, assays, informatic tools and targeted testing required for the innovative testing methods;
(3) to prioritize chemicals for use in more extensive toxicologic examination;
(4) to identify the mechanism(s) associated with chemical-induced alterations in biologic activity in order to characterize the pathways, explore cross-species extrapolation, and examine models for low-dose extrapolation; and,
(5) to achieve the penultimate goal of developing models for prediction of biological responses in humans.

The BSC was tasked with the responsibility for review of the Biomolecular Screening Branch and Tox21 Initiative. In general, the efforts of the Biomolecular Screening Branch and the overall design and implementation of the Tox21 Initiative engendered considerable enthusiasm and support among the members of the BSC with the consensus that this approach offered the greatest potential for ultimate application to predictive human toxicology and risk assessment. While some concerns were identified, this should not be surprising given the breadth and scope of the Tox21 initiative. The discussions of the BSC, while highlighting some concerns, nonetheless reflected considerable enthusiasm and an overwhelming desire for success in this initiative.

Overview of the BSB and Tox21 Initiative
Reviewers: Gina Solomon (lead), Miguel Fernandez, Dana Loomis

Clarity and the public health relevance of the goals of the Tox21 program

The Tox21 program represents an exciting and valuable step forward with considerable potential to improve protection of public health and to advance science. The overall goals of Tox21 are clear, exciting, and forward-looking. The public health relevance is an area that needs more attention, both to assure that the program will remain grounded in human health and in terms of communicating about the program to the public, so that the public understands what is being done in the program and its relevance to their health.

The science has been developing rapidly in this area, and in related fields. Newer “high-content” assays have the potential for delivering thousands of endpoints at once. It would be appropriate to consider whether to add an element to the Tox21 effort that is focused on developing and testing multiplex assays. The difficulty of assaying volatile chemicals in the Tox21 assays was also flagged as a concern, especially since many chemicals of significant public health importance are volatile.

The greatest practical benefits of the new approach will be realized through the ability to screen far more compounds than ever before. Real world epidemiological and exposure data from human populations will provide an essential complement to the rapid assays under development, and should be an integral part of the program (for example, exposure is depicted as a future addition to ToxPi).

Adequacy of the collective scientific and technical capabilities of the participating agencies to achieve the goals of Tox21

The Tox21 collaboration is very strong and contains most of the key partners. However, there was a strong consensus of the reviewers that human data—especially human exposure assessment and epidemiology—needs to be better integrated into the program. There were recommendations to engage the Centers for Disease Control and Prevention (CDC), specifically NIOSH, NCEH, and ATSDR, in the project. These entities have access to highly exposed populations (workers and affected communities) and these partners could provide important information about exposure and priority-setting, and also could potentially facilitate molecular epidemiology studies to assess toxicity pathway perturbations in human populations exposed to chemicals of concern. Although human exposure and epidemiological studies are slow and expensive, they still need to be done, and resources should be directed toward those endeavors.

Suggestions where increased scientific emphasis or resources could be most beneficial:

- More emphasis on 'omic' assays and other multiplex, high-content assays may be useful over the coming years. In particular, it would be worth considering the use of genome-wide association studies (GWAS) as a tool to predict the effects of chemicals.
- Established observational science - epidemiology and exposure assessment, in particular - should not be overlooked in the rush to apply promising new technologies. The Tox21 program should seek to be more grounded in human data and epidemiologic studies – for purposes of priority-setting and exposure/dosing considerations, to incorporate strategies for evaluating perturbations of key pathways in exposed worker groups, and also for reviewing the molecular epidemiology literature to assure that the program includes assays for all of the important toxicity pathways that have been identified in molecular epidemiology studies.
- It will be interesting and important to begin to use the Tox21 tools to begin to assess mixtures and interactive effects. For example, one reviewer suggested looking at how ingested chemicals may interact with the human microbiome to affect their toxicity.
- There appears to be a need to review the enormous flood of data that has emerged from this effort to assure that it is providing a set of information that is useful for prioritization, and for screening-level risk assessments.

Tox21 Working Groups
Reviewers: William Janzen (lead), Lisa Minor, Ruthann Rudel, James Sherley, Justin Teeguarden.

The BSC was very impressed by the accomplishments of the BSB and remains very supportive of the work. This is a major step forward in the field of toxicology and the data being gathered in the Tox21 project MAY (if they set up a program to actually test the hypothesis in the next 10 years) be critical to demonstrating predictive toxicology. There should be every expectation that this project will lead to major breakthroughs.

The teams appear to be working well together and have assembled impressive teams of experts. There is concern about the accountability for decision-making. This is true at all levels of the BSB since all the teams are cross-functional and cross-agency. Each working group needs to have clearly articulated goals that relate to the ultimate goal of Tox21—hazard rankings *in vitro* that predict hazard rankings *in vivo.*

Chemical Selection Working Group (CSWG)

It was noted that there are currently some 80,000 chemicals in commerce, with very little documented toxicity data. It was agreed that the same is probably true of the commercially available compounds.

Another point of discussion was the stereochemistry of the compounds in the library. In general, the compounds are racemic, but in a few cases, stereoisomers have been included in different wells.

Solubility and volatility of test chemicals can affect the concentration in the test well, and it was recognized that current analytical techniques cannot determine the exact concentration in the test well in the actual assay. It was generally agreed that this is a “huge” issue, but that because it requires exact quantitation of very small amounts of compound in low microliter volumes, it is beyond the limits of current analytical technology. The potential use of carrier proteins to increase solubility has not been investigated.

Another point of discussion was the diversity and purity of the compounds that have been chosen. The term diversity is generally used in pharmaceutical research to denote compounds that are substantially different from one another and represent coverage of all possible synthetic chemicals. The compounds for Tox21 were not chosen to create maximal diversity but because of some connection to toxicity or toxicity testing. They should be said to represent a broad representation of toxicology-related compounds rather than a diverse set of compounds. It was suggested that the CSWG consider including mixtures in the library and adding some common nutrients, such as glucose or vitamins, into the mix of chemicals.

The working group has a goal of 90% purity for all compounds and has a quality control (QC) process in place to monitor the purity of a representative set as well as the purity of all active compounds.

The BSC was very supportive of the efforts of the CSWG, in particular, the inclusion of the 100 failed pharmaceutical compounds. Questions were raised about the logistics and QC procedures. These focused on the plate creation methodologies and the need to positively identify the compound and its concentration in all active wells. There was strong support for the creation of a water solubilized library.

Assays and Pathways Working Group (APWG)

The first point of discussion revolved around the use of full-length and ligand-binding domain (LBD) assays in related nuclear receptors. There has been much discussion of that issue within the working group and in Tox21 itself regarding the benefits or drawbacks of using either approach, with two distinct schools of thought. Thus, the decision was made to run both, on both the estrogen receptors (ER) and androgen receptors (AR), and to look to the data to resolve the questions involved. The BSC advocated the use of full-length versus truncated receptors, as they could provide pieces of the puzzle otherwise left missing.

There was concern that the APWG seemed to have been concentrating on the mechanics of the screening, rather than seeing whether it's actually predictive in terms of adverse outcomes and useful in risk assessment. It was recommended that the working group take some model pathways and run them all the way through the system.

There was considerable discussion about the cell lines chosen for screening and their implementation. Coverage of the overall cell space and the chemical space, and how many processes in the cell can be, or need to be assayed. The goal for the screening taking place at the NCGC is to find assays that cover broad space, integrating multiple pathways. Some active compounds may be picked out for ToxCast™, which has the ability to run some 500 assays in a relatively short period of time, compared to a much smaller number in Tox21. As space is covered, the number of assays that are needed can be pared down, as more information becomes available on which assays are necessary and which may not be. It is difficult to estimate the number needed but it is estimated that at least 100 or more assays are needed.

Another question was whether the cell lines had been selected to represent specific organs or organ systems. Early in the process, several different cell types were screened, and the original 13 cell lines screened were chosen because they came from organs that were typical sites of toxicity from xenobiotic exposures. It has been necessary to prioritize, so the number of cell types has remained small to enable comparisons across assays. The HEPG2 cells approach was supported, but there was concern about the choice of culture medium for those assays, due to issues related to mitochondrial activation. It was recommended that, in general, the cell lines be selected keeping in mind the goal of evaluating human health effects.

The BSC was supportive of the APWG but had some criticisms and suggestions. The working group's goals and responsibilities are clearly articulated and align well with the Tox21 mission, with a strong group and a strong group of advisors. There is a concern that the group placed a premature emphasis on the development of high-throughput methods, with assays being chosen largely on the basis of whether they would work in the high throughput format, rather than their predictive value. The working group should consider middle ground, medium throughput assays that may be useful and could help drive innovation in the development of high throughput assays, particularly those with clear linkages to specific tissue level effects or disease states, or of known predictive value.

The plan to use the quantitative high throughput screening (qHTS) approach was supported but it will be important for the group to consider alternate formats and carefully balance cost and time versus productivity in the choice of which assays to miniaturize to the 1536-well format. The APWG also needs to ensure cell quality before and during the screen.

Informatics Working Group (IWG)

The BSC was very supportive and congratulated the IWG on their approach to a daunting challenge. There was concern about the method of dropping outliers in the curve class calculations; for example, there should be a limit on how many outliers can be dropped. The IWG is also encouraged to link as strongly as possible their work on human prediction into the existing FDA quantitative structure activity relationship (QSAR) models.

The BSC thought the IWG needs stated objectives and milestones, and needs to focus more on validating rank ordering of compounds. The CSWG and the IWG should be working closely with the TTWG. The BSC would like to see stated decision points about how and when the decision is made about correlations between *in vitro* and *in vivo* outcomes.

A concern was raised that relative to the resources placed in data generation, the IWG was under resourced. It appeared that that one or two FTE's at most were devoted to the analysis of massive amounts of high dimensional data across the platform. This would be expected to be a bottleneck, limiting progress towards prediction.

Targeted Testing Working Group (TTWG)

In general, there were a number of favorable comments expressing the view that the presentation was well constructed, that incredible progress had been made by the working groups since 2005, and that the presentations were vey exciting and a great start to the program. It was stated that this was absolutely something that should be done.

It was felt that demonstration of some specific applications of the data should be accomplished in the near term. The disease specific pathway approach was viewed favorably, although questions were raised as to the choice of cell types and the eventual need to expand the number of cell types used.

There was significant skepticism about the reverse toxicokinetics approach. The relationship between media and cells is not equivalent to that of blood to tissues. A pharmacokinetics analysis in animals to measure blood levels should be conducted to determine the highest blood level prior to the targeted testing.

Concern was expressed about timing, lack of milestones, and the potentially adverse effects on the program from a productivity perspective. Additional comments focused on the use of assays from a "bottom-up, the biology up" approach with selection focused on sensitivity, specificity, and rank order potency rather than simply being chosen because they are available in a high throughput capacity format. Correlation of results or the predictive value of results from *in vitro* assays with *in vivo* results is less than optimal, and the view was expressed that a focus on compounds which exhibited activity in the cell-based assays be coupled with pharmacokinetic analysis of levels in animals. It was clear from the discussion that the overarching consideration that formed the basis for these concerns is that the program, which is viewed as encompassing all the components for success, i.e., the breadth, depth, collaboration, and leadership, not be compromised by a perceived lack of productivity. Questions regarding the governance of the program were raised. It was pointed out that specific organizational structures, defined meetings, reporting, responsibilities, and decisions on how to proceed with the various components of the program were in place, and that a group at the NIH was responsible for implementing the Government Performance and Response Act to provide oversight on the performance of such large initiatives.

The program boils down to whether or not the TTWG, in close collaboration with the other groups, adequately and appropriately tests the stated hypothesis of the program: that *in vitro* systems can be predictive of *in vivo* outcomes. The most significant concern with the TTWG is that the working group has yet to rise to meet its lofty place in the program. Their hypotheses were very limited, and did not meet the standard set by the program's goals.

Program Goals

In addition to the overall program goal of developing *in vitro* methods that predict *in vivo* outcomes, there was a suggestion to conduct some intermediate and short-term applications of the data that would demonstrate the utility of the program in the short term and provide a platform for communicating with scientists, policy-makers, and the public about the program, in order to ensure its continued support. For example:

- Much frustration has been expressed at the large data gaps we have for commercial chemicals, including many consumer product chemicals. A strength of the biomolecular screening program is in being able to provide

something where we have had nothing. Even though we don't fully understand the implications of all the test findings, it would be a useful exercise to demonstrate ways to use the data anyway. For example, perhaps there are interesting ways to use these data in product formulation, or development of safer/greener chemicals, and in chemical prioritization for further study or regulation. Perhaps they could be used in EPA's Design for the Environment Program in the context of identifying the "best" chemicals in a particular functional class. It would serve the program well to encourage and document these types of activities.

- Another area where data could be used now would be as a basis for selecting chemicals for *in vivo* study and directing the study design, including potentially adding/leaving out certain endpoints or tests to increase efficiency.
- One presenter (David Dix) mentioned that the *in vitro* assays appeared to show mechanisms of concern for reproduction when multi-gen studies for the same chemicals were less sensitive. This finding could be a basis for adding new endpoints to the multi-gen study design in order to increase its sensitivity.
- Can findings from the screening program be used to develop a new "early effect" marker and deploy it in an occupational epidemiology study?
- Consider using HTS to address mixtures questions, e.g., by testing many different herbal preparations of the same product (e.g., ginseng) to see how and why their activities vary and to pick preparations for *in vivo* tests.
- Use data from this program in a specific prioritization effort, e.g., to prioritize revision of occupational exposure limits, or endocrine-disruptor chemical (EDC) testing.
- Conduct a comparison of utility of HTS data and *in silico* data on potential toxicity. Demonstrate that HTPS data can improve in silico methods.

To make these applications happen would require extending the scope of the working groups. These projects would each be a good platform for communication and outreach related to the program.

Tox 21 Activities

Reviewers: Richard Miller (lead), Russel Cattley, Elaine Faustman, Nicholas Jewell, Lisa Minor, Judith Zelikoff

The NTP's BSB Tox21 activities comprise an exciting collection of mostly interrelated, collaborative research initiatives designed to enhance the NTP's ability to characterize hazard, and potentially support translational efforts, and ultimately further inform risk assessment. These activities consist of the *C. elegans* (WormTox) Screening Program, an inter-individual susceptibility collaboration with the University of North Carolina at Chapel Hill (UNC), NTP archival tissue mining, and a nascent bioinformatics-based effort to identify disease and toxicity pathways. There is an overarching expectation that these efforts will become ever more integrated as the activities progress and data emerges to drive the linkages.

Caenorhabditis elegans "Worm Tox"

The WormTox group is considered a world-class laboratory that in many ways is leading the field of *C. elegans* research. The WormTox program is working to leverage the nematode's well developed organ systems, well known conserved genome, and well characterized development and reproductive cycles. The investigators have also established the utility of transgenic nematodes. One of the major thrusts of this activity is in determining the nematode's responsiveness to known toxicants relative to other non-mammalian systems (zebrafish), *in vitro* systems, as well as standard mammalian systems, with emphasis on neurotoxicants and reproductive toxicants.

There are several areas of emphasis with regard to suggestions for the future. The investigators are encouraged to consider focusing on the utility of the nematode model in a problem-solving mode or for hazard characterization, rather than screening, in light of the lower throughput nature of the organ-specific assessments, high potential for transgenic models to be used in this capacity, as well as acknowledged difficulties in understanding absorption, distribution, metabolism, and elimination (ADME) of test chemicals in the model in high throughput mode. In addition, the investigators are encouraged to further strengthen their integrative efforts with related activities in Tox21 and elsewhere in NTP (e.g., the Mouse Methylome Project and APWG). Some specific suggestions underpinning general strategic commentary include consideration of reproductive hormone receptors, 3D and 4D mapping of organ/cell specific responses, and assessment of nephro-, hepato- and respiratory toxicants.

Mechanisms of Inter-individual Susceptibility

A collaborative project with investigators at UNC centers on probing mechanisms of inter-individual susceptibility using population-based experimental approaches, specifically, genomic assessments coupled with *in vitro* models using various toxicants, with special focus on either a human lymphoblastoid cell line or across a series of cell lines against key toxicity mechanisms (e.g., apoptosis). The latter approach can be used to detect subsets of chemicals for which the variability of response is large and can subsequently be explored in follow up genetic studies. In addition, the investigators are working to link the efforts and data coming from the human lymphoblastoid cell line with similar efforts using mouse primary cells or cell lines.

The investigators are aware of, and acknowledge, the major concern of this activity regarding poor metabolic competency of their test model system, which could underpin some of the variability issues that were presented and have been raised in the literature by other researchers in this field. The investigators are encouraged to consider primary human cells, such as primary hepatocytes cultured in a time frame and under conditions where metabolic competency closely approximates *in vivo* metabolic capability.

Mining of the NTP Tissue Archives

Mining of the NTP tissue archives for gene signatures is another major Tox21 activity and seeks to utilize the extensive, well characterized, and annotated studies comprising outcomes from thousands of studies, representing a multitude of chemical classes and toxicity phenotypes. This project has high potential for furthering our understanding of toxicologic and carcinogenic mechanisms, and linking with other Tox21 activities such as the Mouse Methylome Project, inter-individual susceptibility effort, and other *in vitro* efforts, such as ToxCast™. The investigators used samples from the AFB1 bioassay as proof of principle with encouraging preliminary results. This project also has high potential to leverage the recently acquired DrugMatrix® transcriptomic database. The investigators are assessing protein expression in parallel, utilizing an antibody array approach.

Overall, the BSC had strong enthusiasm for this activity. The investigators are encouraged to set prospective performance and decision making/stopping criteria with regard to acceptable yields, reproducibility, and quality. In addition, they are encouraged to provide clarity on whether genes identified represent initiating events or adaptive responses, given that the time points available in most bioassays are weeks to months after the onset of exposure.

The investigators acknowledge that using immunohistochemistry (IHC) to assess protein affords the ability to localize the protein expression to a specific tissue/cell type but have chosen to use the antibody array as a starting place, with the intention that IHC can be used to drill down on specific proteins of interest on a case-by-case basis. As well, the investigators acknowledge that disconnects between protein and mRNA expression will be likely. The BSC encouraged that special attention should be given in the near future to translatable/mechanistic miRNA assessments to complement the mRNA and protein assessments.

Bioinformatics-based Approaches

Lastly, the BSB is applying a bioinformatics-based approach to identify assays that can be used to query human health effects. This activity is in its very early stages and is intending to develop assays by working backwards from disease—gene associations using type I diabetes as a proof of principle. Druggability of the gene product will be used as key criteria for the determining the genes that are ultimately pursued. It is hoped that susceptibility or early stage genes, and relevant *in vitro* assays reflecting these critical genes, can be developed as part of a battery of predictive screening tools.

The investigators should be open-minded about the fundamental underlying premise that the genes determining toxicity represent a subset of the druggable targets. In addition, the investigators should consider chemical signatures as a way to triangulate on determinant genes. And related to this, the investigators should be mindful of tissue specificity of genes, diseases, and pathways of interest as well as whether these genes/pathways represent adaptation.

The Mouse Methylome Project

The Mouse Methylome Project is an ambitious proposal intended as the NTP's first major step towards understanding the impact and toxicologic relevance of chemically induced epigenetic changes by first understanding differences in DNA methylation amongst mouse strains commonly used in toxicologic research. The liver is proposed as the first organ for focus due to its frequency as a target organ for toxicity and carcinogenicity, mouse strain differences in sensitivity, as well as previous evidence suggesting epigenetic components to hepatocarcinogenic mechanisms.

The proposal's specific aims are clearly articulated and the technical approaches generally seem appropriate to achieve the expected outcomes. The investigators are aware of the variability, as well as gender and developmental stage/age dependency of DNA methylation status, and intend to take that into account in the number of animals per strain that are initially assessed, but plan to limit the number of time points on the Tier one assessment. Once promising loci are identified, the investigators, rather than conducting extensive time course studies at the outset, plan to examine specific loci of interest using archival tissues. The investigators are encouraged to continuously bear in mind the dynamicity of DNA methylation as the project moves forward.

One of the major opportunities for furthering this project and integrating it with other efforts as part of Tox21 is in leveraging the extensive archival samples/data that exists in the NTP. However, there may be significant technical challenges in assessing methylation status from archival tissues, and the investigators have acknowledged this and are working to address.

Without overextending the scope of the project in its early phases, the investigators are encouraged to consider other key determinants of the transcriptome, such as histone methylation, miRNAs, and other transcriptional regulatory mechanisms. In addition, assuming the mouse liver as proof of principle has a positive outcome, the investigators should consider target organs/toxicities with a more clear relevance to humans and move to incorporate corresponding translatable endpoints (e.g., miRNAs). While the experimental design, sampling handling, and data acquisition aspects of the proposal are considered sufficient, is the BSC has concern that the biostatistical tools/approach is not as well defined—the principle investigators should work to address this issue before extensive amounts of data are generated.

In summary, the BSC had strong enthusiasm for this project and this proposal is rated moderate to high. Some of the reservations being expressed are as a consequence of uncertainty around specifics of the biostatistical/analytical approach and human relevance of initial outcomes, and these will hopefully be clarified and elaborated upon as early data emerge and the project moves forward.

The Future of Tox21 at the NTP

In summary, the BSB's Tox21 activities offer great promise towards enhancing the NTP's abilities in testing prioritization, refining hazard characterizations, broadening

the range of predictive assays available, as well as bolstering mechanistic understanding of toxic effects detected in routine bioassays. The BSC felt that across the activities, further attention to, and specificity of statistical approaches should be established. As well, greater emphasis on translatability/biomarkers and risk assessment is encouraged. The BSB should be vigilant in its goals to fully integrate the various programs, as an integrated approach moving forward will greatly increase the chances of success of each of the individual projects and ultimately represents the greatest strength of the overall initiative. Overall, this is an exciting initiative that holds great promise for advancing the mission of the NTP.

Discussion: The overall consensus of the BSC is that Tox21, through a memorandum of understanding involving multiple agencies, presents an incredible opportunity having unique attributes and assets with a very rare collection of intellectual and technical capital arrayed around an important central question. The NTP and its partners are in the position of potentially having the greatest impact on predictive toxicology and risk assessment of any initiative to date. There was a great deal of excitement among the members of the BSC, and it is imperative that this excitement not only be continued but be heightened among the participants.

Both the progress and continued excitement will need to be continuously bolstered by a progressive evolution in the structure and dynamics of the Tox21 initiative with the overall goal of achieving the aims of the project. This unique project presents an extraordinary opportunity to combine the considerable physical, intellectual, and leadership resources of the various participant agencies and to engage such resources in a directed manner. The multiple questions and concerns brought forward by the BSC reflect the strong scientific interest, enthusiasm, and desire to see this program succeed. It is envisioned by all, that such comments will assist and facilitate progress as the initiative develops. As with all major scientific initiatives, there is much to be defined and correspondingly much to be learned. The continued progress in technology development, construction of data repositories, as well as data interrogation and analysis will present one of the interesting challenges as the initiative progresses.

The very uniqueness of this initiative is both a strength and a potential weakness. Overall, the program will need to provide leadership, develop cohesion, achieve agreement, and demonstrate progress, while guarding against divisiveness and diversion resulting in a loss of focus. To accomplish this, the program will need to remain dynamic, flexible, selective, and perseverant. The BSC wishes the NTP and participating agencies much success in the accomplishment of the goals of this initiative.